EFFICACY OF PROTEIN SUPPLEMENTATION ON FRAILTY IMPROVEMENT, MUSCLE STRENGTH, AND PHYSICAL PERFORMANCE AMONG PRE-FRAIL OLDER ADULTS IN SELANGOR, MALAYSIA

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the Requirements for the Degree of Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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July 2024

Chairman : Siti Nur'Asyura binti Adznam, PhD
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There will likely be 2 billion people over the age of 65 in the globe by 2050, which will have a significant impact on how health and social care are planned for and provided. Pre-frail people are more vulnerable to increasing frailty, hospitalization, falls, deteriorating disability, and mortality than robust adults, even though they are less vulnerable than frail older people. The efficacy of protein supplement (PS) in improving muscle strength, physical performance, and body composition in older adults has been widely promoted. However, the results of randomized clinical trials in this regard have been inconsistent, and a limited number of studies targeted pre-frailty status. Hence, the objective of the study was to evaluate the impact of protein supplements (PS) on older people's pre-frailty status, muscle strength, physical performance, and dietary intake. This trial's primary outcomes were frailty status and its phenotypes. Secondary outcomes included handgrip strength, physical performance, body composition, and nutritional intake. This study was 12 week-double-blinded, randomized, parallel, placebo-controlled clinical trial with a sample of 40 pre-frail older adults aged 60 years and above, divided into the interventional

group: participants received 30 g of whey protein isolate and the placebo (maltodextrin) group. Assessments were conducted at baseline (week 0), middle (6th week), and at the end of the trial (12th week) and included a Frailty screen, handgrip strength, the Short Physical Performance Battery test (SPPB), and a 3-day dietary record to get the macronutrient change during the trial. The data were analyzed using the Chi-square test, Independent t-test, Cochran's Q test, Mann-Whitney test, and repeated measures ANOVA using SPSS version 26, with significance determined if the p-value was less than 0.05. In the Protein Supplementation Group (PSG), a noteworthy decrease was observed in the number of pre-frail individuals with a score of 2, declining from 70% (n=14) to 15% (n=3) by week 12. However, these changes did not reach statistical significance between the groups. Muscle weakness and low activity were prevalent among participants, with PSG showing some -but not significant- improvement. Specifically, muscle weakness decreased from 40% (n=8) to 20% (n=4), and low activity decreased from 95% (n=19) to 30% (n=6) from baseline to week 12, although these changes were not statistically significant. Furthermore, significant time effects were observed for handgrip strength (HGS), indicating promising enhancements ($p = 0.043$), along with a notable trend towards a positive time*group interaction ($p = 0.095$). Moreover, protein intake demonstrated significant differences in time*group interaction within the interventional group, reflecting positive dietary changes ($p < 0.001$) by the end of the trial. Physical performance tests and body composition did not show significant differences between groups. The study revealed that whey protein supplementation did not produce significant changes in frailty status or physical performance among pre-frail older adults compared to the control group. However, promising improvements were noted in frailty phenotypes and handgrip strength, suggesting that longer intervention periods and larger sample

sizes may yield more noticeable results. The study also highlights the critical role of participants' nutritional status and consistent protein intake in determining the efficacy of such interventions. Despite the lack of statistically significant effects, the comprehensive nature of this study contributes valuable insights to the existing literature, emphasizing the necessity for further exploration of individual responses and considerations of timing and dosage in nutritional interventions for older adults.

Keywords: Physical Performance, Frailty, Older Adults, Protein Supplement, Muscle Strength.

SDG: GOAL 3: Good Health and Well-Being.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KEBERKESANAN SUPLEMENTASI PROTEIN TERHADAP PENAMBAHBAIKAN KEUZURAN, KEKUATAN OTOT, DAN PRESTASI FIZIKAL DALAM KALANGAN WARGA EMAS PRA-UZUR DI SELANGOR, MALAYSIA

Oleh

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Pada tahun 2050, kemungkinan akan ada 2 bilion orang yang berusia lebih dari 65 tahun di seluruh dunia, yang akan memberi kesan yang ketara terhadap cara perancangan dan penyediaan penjagaan kesihatan dan sosial. Warga emas pra-uzur lebih berisiko terhadap peningkatan kelemahan, kemasukan hospital, kemungkinan terjatuh, kehilangan upaya yang merosot, dan kematian berbanding dewasa yang tidak uzur, walaupun mereka kurang berisiko berbanding warga emas yang uzur. Keberkesanan makanan tambahan protein whey (WPS) dalam meningkatkan kekuatan otot, prestasi fizikal, dan komposisi tubuh badan warga emas telah dipromosikan secara meluas. Walau bagaimanapun, keputusan ujian klinikal rawak berkenaan dengan perkara ini tidak konsisten dan hanya sebilangan kecil kajian memberi tumpuan kepada status pra-uzur. Oleh itu, objektif kajian ini adalah untuk menilai impak suplemen protein (PS) terhadap status pra-uzur, kekuatan otot, prestasi fizikal dan pengambilan diet dalam kalangan warga emas. Hasil utama ujian ini adalah status keuzuran dan ciri - ciri fenotipnya. Hasil kedua termasuk kekuatan genggaman tangan, prestasi fizikal, komposisi badan, dan pengambilan nutrisi. Kajian ini adalah ujian

klinikal berbilang buta, rawak, selari, terkawal plasebo dengan tempoh 12 minggu dengan sampel 40 warga emas pra-uzur lemah yang dibahagikan kepada kumpulan intervensi: peserta menerima 30 g protein whey terasing dan kumpulan plasebo (maltodekstrin). Penilaian dilakukan pada permulaan (minggu 0), pertengahan (minggu ke-6), dan pada akhir ujian (minggu ke-12) dan termasuk saringan keuzuran, kekuatan genggaman tangan, ujian Bateri Prestasi Fizikal Pendek (SPPB), dan rekod pemakanan selama 3 hari untuk mendapatkan perubahan makronutrient sepanjang tempoh ujian. Data dianalisis menggunakan ujian Chi-Square, ujian-t sampel tidak bersandar, ujian Q Chocran, ujian Mann-Whitney dan ANOVA ukuran berulang menggunakan SPSS versi 26, dengan signifikan ditentukan melalui nilai p yang kurang daripada 0.05. Tujuan utama kajian ini adalah untuk menilai impak Suplemen Protein Whey (WPS) terhadap status keuzuran dan fenotipnya. Dalam Kumpulan Suplemen Protein (PSG), terdapat penurunan yang ketara dalam bilangan individu pra-uzur dengan skor 2, yang menurun daripada 70% (n=14) kepada 15% (n=3) menjelang minggu ke-12. Walau bagaimanapun, perubahan ini tidak mencapai signifikan statistik antara kumpulan. Kelemahan otot dan aktiviti rendah adalah lazim di kalangan peserta, dengan PSG menunjukkan sedikit peningkatan. Secara khususnya, kelemahan otot berkurangan dari 40% (n=8) kepada 20% (n=4), dan aktiviti rendah berkurangan daripada 95% (n=19) kepada 30% (n=6) dari titik permulaan hingga minggu ke-12, walaupun perubahan ini tidak signifikan secara statistik. Selain itu, terdapat kesan masa yang signifikan yang diperhatikan untuk kekuatan cengkaman tangan (HGS), menunjukkan peningkatan yang memberansangkan ($p = 0.043$), bersama dengan trend positif terhadap interaksi masa*kumpulan ($p = 0.095$). Selain itu, pengambilan protein menunjukkan perbezaan yang signifikan dalam interaksi masa*kumpulan di dalam kumpulan intervensi, mencerminkan perubahan pemakanan yang positif ($p < 0.001$)

menjelang akhir ujian. Manakala, ujian prestasi fizikal dan komposisi badan tidak menunjukkan perbezaan yang signifikan antara kumpulan. Kajian ini menunjukkan bahawa suplemen protein whey tidak menghasilkan perubahan yang signifikan dalam status keuzuran atau prestasi fizikal dalam kalangan warga emas pra-uzur berbanding dengan kumpulan kawalan. Walau bagaimanapun, terdapat peningkatan yang memberangsangkan didilihat dalam fenotip keuzuran dan kekuatan genggaman tangan, yang mencadangkan bahawa tempoh intervensi yang lebih panjang dan saiz sampel yang lebih besar mungkin akan memberikan hasil yang lebih ketara. Kajian ini juga menekankan peranan penting status pemakanan peserta dan pengambilan protein yang konsisten dalam menentukan keberkesanan intervensi sebegini. Walaupun tiada kesan yang signifikan secara statistik, kajian ini menyumbang pandangan berharga kepada literatur sedia ada, menekankan keperluan untuk penyelidikan lanjut terhadap respons individu dan pertimbangan masa dan dos dalam intervensi pemakanan untuk orang tua.

Kata Kunci: Prestasi Fizikal, Kelemahan, Dewasa Lebih Tua, Tambahan Protein, Kekuatan Otot.

SDG: MATLAMAT 3: Kesihatan dan Kesejahteraan Baik.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

PS	Protein Supplementation
RCT	Randomized Controlled Clinical Trial
PSG	Protein Supplement Group
CG	Control Group
SPPBT	Short Physical Performance Battery Test
BMI	Body Mass Index
MUAC	Mid-upper Arm Circumference
CC	Calf Circumference
TBF	Total Body Fat
SMM	Skeletal Muscle Mass
LLM	Leg Lean Mass
HIC	High-income Countries
COPD	Chronic Obstructive Pulmonary Disease
CKD	Chronic Kidney Diseases
DM	Diabetes Mellitus
RT	Resistance Training
1RM	1-Rebitition Maximum
LBM	Lean Body Mass
WPS	Whey Protein Supplement
MM	Muscle Mass
6MWT	6-minutes Walking Test
SPIRIT	Standard Protocol Items: Recommendation for Clinical Trial
FMC	Family Medicine Clinic
ACCI	Age-adjusted Charlson Co-morbidity Index
ITT	Intention-to-treat Analysis

ANOVA	Analysis of Variance
GLM	General Linear Model
GCP	Good Clinical Practice
CONSORT	Consolidated Standards of Reporting Trials
HGS	Handgrip Strength
GS	Gait Speed
CST	Chair-stand Test
4MWT	4-meters Walking Test
RDA	Recommended Dietary Allowance

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

1.1.1 Frailty

Frailty is characterized as a clinically identifiable state in which older persons exhibit greater vulnerability due to age-related decreases in physiologic reserve and function across several organ systems, making it more difficult for them to manage chronic or acute stresses (Corral-Pérez et al., 2023).

The phenotype model of frailty developed by Fried and colleagues (2001) considers frailty as a biological syndrome and classifies an individual as frail when three or more of the following five physical components are present: shrinking (unintentional weight loss of 4·5 kg or more in the last year), weakness (low grip strength), exhaustion (self-reported), slowness (slow walking speed), and low physical activity. A classification of pre-frailty is given when only one or two of these physical components are present; robust is the classification given to an individual with no frailty components present (Fried et al., 2001). These indicators have been found to be risk factors for disability and mortality among older adults (Dodds & Sayer, 2016). The causes of frailty syndrome are not fully known. As the person advances in age, their physiological reserves decline. There are many risk factors of frailty syndrome. Well-known risk factors such as high age and sex (women) and several social and behavioral factors (lower education, lower marital status, living arrangements, bad lifestyle, smoking status, and alcohol use) are significantly associated with frailty (Leng et al., 2014).

According to estimates, 10.7 % of community-dwelling older adults in developed countries are frail, and 42% are in the transitional state of pre-frailty, in which a person has some frailty characteristics but can respond to injury, disease, or stress with a chance of complete recovery (Frost et al., 2017). Consistent with the global trend, frailty among Malaysian seniors has arisen as a source of worry. Despite the scarcity of published local studies, a large-scale study conducted among community-dwelling elderly in an urban area of Malaysia discovered that pre-frailty and frailty afflicted 72.8 % and 15.9 % of older persons, respectively (Nohan et al., 2020).

Though less susceptible than frail older individuals, pre-frail persons are at a higher risk of increasing frailty, hospitalization, falls, deteriorating disability, and mortality than robust adults (Corral-Pérez et al., 2023; Fried et al., 2001; Werner et al., 2022). As the number and proportion of individuals aged 60 and over rises in populations throughout the world (World Health Organization, 2015), there is growing concern about rising health and social care expenditures.

1.1.2 Protein Intake and Muscle Health

Adequate protein intake is essential for preserving muscle mass and strength in aging adults. The standard recommended dietary allowance (RDA) for protein, set at 0.8 g/kg/day, is considered insufficient for older adults due to age-related anabolic resistance, which reduces the efficiency of muscle protein synthesis (MPS). Recent expert guidelines suggest that older adults should aim for higher protein intakes, between 1.2 to 1.5 g/kg/day, to effectively maintain muscle health and mitigate muscle loss with aging (Bauer et al., 2013; Deutz et al., 2014). This recommendation accounts

for the physiological decline in muscle protein synthesis response to dietary protein, which can be countered by increased intake of protein-rich foods or supplements

Despite these recommendations, many older adults struggle to meet even the basic RDA due to reduced appetite, physical limitations, and other age-related factors (Landi et al., 2013). Whey protein supplementation, known for its high digestibility and leucine content, has been shown to be particularly effective in stimulating muscle protein synthesis, both at rest and after resistance exercise (Devries & Phillips, 2015; Morton et al., 2017). Evidence suggests that whey protein offers superior benefits over other protein sources such as soy and casein due to its rapid absorption and high leucine concentration, which is a critical amino acid for muscle protein synthesis (Phillips, 2016).

Focusing on pre-frail older adults, who are in a transitional stage between robust health and frailty, is crucial because early intervention can help prevent further decline. Targeted protein supplementation strategies, including whey protein, provide a simple, cost-effective method to enhance dietary protein intake, support muscle function, and potentially reverse the progression towards frailty (Fried et al., 2001; Landi et al., 2016). This underscores the need for strategic nutritional approaches to support healthy aging in this vulnerable population.

1.2 Problem Statement

Recently, many researchers have focused on protein supplements for older adults in an attempt to improve their quality of life. Observational studies show that a high dietary protein intake is associated with a lower loss of lean body mass and less frailty

compared to a low protein intake (Houston et al., 2018). Data from stable isotope studies show that the intake of dietary protein and/or amino acids stimulates muscle protein synthesis and decreases muscle protein breakdown, resulting in net muscle protein balance and muscle mass accretion (Pennings et al., 2011). However, increasing dietary protein presents an increase in muscle mass after at least 3 months of protein supplementation (Bauer et al., 2013; Rondanelli et al., 2013). A similar discrepancy exists with muscle strength, where beneficial effects are observed (Tieland et al., 2012).

According to the United Nations World Ageing Population report (2017), the proportion of individuals aged 60 and above in Malaysia is projected to rise to 23% by 2050. This demographic shift, which would make nearly a quarter of the population older adults, underscores the importance of addressing age-related health issues. Additionally, over 22% of Malaysian elderly men and 20% of elderly women are living independently or with a spouse, increasing the risk of poor dietary habits, low food intake, and physical inactivity, all of which contribute to frailty. Given these trends, there is a pressing need to address pre-frailty among Malaysian older adults through targeted nutritional interventions, such as protein supplementation (PS).

To our knowledge, most nutritional and educational interventions in Malaysia have focused on frail older adults (Murukesu et al., 2020; Suffian et al., 2020), with fewer studies targeting pre-frail individuals (Badrasawi et al., 2016). There is currently a lack of research on the specific use of protein supplements to improve frailty phenotypes and related outcomes—such as body composition, nutritional status, physical activity, muscle mass, and strength—in pre-frail Malaysian older adults. This study seeks to address this gap by investigating the effects of targeted protein supplementation on

pre-frailty improvement, offering the potential for health promotion and disability prevention.

Pre-frailty represents a critical intervention opportunity because older adults in this condition are more likely to revert to robust health compared to those who are already frail (Gill et al., 2006), Effective interventions at this stage could reduce the likelihood of further deterioration and dependency, improve health outcomes, and minimize the need for disability support and caregiving (Frost et al., 2017). Nevertheless, the majority of previous studies targeted frail or sarcopenic older adults, and a tiny number of studies test the effect of protein supplements on prefrail older adults, which is the primary focus of this study.

1.3 The Study Significance

This study is deemed necessary for several reasons, as evidenced by the existing literature. Firstly, there is a notable scarcity of research focusing on the effects of protein supplements on frailty phenotypes, which constitutes the primary outcome of this investigation. Additionally, studies targeting the enhancement of health status among pre-frail older adults, the primary population under examination in this study, are limited. Furthermore, the controversy surrounding the impact of protein supplements on muscle strength in older adults underscores the significance of this research, which aims to evaluate the efficacy of protein supplementation in enhancing muscle strength. Moreover, the existing body of research lacks sufficient randomized controlled trials (RCTs) to substantiate the need for revising the recommended dietary guidelines for protein intake among older adults. Lastly, to date, there has been a notable absence of frailty intervention studies specifically addressing the utilization of

protein supplements to ameliorate frailty status, phenotypes, and other relevant outcomes, such as body composition, nutritional status, physical activity level, muscle mass, and strength, among elderly individuals in Malaysia.

The proposed pre-frailty intervention can be easily adapted to regular practice in senior care health service environments and realistically utilized in everyday life. If this intervention is demonstrated to be successful, it holds significant potential for benefiting the elderly population by preventing the transition to frailty, ultimately improving their quality of life and reducing the burden on healthcare systems.

Avoiding frailty will play a significant role in supporting healthy and active ageing and has the potential to reduce negative health outcomes such as fall rates, hospitalization, and institutionalization, as well as the accompanying financial expenses. Improved frailty outcomes may also lead to enhanced function and quality of life for older persons, their family, and caregivers.

Furthermore, the study may point toward the application of dietary protein supplementation as a promising nutritional strategy to improve physical performance, attenuate the progression of frailty, and delay the onset of disability. This study may provide researchers with the opportunity to investigate the effect of greater dietary protein intake on muscular strength and physical performance.

Results from this study will add to the body of knowledge for health professionals, health promoters, and policymakers in this field, in creating strategies for any associated intervention program among the elderly to enhance health status.

1.4 Research Question

Does PS intervention significantly improve frailty status, muscle strength, physical performance, dietary intake and body composition of elderly people?

1.5 Research Objectives

1.5.1 Main Objective

To study the differences in frailty status, scores, and phenotypes between and within the protein-supplemented group (PSG) and control group (CG) for a duration of 12 weeks in the prefrail older adults.

1.5.2 Secondary Objectives

To determine the level changes between and within the interventional group (PSG) and control group (CG) for a duration of 12 weeks in the prefrail older adults in:

- Physical performance (SPPBT).
- Muscle strength including handgrip strength (HGS)
- Body Composition including anthropometric measurements (BMI, body part circumferences [mid upper arm circumference (MUAC) and calf circumference (CC)], total body fat percentage (%TBF), skeletal muscle mass SMM, and leg lean mass LLM).
- Dietary Intake including energy, carbohydrate, protein, and fat intake.

1.6 Research Hypotheses

a) There is a significant difference in frailty status, scores, and phenotypes in the interventional group (PSG) and control group (CG) from pre-intervention and post-intervention.

b) There is a significant difference in Body composition in the interventional group (PSG) and control group (CG) from pre-intervention and post-intervention.

c) There is a significant difference in muscle strength in the interventional group (PSG) and control group (CG) from pre-intervention and post-intervention.

d) There is a significant difference in the level of physical performance in the interventional group (PSG) and control group (CG) from pre-intervention and post-intervention.

e) There is a significant difference in energy intake in the interventional group (PSG) and control group (CG) from pre-intervention and post-intervention.

1.7 The Theoretical Framework and Flowchart of the Study

Figure 1.1 presents the conceptual framework of the study, showing how multiple factors contribute to frailty and sarcopenia, along with potential intervention. Inadequate nutritional intake is a key factor, leading to sarcopenia characterized by muscle loss and poor muscle quality, which impairs physical function. Whey protein supplementation is highlighted as a strategy to improve muscle quality and reduce the risk of frailty. Besides nutrition, other factors such as physical inactivity, chronic diseases (e.g., diabetes, cardiovascular conditions), and polypharmacy (use of multiple

medications) further exacerbate frailty by contributing to muscle weakness, fatigue, and functional decline. Psychosocial aspects like social isolation and depression can lead to sedentary behavior and poor diet, worsening physical decline. A critical aspect of frailty is the accumulation of deficits, where multiple physical, cognitive, and behavioral impairments gradually build up, leading to a cycle of reduced mobility, increased disability, and loss of autonomy.

Figure 1.2 outlines the trial design comparing two groups of pre-frail outpatient older adults: the Interventional Group (PSG) and the Control Group (CG). The Interventional Group receives a daily whey protein supplement (30g/day), while the Control Group receives a placebo supplement (30g/day). Both groups are monitored via daily message reminders, and retention of sachets is checked from the 6th to the 12th week to ensure compliance. Data collection is conducted at three time points: baseline, the 6th week, and the 12th week, with follow-ups for any adverse effects.

The primary outcomes of the trial focus on frailty status, scores, and phenotypes, based on the Fried criteria, including indicators such as weight loss, fatigue, muscle weakness, walking speed, and low activity. The secondary outcomes are categorized into four areas:

1. Muscle Strength, measured by handgrip strength.
2. Physical Performance, assessed through the Short Physical Performance Battery (SPPB), which includes balance tests, gait speed tests, and chair-rise tests.

3. Body Composition, evaluated by metrics like body weight, BMI, muscle mass, total body fat, leg lean mass, calf circumference (CC), and mid-upper arm circumference (MUAC).
4. Dietary Intake, which records energy, carbohydrate, protein, and fat consumption.

This design aims to assess the effectiveness of whey protein supplementation in improving frailty-related parameters compared to a placebo.

The study outcomes of frailty improvement, muscle strength, and physical performance were chosen because they address the key aspects of physical decline in older adults. Frailty improvement is essential as it reduces vulnerability to adverse health outcomes, improving quality of life. Muscle strength is directly linked to physical function, independence, and reduced risk of falls, while physical performance measures like gait speed and balance are critical indicators of mobility and functional health.

Dietary intake was included as a secondary outcome to monitor whether the intervention effectively supported the primary goals by ensuring adequate protein consumption, which is crucial for muscle maintenance and recovery. Thus, the study's design targets both immediate physical improvements and long-term nutritional adequacy, offering a holistic approach to combating frailty.

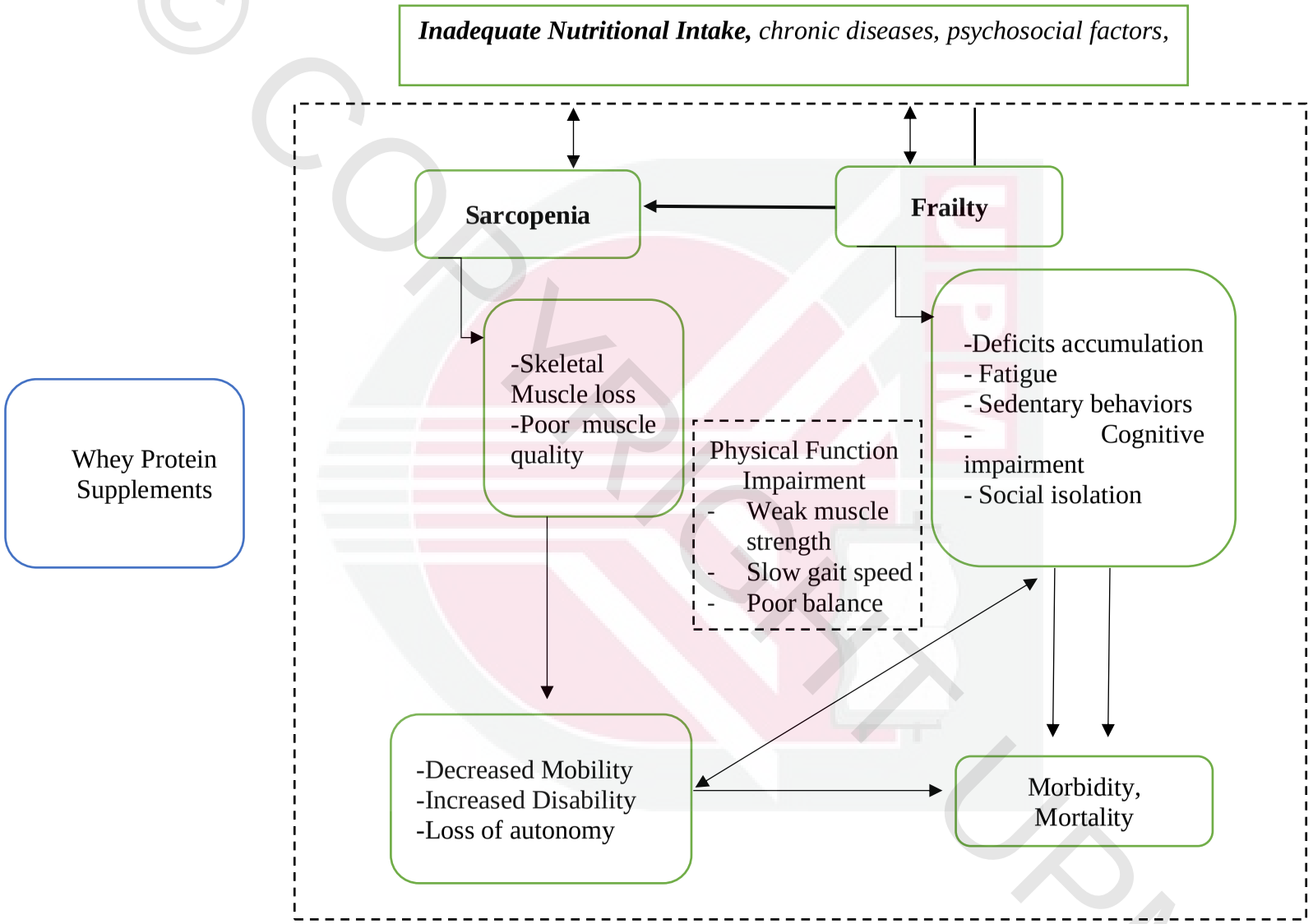


Figure 1.1: The theoretical framework of the study (Source: Cesari et al., 2014; Tessier & Chevalier, 2018).

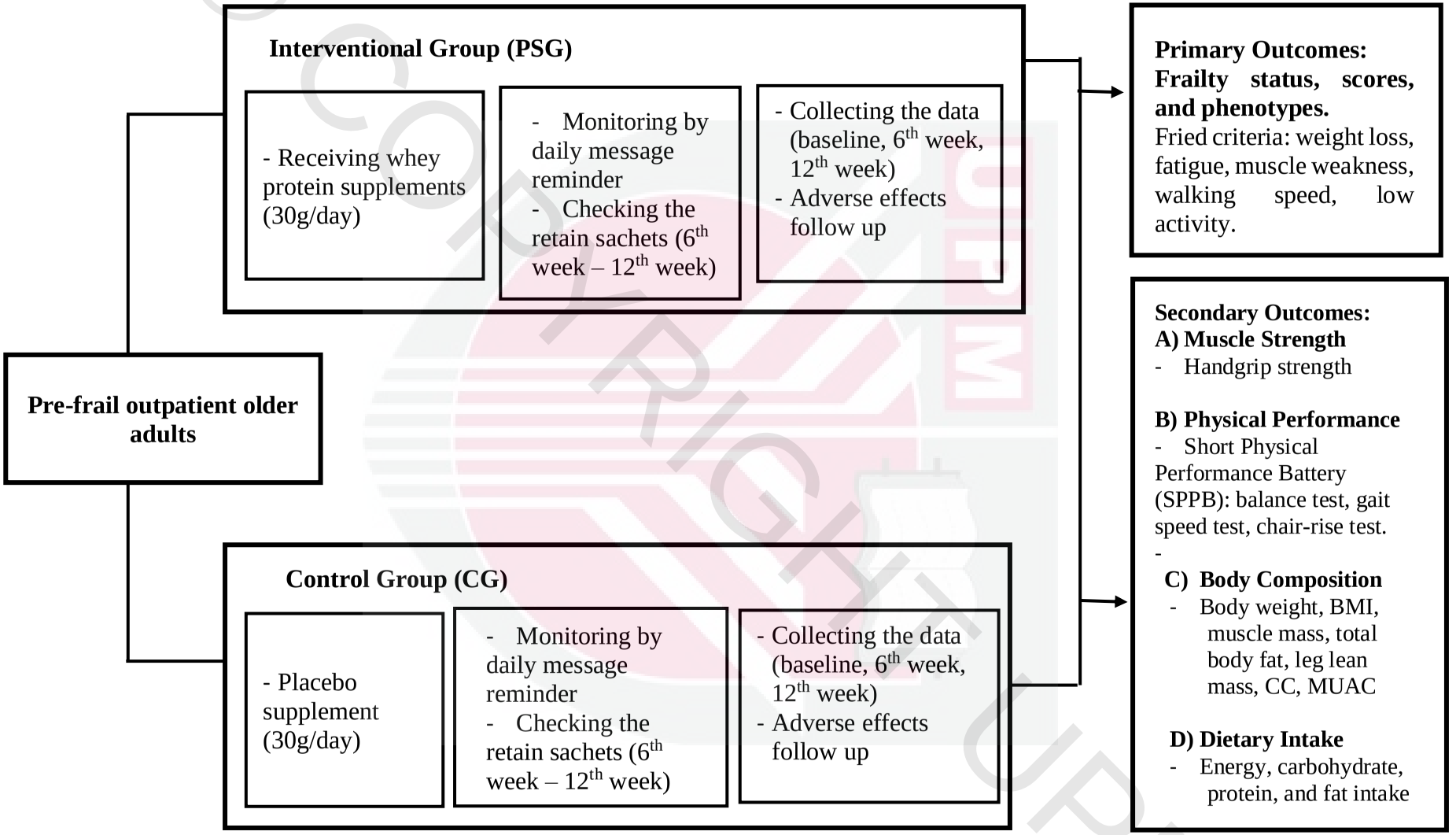


Figure 1.2: The flow chart of the trial.

CHAPTER 2

LITERATURE REVIEW

Frailty is a significant public health problem in older adults; these issues affect their physical function and quality of life, and increase their risk of falls, fractures and hospitalization (Corral-Pérez et al., 2023). Older individuals classified as pre-frail or frail face heightened susceptibility to various challenges, including limitations in physical functioning and mobility, a higher propensity for falls, feelings of loneliness, reduced social engagement and quality of life, disability, increased likelihood of hospitalization, institutionalization, and ultimately, mortality, in comparison to those considered robust (Werner et al., 2022).

2.1 Pre-frailty Prevalence

Prefrailty is an intermediary stage between frailty and non-frailty/strength that has a greater chance of developing frailty (Xue, 2011). Since the frailty state is determined using a variety of assessment methods, the majority of assessment methods have their own prefrailty status cutoffs. For Fried's phenotype, which we are using in our study, meeting one to two criteria out of five (shrinking, weakness, exhaustion, slowness, and low physical activity) is deemed prefrail (Fried et al., 2001). Prefrailty is also related to worse health outcomes, similar to frailty. A recent meta-analysis based on six prospective cohort studies indicated that prefrail patients had an elevated risk for the start of cardiovascular illnesses (Siriwardhana et al., 2018). Prefrail people are more prone to have persistent and novel depression symptoms, according to yet another longitudinal research (Feng et al., 2014).

In high-income countries (HIC), a significant amount of study on frailty has been undertaken. According to a 2012 systematic review included twenty-one studies applied Fried Frailty Index FFI method (Collard et al., 2012), the weighted prevalence of frailty in HICs is 10.7% and that of prefrailty is 41.6%. Another systematic review (Siriwardhana et al., 2018) conducted to study the prevalence of frailty and prefrailty in middle- and lower-income countries according to Fried phenotypes stated that the prevalence of frailty and prefrailty in community-dwelling older adults in these countries is 17.4% and 49.3%, respectively. These estimates appeared significantly higher than high-income countries' statistics, which may relate to the different levels of healthcare services and life expectancy.

According to the living status, the overall prevalence of pre-frailty among geriatric outpatients in more than 21 countries, as summarized by (Doody et al., 2022) is 25.8% and 41.6% as reported for community-dwelling older adults (Collard et al., 2012), and estimated to reach in nursing home residents at 40.2% (Kojima, 2015).

Malaysia seems to have the highest prevalence of frailty and prefrailty when compared to other Asian countries, as concluded by a scoping review conducted by (Embong et al., 2021), who retrieved a total of ten studies and found that the prevalence of pre-frailty is in the range of 57.9% and 72.8% while frailty was ranged from 5 to 56.5%. The scoping review by Embong et al. (2021) identifies key risk factors to this prevalence that include body impairments such as anthropometric and body compositions and body weakness, activity limitations, and personal factors such as gender (female), low income, unmarried, hospitalization in the previous years, poor self-rated health, co-morbidities, higher overnight fasting, higher energy intake, and depending on mobiles. which collectively contribute to a higher risk of frailty among

Malaysian older adults. Since Malaysia is moving very fast to become an aging population, which is estimated to reach 15% of the total population by 2030 (Embong et al., 2021), it is important to identify the possible healthcare and rehabilitation strategies and nutritional interventions to achieve successful and healthy aging.

Protein is essential for maintaining muscle health as it stimulates muscle protein synthesis, helping to preserve muscle mass even as aging diminishes the body's natural ability to build and repair muscle tissue (Phillips et al., 2016). Adequate dietary protein intake is particularly important for older adults because it helps counteract the muscle loss associated with aging. Studies suggest that higher protein consumption can contribute to better muscle strength, enhanced physical function, and reduced frailty, underscoring its significance in elderly nutrition (Bauer et al., 2013). Given that muscle health directly impacts physical performance and quality of life, the role of protein becomes vital not only in treating frailty and sarcopenia but also in preventive strategies aimed at maintaining muscle integrity during aging.

2.2 Protein Intake for Aging

The protein requirement for healthy people is defined as the smallest quantity of dietary protein intake required to balance nitrogen losses from the body and hence maintain body protein mass in individuals in energy balance who engage in moderate physical activity (Traylor et al., 2018). From another perspective, the human skeletal muscle protein cycle consists of both muscle protein synthesis and degradation (Reule et al., 2017). On the one hand, muscle hypertrophy occurs when the rates of protein synthesis exceed the rates of protein breakdown, which may be elicited by hyperaminoacidemia induced by dietary protein intake; on the other hand, an

inadequate protein intake results in a lower protein synthesis rate, which leads to net protein breakdown and muscle catabolism.

Numerous factors may influence protein intakes in older adults as shown in Figure (2.1), including reduced energy needs, genetic predispositions to low appetite, age- and disease-related anorexia, physical and mental disabilities that limit acquiring and preparing food, changes in food preference, dysphagia, dental issues, and food insecurity due to financial and social limitations; consequently, collaborating to muscle catabolism (Deutz et al., 2014; Price et al., 2019). In addition, data suggests that the anabolic response to hyperaminoacidemia may be decreased in older individuals (Coelho-Júnior et al., 2018), indicating that older persons need to ingest more protein than younger ones in order to sustain muscle protein synthesis. In addition, older persons have greater protein requirements to counteract the elevated metabolism of inflammatory illnesses such as heart failure, chronic pulmonary disease (COPD), or chronic kidney disease (CKD) on dialysis as shown in Figure (2.2) (Bauer et al., 2013).

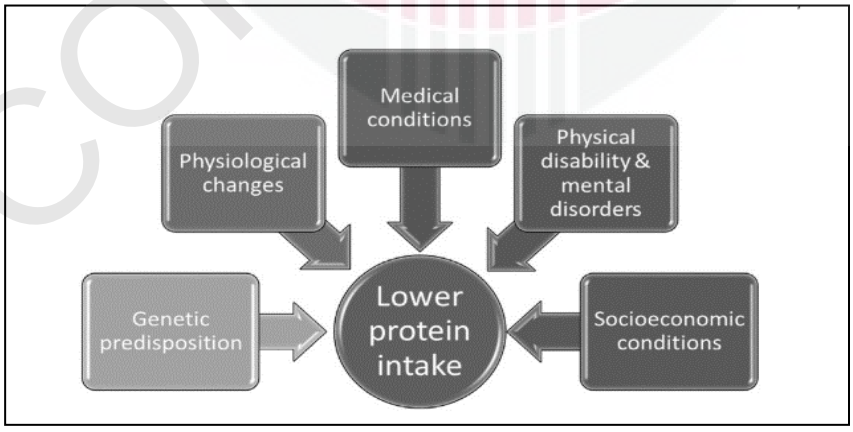


Figure 2.1: Factors leading to lower protein intake in older persons (Source: Bauer et al., 2013).

Nonetheless, inadequate protein consumption over time leads to a condition known as sarcopenia (Tessier & Chevalier, 2018), which is characterized by severe muscular atrophy, daphnia, and diminished physical function, all of which are included in the definition of frailty.

Besides sarcopenia, there is further evidence that protein consumption is connected with dementia, global cognitive scores, visuospatial ability, nonverbal memory, and logical memory in older persons, all of which are associated with frailty (Coelho-Júnior et al., 2018). In addition, it is found that adults not meeting the protein recommendation are more likely to have lower intakes of several micronutrients, including fiber, thiamin, niacin, vitamin B6, folate, choline, vitamin C, vitamin B12, vitamin A, vitamin D, vitamin E, vitamin K, zinc, calcium, phosphorus, magnesium, iron, copper, and selenium (Price et al., 2019).

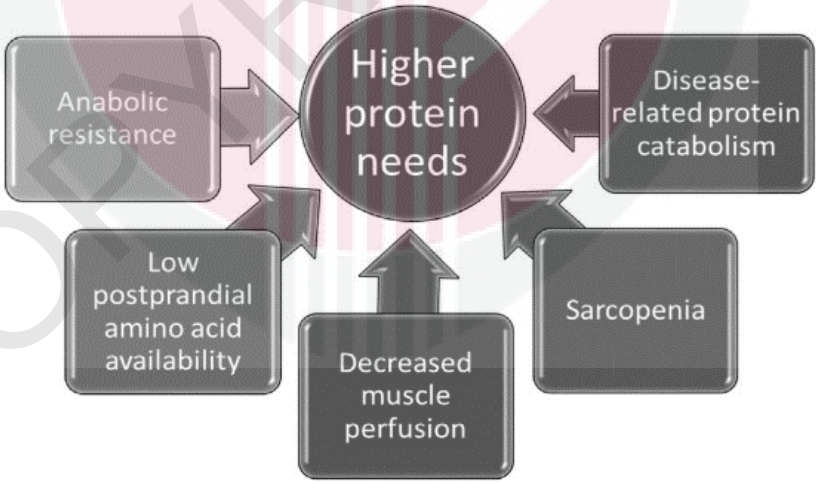


Figure 2.2: Factors leading to higher protein needs in older persons (Source: Bauer et al., 2013).

The prevalence of inadequate protein intake among Malaysian older adults has been a concern. Studies have shown that many do not meet the recommended dietary intake for protein. According to the Malaysian Adult Nutrition Survey (MANS), less than half of adults achieved the recommended intake for macronutrients, including protein. Only 40.4% of adults met the protein intake requirements, and a substantial portion of the population consumed less than the recommended amounts, which could lead to issues like muscle loss and frailty as they age (Mirnalini et al., 2008).

The European Society for Clinical Nutrition and Metabolism (ESPEN) has provided updated guidelines recommending a daily protein intake of 1.0–1.2 g/kg of body weight for healthy older adults. For those who are malnourished or experiencing acute or chronic illnesses, higher intakes of 1.2–1.5 g/kg/day are advised to ensure sufficient muscle maintenance and metabolic function (Deutz et al., 2014). Furthermore, ESPEN emphasizes the need for high-quality protein sources, particularly those rich in essential amino acids like leucine, which is crucial for muscle protein synthesis (Deutz et al., 2014).

The challenges faced by older adults, such as reduced appetite, difficulty in chewing, and social isolation, often lead to inadequate protein consumption. Addressing these barriers through targeted dietary interventions and supplementation strategies is key to promoting muscle health and reducing the risk of frailty in this population (Volpi et al., 2013).

In general, older adults who do not meet their protein needs have a much higher likelihood of having lower micronutrient intakes (on the day of intake), and nutrient deficiencies, when combined with a low protein intake, may increase the risk of

common issues in older adults, including falls, pressure sores, osteomalacia, osteoporosis, hip fractures, muscle weakness, and mortality (Coelho-Júnior et al., 2018; Price et al., 2019). When it comes to the relation between frailty and protein intake, (Shikany et al., 2014), in their cross-sectional study, observed that protein consumption was negatively related to the risk of switching from robust to pre-frail condition across a period of 4.6 years.

In an effort to prevent sarcopenia and maintain physical function and long-term optimal health, large international groups of experts issued a consensus in 2013 and 2014 to increase protein recommendations to 1.0–1.2 g/kg/day for healthy people, 1.2 g/kg/day for active people, and 1.2–1.5 g/kg/day for those with chronic or acute diseases (except renal) (Bauer et al., 2013). This consensus was founded on information from metabolic, interventional, and observational research that was available at the time, and it is still supported by more recent evidence, which has been thoroughly reviewed elsewhere (Traylor et al., 2018). However, further randomized clinical studies are required to corroborate the evidence of the optimum protein consumption for older persons and to determine whether increased protein intake can measurably improve the functional outcomes of older adults (Bauer et al., 2013).

2.3 Protein and Amino Acids Supplements as a Therapeutical Approach for Frailty

Despite the evidence that protein supplementation can improve physical function and reduce frailty in older adults, there is still a need for more research to fully understand the effects of different types of protein on frailty and sarcopenia. Different sources of protein, such as whey, soy, and milk protein, have different amino acid profiles and rates of digestion and may have different effects on muscle protein synthesis and

physical performance. Furthermore, it is not clear which type of protein is the most effective or safe for older adults, and what the optimal amount of protein supplementation is for reducing the risk of frailty and sarcopenia.

Therefore, a literature review was carried out to comprehensively overview the available literature on the effects of different types of protein supplementation on frailty and sarcopenia in older adults. The review aims to shed light on the different types of protein that have been used in studies to improve frailty and sarcopenia and to provide insights into the potential advantages and disadvantages of each type of protein. The ultimate goal of this review is to provide guidance for older adults and healthcare professionals in choosing the most effective and safe protein supplement regimen for reducing the risk of frailty and sarcopenia.

2.3.1 Study Characteristics

Eighty-one full article studies used eleven different types of protein and AA supplementation with a total of 5319 participants as shown in Table (2.1). Population areas included the Americas (35 RCTs), Europe (33 RCTs), Asia (12 RCTs), and Oceania (2 RCTs). Regarding the population types which is presented in Figure (2.3), 43 RCT populations are healthy community dwellers, 12 studies targeted pre-frail, frail, malnourished, or sarcopenic older adults, while 11 trial populations were older adults with special conditions or diseases such as (arthritis, Osteopenia, Osteoporosis, Type2 DM, Heart Failure, or obesity), 7 studies of total 83 studies population were postmenopausal women, 6 studies for low-activity or sedentary older adults, and 4 studies for inpatient, post-hospitalized or in residential care centres. 23 studies were

specified for older women, 13 studies for older males while the rest studies (n=47) targeted both male and female participants.

When it comes to the training programs combined with protein supplementation consumption, 58 trials used resistance training program, 39 of the total 60 applied supervised whole body resistance training for the participants graded between low, moderate and high intensity training. 8 Studies conducted training programs for upper and/or lower limps exclusively. 7 other studies used different types of training such as (exergames, walking, balance training, endurance training...etc.), and 4 trials applied self-administered, home-based training. On the other hand, 24 studies tested the effect of protein supplementation alone without training. Other specific characteristics are described in table 2.1.

2.3.2 Supplements Characteristics

2.3.2.1 Types of Protein and AAs Supplements

A variety of protein and amino acids supplements were investigated across studies and were grouped into the following categories: The most widely used type in these RCTs is Whey protein (n=33), as shown in Figure (2.4), followed by Creatine (n=12), Milk Protein (n=11), Leucine (n=8), Essential Amino Acids (EAA) (n=6), Soy (n=4) and other types such as Citrulline (n=2), L-Carnitine (n=2), Collagen Peptides (n=1), L-Arginine (n=1), Beta-Alanine (n=1) respectively. Each supplement measured different outcomes as elaborated in Figure (2.5) classified among three categories which are muscle strength, physical performance and body composition.

2.3.2.2 Whey Protein Supplements Dosage, Timing, and Resistance Training

Eight studies investigated the effects of whey protein on muscular strength, physical performance and body composition, and 25 studies examined the effect of whey protein supplementation alongside resistance exercise on the aforementioned measures. We classified whey protein consumption in the included studies as high (≥ 40 g/day), medium (30–39 g/day) or low (< 30 g/day). Nine studies employed a high amount of whey protein (Bell et al., 2017; Björkman et al., 2020; Gerontol et al., 2012; Griffen et al., 2022; Jadczak et al., 2021; Kerstetter et al., 2015; Mojtahedi et al., 2011; Neske & Azevedo, 2022; Oikawa et al., 2020), whereas 7 and 12 trials used a medium (Fernandes et al., 2018; Junior et al., 2017; Michel et al., 2022; Nabuco, Tomeleri, Fernandes, et al., 2019; Nabuco, Tomeleri, Junior, et al., 2019; Villanueva et al., 2014; Zhu et al., 2015) and low dose (Amasene et al., 2019, 2022; Arnarson et al., 2013; Atherton et al., 2020; Biesek et al., 2021; Björkman et al., 2011; Gade et al., 2019; Holwerda et al., 2018; Karelis et al., 2015; Mertz et al., 2021a; Mori & Takudo, 2018; Niccoli et al., 2017), respectively. Furthermore, 3 trials employed a technique to determine protein consumption to achieve whey protein supplementation of 1.2–1.5 g/kg/day.

Regarding the 30g dosage of whey protein, this specific amount has been widely studied and found to be effective in maximizing muscle protein synthesis, especially in older adults. Aging muscles often exhibit "anabolic resistance," meaning they are less responsive to smaller protein doses, and hence, a higher intake is necessary to stimulate MPS. Research by Moore et al. (2014) indicates that consuming around 25-30g of whey protein is sufficient to elicit a robust anabolic response, particularly because of the leucine content. Whey protein is rich in leucine, an essential amino acid

that acts as a potent stimulator of MPS, making it more effective than lower doses (e.g., 10-20g), which may not provide the same benefit in older populations (Moore et al., 2014). This is further supported by Devries & Phillips (2015), who reviewed the literature and concluded that a 30g dose was more beneficial for muscle protein synthesis, both at rest and following exercise, due to its optimal amino acid profile (Devries & Phillips, 2015).

Additionally, guidelines from the European Society for Clinical Nutrition and Metabolism (ESPEN) suggest that older adults should aim for higher protein intakes of 1.2-2.0 g/kg body weight per day, with each meal providing at least 25-30g of protein to counteract muscle loss effectively (Bauer et al., 2013). The 30g dose aligns well with these recommendations, ensuring that the elderly can achieve sufficient protein intake to stimulate muscle growth and maintenance, addressing the challenges posed by age-related sarcopenia and frailty.

The studies that tested the effect of whey protein supplements combined with RT mostly applied full-body training (n = 19). Another 4 studies focused on upper and lower extremities in their training programmes (Amasene et al., 2019, 2022; Gade et al., 2019; Mori & Takudo, 2018) and 2 studies applied home-based training (Björkman et al., 2011, 2020). Finally, (Biesek et al., 2021) investigated the effectiveness of exergames when combined with whey protein on muscle strength and physical performance of older adults.

Whey protein supplement timing was linked to the RT programme in 16 of the 33 studies. In 9 of them, supplements were consumed after training on the training days only (Amasene et al., 2019, 2022; Arnarson et al., 2013; Atherton et al., 2020;

Björkman et al., 2020; Fernandes et al., 2018; Junior et al., 2017; Nabuco, Tomeleri, Fernandes, et al., 2019; Nabuco, Tomeleri, Junior, et al., 2019), while in the remaining 7 studies, the researchers instructed the participants to also consume whey protein supplement either after training or during meal times on non-training days (Biesek et al., 2021; Gade et al., 2019; Gerontol et al., 2012; Holwerda et al., 2018; Karelis et al., 2015; Michel et al., 2022; Villanueva et al., 2014). Additionally, 11 studies linked the intake of whey protein supplement with regular meal times (Bell et al., 2017; Björkman et al., 2011; Griffen et al., 2022; Kirk et al., 2019, 2020; Krause et al., 2019; Mertz et al., 2021b; Mojtahedi et al., 2011; Neske & Azevedo, 2022; Niccoli et al., 2017; Zhu et al., 2015).

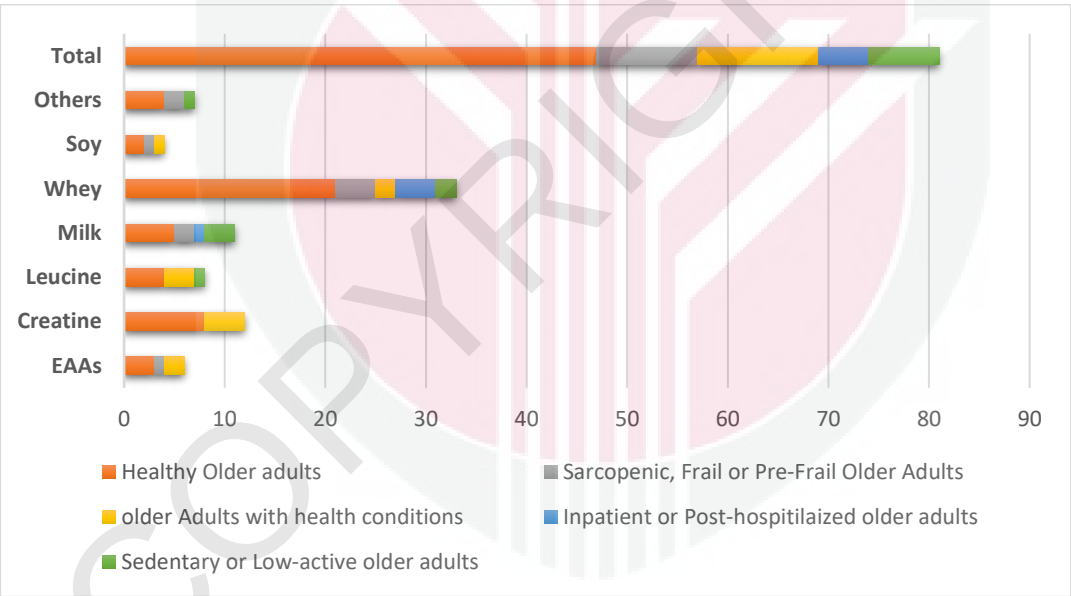


Figure 2.3: Types of population in the included studies of the literature.

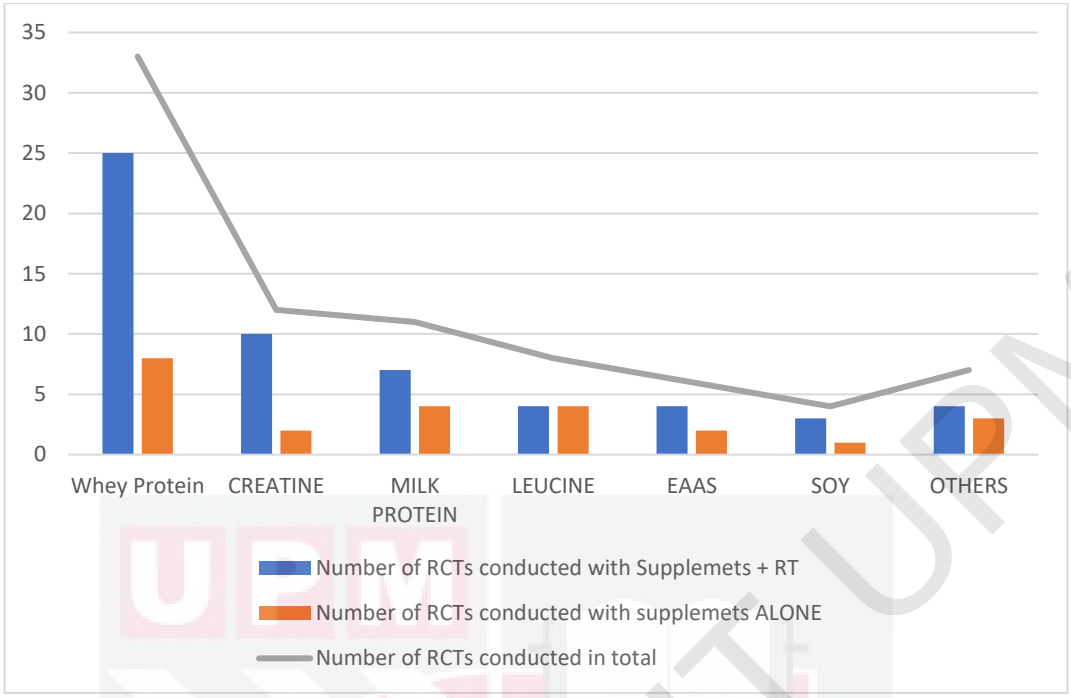


Figure 2.4: Number of studies used different types of supplements, RCT: Randomized Clinical Trial, RT: resistance training.

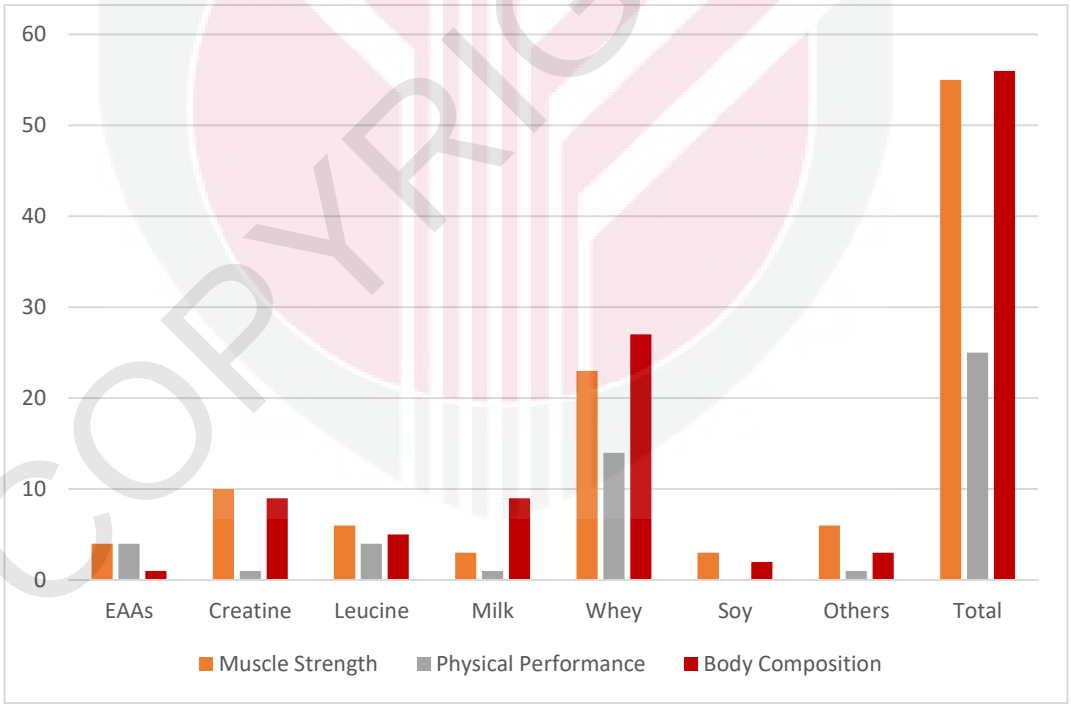


Figure 2.5: The number of studies that used different outcome measures

The choice of a 12-week duration for this study is supported by previous wide research applied this period of time as elaborated in Figure (2.6), demonstrating that this time frame is sufficient to observe significant changes in muscle mass, strength, and physical performance following protein supplementation. Muscle adaptation and improvements in muscle protein synthesis (MPS) typically require consistent protein intake over several weeks. Studies such as Tieland et al. (2012) have found that a 12-week period is effective for older adults, showing marked improvements in muscle strength and physical performance when they were supplemented with 15-30g of protein. This duration aligns with the cycle of muscle protein synthesis and adaptation, allowing researchers to capture not just immediate but sustained effects on physical function and muscle health. Additionally, Rondanelli et al. (2016) highlighted that a 12-week intervention allows enough time to assess the impact of nutritional supplements on functional outcomes, such as muscle mass and strength, making it a suitable choice for studies aiming to counteract age-related muscle deterioration. This time frame also aligns well with the physiological processes of muscle hypertrophy, enabling researchers to track both short-term and mid-term effects on physical health.

Thus, the combination of a 12-week duration and a 30g whey protein dose offers an effective approach for intervention studies aiming to mitigate muscle deterioration in older adults. These parameters have been shown to provide enough time for meaningful physiological changes and to deliver an optimal level of protein that can stimulate muscle synthesis, leading to improved muscle mass, strength, and overall physical performance.

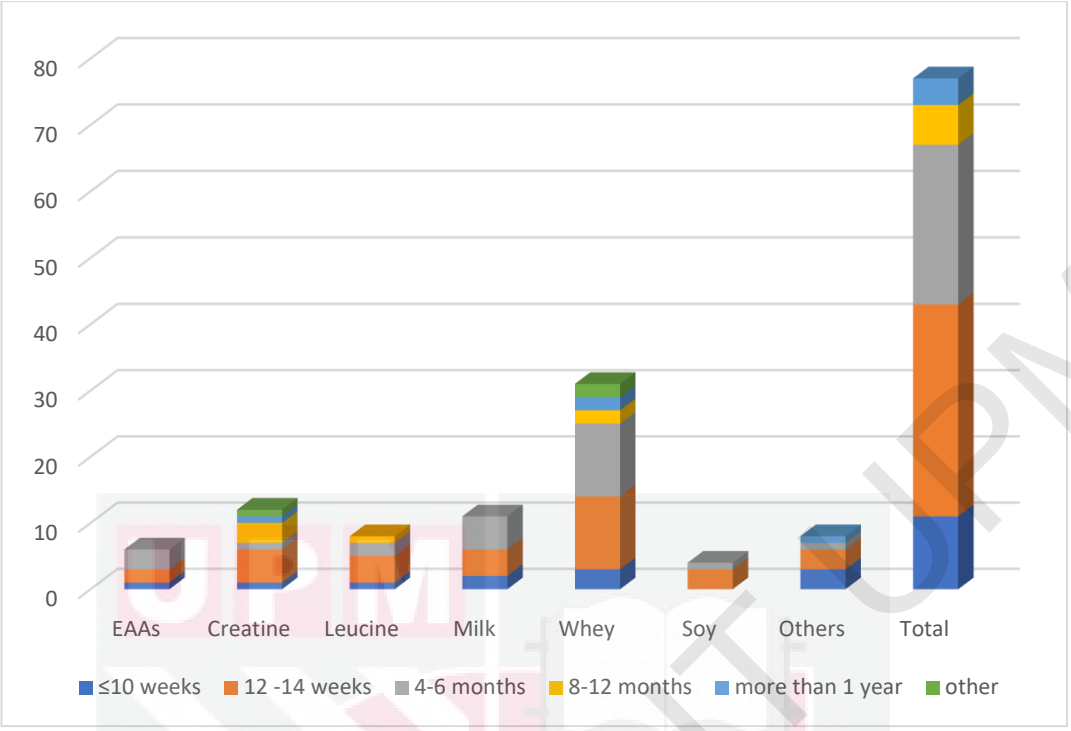


Figure 2.6: The programs duration of the included studies of the literature.

Table 2.1: Protein and Amino Acids Interventional Studies

Supple-ment Type	Studies	Country	Design	Duration	Population Type	n (f)	Age	RT	PS dose	Timing	Placebo type	Main Outcomes
EAAs	(Bonnefoy et al., 2012)	France	RCT	4 M	Community-dweller older people	102	> 80 years old	HEX	10 g	AT	NA	↑GS
	(Ikeda et al., 2018)	Japan	SB	1 M	Women with arthritis	55 f	64.2±9.4	HEX	6 g	BT	Starch	ND HS, ND GS
	(Kawada et al., 2013)	Japan	SB	6 M	Healthy elderly people	29(17)		FBRT 2d/w	3g and 6 g	AB	exercise alone	↑GS
	(Kim et al., 2012)	Japan	SB	3 M	Sarcopenic women	155 f	> 75	FBRT 2d/w	6 g	NR	health education	↑ (KE, Leg MM, GS)
	(Markofski et al., 2019)	USA	DB	24 W	Non-frail, independent, older adults	45(30)	65-82	Progressive Aerobic RT 3d/w	15g	BM	NR	↑LE
	(Pineda-Juárez et al., 2016)	Mexico	RCT	3 M	Patients with heart failure	66(27)	58-84	FBRT 2d/w	10 g	AT, AB	exercise alone	ND (HS, BC)

Table 2.1: Continued

Supple-ment Type	Studies	Country	Design	Duration	Population Type	n (f)	Age	RT	PS dose	Timing	Placebo type	Main Outcomes
Creatine	(Aguiar et al., 2013)	Brazil	DB	12 W	Older women	18 F	64.9 ± 5.0 years	FBRT 3d/w	5 g	AT	Maltodextrin	↑ (BP, KE, BIC, FFM, MM)
	(Baker et al., 2016)	Canada	DB, Crossover	2 d*	Healthy elderly men	9m	54.8 ±4.3	Leg and chest press 3d/w	20 g	BT	Maltodextrin	ND (CP, LP)
	(Bemben et al., 2010)	USA	DB	14 W	healthy men	42 M	48-72 years	ULLRT 3d/w	5g	AT	Glucose	ND (BP, KE, BIC, LP, LBM)
	(Bernat et al., 2012)	Canada	DB	8 W	Untrained healthy aging males	27 M	> 50	HVRT 2d/w	0.1g/kg	Non- train day	Maltodextrin	↑ (LP, KE, KF)
	(D. G. Candow et al., 2021)	Canada	DB	12 M	Healthy men	38M	> 50	FBRT 3d/w	0.1g/kg	BT & AT	Maltodextrin	ND (MS, LBM, FM)
	(D. G. Candow et al., 2014)	Canada	DB	12 W	Healthy elderly people	9(13)	50-64	FBRT 3d/w	0.1g/kg~ 8g	BT vs AT	Rice flour	↑ (LP, CP, LBM)
	(D. Candow et al., 2015)	Canada	DB	32 W	Healthy elderly people	64(38)	50-71	FBRT 3d/w	0.1g/kg	BT or AT only	Maltodextrin	↑ (LP, CP, LTM)
	(Gualano et al., 2014)	Brazil	DB	24 W	Postmenopausal women with osteopenia or osteoporosis	60F	≥ 60 years	FBRT 2d/w	5 g	AL	Dextrose	↑ (BP, LBM)
	(Johannsmeyer et al., 2016)	Canada	DB	12 W	Healthy elderly people	17(14)	57.6 ± 5.0	FBRT 3d/w	0.1g/kg	BT & AT& with meals	Maltodextrin	↑ (BM, MM), ND (LP, CP)
	(Lobo et al., 2015)	Brazil	DB	12 M	Postmenopausal osteopenic women	109f	58(6)	NA	1g	NR	Dextrose	ND (TUG, CST, LBM, FM)
	(Neves et al., 2011)	Brazil	DB	12 W	Postmenopausal women with knee OA	24F	50-65	LLRT 3d/w	20 g/d for 1 wk, 5 g/d thereafter	Daily dose	Dextrose	↑ (CST, Leg LM) ND LP

Table 2.1: Continued

Supplement Type	Studies	Country	Design	Duration	Population Type	n (f)	Age	RT	PS dose	Timing	Placebo type	Main Outcomes
Leucine	(Ispoglou et al., 2016)	UK	DB	3 M	Community-dweller older adults	36(14)	65–75 years	NA	11-21g	ME	Lactose	↑ (CST, 6MWT, LTM)
	(Y. Kang et al., 2020)	Korea	DB	12 W	Community-dweller older adults	120(87)	50-80	NA	20 g protein, 3g leucine	Twice a day	Iso- CHO	↑ LBM
	(Leenders et al., 2011)	Netherlands	RCT	6 M	Type-2 diabetic patients	60 M	71 ± 1	NA	7.5 g/d alone	MLE	Wheat flour	ND (LP, LE, BC)
	(Murphy et al., 2021)	Ireland	DB	24 W	Community-dweller older adults	107(55)	≥65	NA	21 pr (6leu)	AB, L or E	Maltodextrin	ND (HS, KE, TUG, SPPB)
	(Reule et al., 2017)	Germany	DB	12 W	Low-active older adults	48(24)	> 60	MIRT T 3d/w	3.2 g/d with AA	NM	NM	↑ Strength lose
	(Trabal et al., 2015)	Spain	DB	12 W	Community-dweller older adults	30 M	> 70	LLRT, BALANCE	10 g/d only	L,E	Maltodextrin	↑ (LP, CST, TUG)
	(Yamamoto et al., 2021)	Japan	RCT	48 W	Type-2 diabetic patients	53(25)	> 70	RT at home with elastic bands	6 g/d AA, 2.4 LU	AB, AL	NA	↑ KE, ND (HS, GS, MM)
	(Yoshimura et al., 2019)	Japan	DB	8 W	Post-stroke older patients with sarcopenia	44 (30)	≥ 65	LIRT	3 g/d with AA	AT	NA	↑ (HS, PF, MM)

Table 2.1: Continued

Supplement Type	Studies	Country	Design	Duration	Population Type	n (f)	Age	RT	PS dose	Timing	Placebo type	Main Outcomes
Milk Protein	(Aas et al., 2020)	Norway	RCT	10 W	Mobility-limited older adults	22	85 ± 6 years	ST heavy 3d/w	34 g/d	AT, ME	NA	↑ (KE, Leg LM)
	(Bouillanne et al., 2013)	France	RCT	6 W	Malnourished or at-risk elderly inpatient	63 (46)	> 80	NA	1.3 g/kg/d, 71% CASEIN	Pulse Diet (at noon)	Spread diet	↑ (LM, ASM) ND HS
	(Carlsson & Haglin, 2011)	Sweden	DB	3 M	In residential care with severe physical or cognitive impairment	177(131)	65-99	NA	7.4 g	NR	NR	ND MM
	(Francis et al., 2017)	Ireland	SB	12 W	Healthy older women	57 F	61.1 ± 5.1	ULRT 3d/w	0.33 g/kg	BL	NA	↑ (Leg LM, GS, KE), ND GS
	(Gryson et al., 2014)	France	DB	16 W	Healthy sedentary men	48 M	60.8 ± 0.4 years	FBRT 3d/w	10 g	AT or AB	NR	↑ (FFM, MM, FM)
	(Nakayama et al., 2021)	Japan	DB	6 M	Healthy older adults	122 (92)	60-84	low-mod RT daily	10 g/d	AT	isocaloric drink	↑ (LBM, FM), ND (HS, KE, GS, TUG, CST)
	(Norton et al., 2016)	Ireland	SB	24 W	Healthy older adults	60	61±5	NA	0.165 g/kg	BL	Maltodextrin	↑ LTM
	(Ottestad et al., 2017)	Norway	DB	12 W	Community-dweller older adults	36 (24)	≥ 70 years	NA	40 g/d	B, E	isocaloric drink	ND (LP, CP, MM, PF)
	(Ten Haaf et al., 2020)	Netherlands	DB	12 W	Physically active older adults	116 (21)	67–73	4 days walking	31 g/d	B, AT	isocaloric drink	↑ (LBM, FM), ND (GS, CST, TUG)
	(Tieland, Dirks, et al., 2012)	Netherlands	DB	24 W	Frail elderly people	62 (41)	78 ± 1	FBRT 2d/w	30 g/d	BL	0g protein	↑ LBM, ND (LE, SPPB)
	(Tieland, van de Rest, et al., 2012)	Netherlands	DB	24 W	Frail elderly people	65 (36)	> 70	NA	30 g/d	BL	0g protein	↑ SPPB, ND (LE, MM)

Table 2.1: Continued

Supple-ment Type	Studies	Country	Design	Duration	Population Type	n (f)	Age	RT	PS dose	Timing	Placebo type	Main Outcomes
Whey Protein	(Amasene et al., 2019)	Spain	RCT	24 W	Post-Hospitalized Older Adults	29(14)	82.9(5.6)	ULLR 2d/w	20 g	AT	Maltodextrin	ND (HS, CST, 6MWT, BC)
	(Amasene et al., 2022)	Spain	RCT	24 W	Post-Hospitalized Older Adults	41(22)	82 (5.89)	ULLR 2d/w	20 g	AT	NM	ND (HS, BC)
	(Arnarson et al., 2013)	Iceland	RCT	12 W	Community-dwelling older people	161(94)	65-91	FBRT 3d/w	20 g 3t/w	AT	isocaloric CHO	ND (LBM, TUG, MS, 6MWT)
	(Bell et al., 2017)	Canada	DB	20 W	Healthy older men	49m	73(1)	FBRT 3d/w	30x2 g	AB, BS	Maltodextrin	↑ (CP, SP, LP, LBM)
	(Björkman et al., 2011)	Finland	DB cross over	16 W	Polymyalgia Rheumatica Patients	47 (42)	mean 69.5 years	HEX	NR	BM	casein	↑MM, ND HS
	(Björkman et al., 2020)	Finland	RCT	12 M	Community-dwelling people with sarcopenia.	218	>74 y	HEX	2x20 g	AT	isocaloric milk	ND (HS, SPPB)
	(Gerontol et al., 2012)	Canada	RCT	6 M	Community-dwelling older people	80(47)	70-85	FBRT 3d/w	40 g	M, E, AT	Maltodextrin	ND (1RM, LBM, CST, SPPB)
	(Fernandes et al., 2018)	Brazil	RCT	26 W	Pre-conditioned older women	32f	67(4)	FBRT 3d/w	35 g	AT	Maltodextrin	↑ LST
	(Griffen et al., 2022)	UK	DB	12 W	Healthy older men	36m	67(1)	FBRT 2d/w	25x2	B, L	Maltodextrin	↑ GS, ND (LP, LE, 6MWT, FFM)
	(Holwerda et al., 2018)	Netherlands	DB	12 W	Healthy older men	41m	70(1)	FBRT 3d/w	21 g	AT, BS	placebo 0p	ND (LP, LE, MM)
	(Jadczak et al., 2021)	Australia	DB	16 W	Community-dwelling pre-frail and frail older adults	70(47)	73.34±6.85	NA	2x20 g	AT, BM	Rice protein	ND (GS, SPPB, TUG, HS)

Table 2.1: Continued

Supplement Type	Studies	Country	Design	Duration	Population Type	n (f)	Age	RT	PS dose	Timing	Placebo type	Main Outcomes
	(Karelis et al., 2015)	Canada	DB	135 D	Non-frail older adults	99(76)	65-88	FBRT 3d/w	20 g	BL, AT	Casein	↑ (LP, KE), ND LBM
	(Kerstetter et al., 2015)	USA	DB	18 M	Healthy older adults	208(186)	60-70	NA	45 g	NM	Maltodextrin	↑ FFM
	(Kirk et al., 2020)	UK	SB	16 W	Community-dwelling older people	100(52)	69(6)	FBRT 3d/w	1.5g/kg	BLD	NR	ND (MM, FM)
	(Kirk et al., 2019)	UK	SB	16 W	Non-frail community-dwelling older people	46(25)	68(5)	FBRT 3d/w	1.5g/kg	BLD	Exercise alone	ND (LP, CP, SPPB, 6MWT)
	(Krause et al., 2019)	Ireland	SB	12 W	Healthy sedentary older adults	38(20)	63.5 ±4.4	FBRT 3d/w	0.165/kg	BL	Maltodextrin	ND (LBM, CST, 10MWT, HS), ↑ FM
	(Michel et al., 2022)	USA	RCT	10 W	Healthy community-dwelling older adults	18(11)	69.7± 8.23	FBRT 3d/w	25-50 g	AT/BET. M	0.8-1g/kg	ND BC
	(Mojtahedi et al., 2011)	USA	DB	6 M	Overweight or obese older women	31f	65.2±4.6	FE, low-mod of walking intensity	2x25 g	BL, BD	Maltodextrin	ND (KE, LST)
	(Mori & Takudo, 2018)	Japan	SB	24 W	Healthy community-dwelling older women	81f	65-80	ULLRT 2d/w	22.3 g	NM	Exercise only	↑ (HS, GS, MM)
	(Nabuco, Tomeleri, Junior, et al., 2019)	Brazil	DB	12 W	Older women	70f	≥60 y	FBRT 3d/w	35 g	AT only	Maltodextrin	↑ (LST, FM)

Table 2.1: Continued

Supplement Type	Studies	Country	Design	Duration	Population Type	n (f)	Age	RT	PS dose	Timing	Placebo type	Main Outcomes
	(Nabuco, Tomeleri, Fernandes, et al., 2019)	Brazil	DB	12 W	Sarcopenic obese older women	26f	≥60 y	FBRT 3d/w	35 g	AT	Maltodextrin	↑ (LST, FM), ND (CP, KE)
	(Niccoli et al., 2017)	Canada	DB	Length of stay 20-30d	Frail Hospitalized Geriatric Patients	47(22)	681.3(1)	NA	24 g	BLD	Usual care	↑ (HS, KE), ND (GS, TUG)
	(Oikawa et al., 2020)	Canada	DB	36 d	Inactive older adults	31(15)	65-80	NA	30x2 g	twice	CP	ND BC
	(Park et al., 2018)	Korea	DB	12 W	Undernourished prefrail and frail older adults	120	70-85	NA	1.2-1.5g/kg	NR	Maltodextrin	↑ (GS, ASM), ND (HS, SPPB, TUG)
	(Neske & Azevedo, 2022)	Brazil	DB	12 W	Healthy older adults	31	60-80	FBRT 2d/w	2x20 g	BD	Maltodextrin	ND (KE, TUG, LBM)
	(Villanueva et al., 2014)	USA	RCT	12 W	Healthy older men	22m	68(6.1)	FBRT 3d/w	35 g	M, AT	Exercise only	ND (BP, LBM, FM)
	(Weisgarber et al., 2015)	Canada	DB	10 W	Healthy postmenopausal women	12f	57(4.7)	Unilateral RT 2d/w	40g	AT	Maltodextrin	ND (KE, LE, BCURL)
	(Zhu et al., 2015)	Australia	DB	2 Y	Healthy older women	110f	74.3(2.7)	NA	30 g	BB	Isocaloric milk	ND (HS, KE, TUG, MM)
	(Gade et al., 2019)	Denmark	DB	During admission & 12 W after discharge	Inpatient older adult	148 (100)	> 70	Low-Intensity LLRT, daily	27.5 g/d	AT, AB	isocaloric drink	ND (LBM, HS, CST)

Table 2.1: Continued

Supplement Type	Studies	Country	Design	Duration	Population Type	n (f)	Age	RT	PS dose	Timing	Placebo type	Main Outcomes
	(Biesek et al., 2021)	Brazil	RCT	12 W	Pre-frail older women	90 F	71.2 4.5	Exergames 3d/w	21 g/d	AT, M or N or E	Maltodextrin	ND (HS, ASM), ↑ (Frailty, Fatigue)
	(Junior et al., 2017)	Brazil	DB	12 W	resistance-trained older women (pre-trial)	31 F	67.4 ± 4.0 years	FBRT 3d/w	35 g/td	AT	Maltodextrin	↑ (KE, BIC, CP, SMM)
	(Mertz et al., 2021a)	Denmark	RCT	1 Y	Community dweller older adults	208 (99)	> 65	Light vs heavy RT	20 g	M, N	CHO	↑ KE
	(Atherton et al., 2020)	UK	SB	10 W	healthy older males	18 M	70.5±5.1	10 RM 3d/w	20 g vs 40 g	AT	NA	40g: ↑ (CP, SP, LP, FM)
Soy Protein	(Christie et al., 2010)	Italy	DB	3 M	postmenopausal women	39 F	> 46	NA	20 g	M, E	casein	↑ BF
	(Orsatti et al., 2018)	Brazil	DB	16 W	Postmenopausal women	32 F	> 46	FBRT 3d/w	25 g	AT or	Maltodextrin	↑ (BP, KE)
	(Shahar et al., 2013)	Malaysia	quasi–experimental	12 W	sarcopenic elderly Malays	65 (18)	60–74 years	home ex 2d/w	1.5g/kg/d	Once a day	NA	↑ MS, ND (FFM, BF)
	(Shenoy et al., 2013)	India	RCT	12 W	Osteopenic/osteoporotic postmenopausal women	60 f	45-65	FBRT 4d/w	40 g/d	Twice a day	NA	↑ (KE, KF)

Table 2.1: Continued

Supplement Type	Studies	Country	Design	Duration	Population Type	n (f)	Age	RT	PS dose	Timing	Placebo type	Main Outcomes
Collagen Peptides	(Zdzieblik et al., 2015)	Germany	DB	12 W	Sarcopenic older male	53 M	72·2±SD 4·68	FBRT 3d/w	15 g/d	AT or same time	Silica	↑ (QS, FFM, FM)
L-Citrulline	(Buckinx et al., 2019)	Canada	DB	12 W	Sedentary obese older adults	73 (40)	> 60	HIIT 3d/w	10 g/d	L	Maltodextrin	↑ (KE, FM)
	(Caballero-García et al., 2021)	Spain	DB	6 W	Healthy older adults	44 (26)	60-73	Balance, aerobic exercises	3 g/d	NR	lactose and starch	↑ GS
L-Carnitine	(Badrasawi et al., 2016)	Malaysia	DB	10 W	Pre-frail elderly people	50 (27)	> 60	NA	1.5g/kg/d (500mg)	NR	corn starch	↑ (Frailty, HS)
	(Sawicka et al., 2018)	Poland	DB	24 W	Healthy older adults	28 F	65-70	NA	1500 mg	After main meals	isonitrogenous	ND (BC, KE, KF)
L-Arginine	(Aguiar et al., 2016)	Brazil	DB	2 W	Physically active older women	20 F	20 F	NA	8g/d			ND (KE, KF)
Beta-Alananine	(Bailey et al., 2018)	Country	DB	12 W	Community dwellers older adults	27	60–82 years	ERT 3d/w	3.2 g/d	Timing	Maltodextrin	ND (CP, LP)

*: 2 separate occasions (7 days apart), RCT: Randomized Controlled trial, DB, double-blinded trial, SB: single-blinded, NA: not-applicable, NR: not-reported, M: months, W: weeks, FBRT: Full-Body Resistance training, ULLRT: Upper and lower limps resistance training, HEX: home-based exercises, HVRT: High-velocity resistance training, ERT: Endurance resistance training, BT: before training, AT: after training, AB: after breakfast, BM: between meals, BS: before sleep, BL: before lunch, AL: after lunch, BLD: before lunch and dinner, M: at morning, E: at evening, B: at breakfast, L: at lunch, N: at noon, CHO: Carbohydrate, ↑: Significant improvement, ND: No significant difference, MS: Muscle strength, BC: body composition, SPPB: Short-Physical Performance Battery test, HS: Handgrip strength, CST: chair-stand test, GS: Gait speed, 1RM: 1 repetition maximum, BP: Bench press, BIC: Biceps curl, SP: Shoulder press, CP: Chest press, KE: Knee extension, KF: Knee flexion, LP: leg press, LE: leg extension, TUG: Time-up-and go test, BM: Body mass, LBM: lean body mass, MM: muscle mass, FFM: Fat-free mass, FM: fat mass, ASMM: appendicular skeletal muscle mass, QS: quadriceps strength, 6MWT: 6-min walk test, 10MWT: 10-min walk test, PF: physical function, ASM: Appendicular skeletal muscle mass.

2.3.3 Effect of Protein Supplements and EAAs on Outcome Measurements

A) Whey protein

Whey protein supplementation did not increase the adaptive response to resistance training in terms of muscle strength (1RM of chest press, bench press, leg press, or knee extension) (Gerontol et al., 2012; Griffen et al., 2022; Holwerda et al., 2018; Kirk et al., 2019; Mojtahedi et al., 2011; Nabuco, Tomeleri, Fernandes, et al., 2019; Neske & Azevedo, 2022; Villanueva et al., 2014; Weisgarber et al., 2015) or handgrip strength (Amasene et al., 2019, 2022; Biesek et al., 2021; Björkman et al., 2011, 2020; Gade et al., 2019; Krause et al., 2019). However, some studies reported a beneficial effect of RT following whey protein supplements in muscle strength such as leg press, knee extension, chest press, or handgrip strength (Atherton et al., 2020; Bell et al., 2017; Junior et al., 2017; Karelis et al., 2015; Mertz et al., 2021a; Mori & Takudo, 2018).

In terms of physical performance, gait speed tended to be improved after WPS combining with RT(Griffen et al., 2022; Mori & Takudo, 2018) but no other measurements such as TUG, CST, and 6MWT (Amasene et al., 2019; Arnarson et al., 2013; Kirk et al., 2019; Neske & Azevedo, 2022). In addition, LBM showed no significant improvement when consuming WPS during RT (Arnarson et al., 2013; Gade et al., 2019; Gerontol et al., 2012; Karelis et al., 2015; Krause et al., 2019; Neske & Azevedo, 2022; Villanueva et al., 2014), neither lean soft tissues LST(Michel et al., 2022; Mojtahedi et al., 2011) nor muscle mass MM (Biesek et al., 2021; Holwerda et al., 2018; Kirk et al., 2020) or fat mass (Kirk et al., 2020; Villanueva et al., 2014). However, other studies showed an additional effect of WPS during RT in LBM improvement (Bell et al., 2017), LST (Fernandes et al., 2018; Nabuco, Tomeleri,

Fernandes, et al., 2019; Nabuco, Tomeleri, Junior, et al., 2019), MM (Björkman et al., 2011; Junior et al., 2017; Mori & Takudo, 2018),and FM (Atherton et al., 2020; Krause et al., 2019; Nabuco, Tomeleri, Fernandes, et al., 2019; Nabuco, Tomeleri, Junior, et al., 2019).

When it comes to non-RT studies, WPS showed no improvement in handgrip strength (Jadczak et al., 2021; Park et al., 2018; Zhu et al., 2015) nor knee extension (Zhu et al., 2015). However, same measurements were improved after WPS in a study conducted by Niccoli et al 207 (Niccoli et al., 2017). Physical performance and muscle mass showed no improvement in older adults consuming WPS despite the duration or the dose (Jadczak et al., 2021; Niccoli et al., 2017; Park et al., 2018; Zhu et al., 2015). However, fat-free mass (Kerstetter et al., 2015) , gait speed and appendicular skeletal muscle mass ASM (Park et al., 2018) were improved after WPS consumption in older adults.

B) Creatine

When it comes to muscle strength, creatine supplementation combined with resistance training shows an improvement in upper body strength measured by 1 RM chest press (D. Candow et al., 2015; D. G. Candow et al., 2014), biceps curl (Aguiar et al., 2013) and bench press (Aguiar et al., 2013; Gualano et al., 2014), for the lower body strength creatine supplements with RT improved 1 RM leg press (Bernat et al., 2012; D. Candow et al., 2015; D. G. Candow et al., 2014), and knee extension (Aguiar et al., 2013; Bernat et al., 2012) in healthy aging males and postmenopausal women. However, in other studies, creatine supplementation does not provide additional benefits to resistance training in terms of upper muscular strength measured by 1 RM

chest press (Baker et al., 2016; D. G. Candow et al., 2021), bench press (Bemben et al., 2010), and biceps curl (Bemben et al., 2010) nor lower body strength such as knee extension (Bemben et al., 2010), leg press (Baker et al., 2016; Bemben et al., 2010), and hack squat (D. G. Candow et al., 2021) in older adults.

Physical function improvement after creatine supplement consumption has been measured in 4 studies out of 12 creatine trials. Two studies prove the positive effect of creatine supplement when combined with resistance training in terms of peak torque and balance board time-to-completion (Bernat et al., 2012) in healthy older males and the timed-stands test (Neves et al., 2011) in older women with knee osteoarthritis. However, another two studies didn't observe any effect of creatine supplementation alone in muscle function assessed by timed-up-and-go and timed-stands tests (Lobo et al., 2015; Sales et al., 2019) in osteopenic postmenopausal women. Creatine supplementation with resistance training improves some body composition measurements such as lean body mass (Baker et al., 2016; D. Candow et al., 2015; Gualano et al., 2014; Johannsmeyer et al., 2016), muscle mass (Johannsmeyer et al., 2016), fat mass (Candow et al., 2015), and bone mineral density BMD (Johannsmeyer et al., 2016) in healthy elderly. On the other hand, other studies confront these results and didn't see any significant changes in lean body mass by creatine supplements alone (Lobo et al., 2015) or when combined with resistance training (Bemben et al., 2010; Candow et al., 2021; Sales et al., 2019) in older adults. In addition, no greater effect by creatine supplements alone or besides RT was observed in fat mass (Candow et al., 2021; Lobo et al., 2015) nor BMD (Candow et al., 2021; Lobo et al., 2015; Sales et al., 2019) in healthy aging males and osteopenic postmenopausal women compared to placebo.

C) Milk Protein

Milk protein supplements whether alone (Tieland, van de Rest, et al., 2012) or combined with RT (Francis et al., 2017) results a positive effect in terms of physical performance, leg press (Aas et al., 2020) and Knee extension (Francis et al., 2017), However, (Ottestad et al., 2017) didn't see any additional effect of milk protein in these measurements or handgrip strength in a trial conducted by using pulse feeding (Bouillanne et al., 2013). When it comes to body composition, milk protein proved its significant effect in lean mass improvement (Bouillanne et al., 2013; Nakayama et al., 2021; Norton et al., 2016; ten Haaf et al., 2019; Tieland, van de Rest, et al., 2012), fat free mass (Gryson et al., 2014), and leg tissue mass (Francis et al., 2017).

Appendicular skeletal muscle mass also improved after pulse feeding of milk protein in malnourished older adults (Bouillanne et al., 2013) while another study showed a significant improvement of muscle mass in sedentary elderly people when consuming milk protein besides resistance training (Gryson et al., 2014). Contrarily, (Carlsson & Haglin, 2011; Ottestad et al., 2017) didn't notice any additional effect of milk protein with concomitant training on muscle mass in older adults. In addition, fat mass significantly improved in older adults when milk protein consumed with low to moderate intensity exercises (Gryson et al., 2014; Nakayama et al., 2021; ten Haaf et al., 2019).

D) Leucine

The effect of leucine or leucine enriched supplement on muscle strength or physical performance in the included studies is controversial. Handgrip strength significantly improved after leucine consumption (Ispoglou et al., 2016) or besides low intensity

training(Yoshimura et al., 2019) and lower limb training (Trabal et al., 2015). However, muscle strength improvement hasn't been seen after leucine consumption in terms of handgrip strength and knee extension (Murphy et al., 2021; Yamamoto et al., 2021) or leg press (Leenders et al., 2011). Two trials reported no significant effect of leucine consumption on physical performance in terms of gait speed (Yamamoto et al., 2021) or short physical performance battery test SPPBT and timed up-and go test (Murphy et al., 2021). Contrarily, another two studies proved leucine supplementation efficacy on Functional Independence Measure (FIM-M) (Yoshimura et al., 2019) and 30-s arm-curl test, 30-s chair-stand test (30-CST), 6-mins walking test (Ispoglou et al., 2016).

For body composition, lean body mass improved significantly after using leucine supplement in community dwellers (Y. Kang et al., 2020), same as lean tissue mass (Ispoglou et al., 2016) and appendicular muscle mass (Yoshimura et al., 2019). On the other hand, no effect of leucine was seen in type 2 diabetic older adults in terms of fat-free mass (Leenders et al., 2011) or muscle mass (Yamamoto et al., 2021)and lean body mass in community dwelling older adults (Murphy et al., 2021).

E) EAAS

The main outcome of the trials with BCAAs was walking speed which improved and healthy older adults during resistance training (Bonnefoy et al., 2012; Kawada et al., 2013) but not in women with arthritis during self-administered training (Ikeda et al., 2018). In addition, walking time was improved after taking BCAAs supplements with full body training in sarcopenic women (Kim et al., 2012). In terms of muscle strength, results showed significant improvements measured by knee extension in sarcopenic

women (Kim et al., 2012), or leg extension in non-frail older adults (Markofski et al., 2019), and hip abductor strength rate in women with arthritis, but not handgrip strength (Ikeda et al., 2018). Regarding body composition, muscle mass didn't improve after consuming BCAAs supplements with aerobic exercise in non-frail, independent, healthy older adults (Markofski et al., 2019). However, leg muscle mass improved in sarcopenic women after full body training plus BCAAs supplements (Kim et al., 2012).

F) Other Supplements

Soy protein reduced total and subcutaneous abdominal fat when consumed 20g per day in postmenopausal women (Christie et al., 2010) and improved muscle strength when combined with resistance training in terms of knee extension (Orsatti et al., 2018; Shenoy et al., 2013), bench press (Orsatti et al., 2018), and arm curl test (Shahar et al., 2013) of older adults. Muscle strength had no significant improvement when consuming L-Carnitine (Sawicka et al., 2018), L-Arginine (Aguiar et al., 2016), or combining Beta-Alanine with RT (Bailey et al., 2018) in healthy older adults, except one study reported handgrip strength improvement after L-Carnitine consumption by pre-frail elderly people (Badrasawi et al., 2016). However, Collagen peptides supplement during RT showed an improvement in body composition of sarcopenic older males in terms of fat-free mass and fat mass (Zdzieblik et al., 2015). In addition, L-Citrulline showed a beneficial effect on handgrip strength, knee extension and fat mass of sedentary obese older adults when consumed beside high-intensity training (Buckinx et al., 2019) and improved walking speed of older adults when combined with balance and aerobic exercises (Caballero-García et al., 2021).

2.4 Frailty Assessment Tools

Frailty is a multidimensional syndrome marked by a decline in physiological reserves, leading to increased vulnerability to adverse health outcomes. Various tools have been developed to assess frailty, each with distinct characteristics. One of the most commonly used is the Fried Frailty Phenotype (FFP), which defines frailty based on five criteria: unintentional weight loss, self-reported exhaustion, weakness (measured by grip strength), slow walking speed, and low physical activity. Individuals meeting three or more criteria are classified as frail, while those with one or two are considered prefrail (Fried et al., 2001). The FFP is advantageous for its simplicity, objective measurements, and widespread validation, allowing for easier comparisons across studies and interventions. This focus on physical aspects is a key reason it was selected for this study, given the interest in protein supplementation and physical outcomes.

An alternative approach is the Frailty Index (FI), which considers a wider range of health deficits, including chronic diseases, cognitive impairment, and disabilities. Developed by Rockwood and Mitnitski (2007), the FI is based on the concept that frailty is an accumulation of deficits. It is comprehensive and provides a detailed assessment of an individual's overall health status, but it requires extensive data collection and can be time-consuming (Rockwood & Mitnitski, 2007). Another commonly used tool is the Clinical Frailty Scale (CFS), a 9-point scale that allows clinicians to quickly assess frailty based on clinical judgment, categorizing individuals from "very fit" to "severely frail" (Rockwood et al., 2005). Although practical in clinical settings, the CFS is more subjective than the FFP or FI and may lack the precision required for research settings. In this study, the FFP was chosen due to its

established validity in measuring physical frailty, ease of use, and suitability for assessing interventions targeting physical frailty features.

2.5 Muscle Strength Assessment

Muscle strength is a key indicator of physical function and frailty, and several methods are available to assess it. The handgrip strength test is one of the most commonly used, where participants are asked to squeeze a dynamometer with maximum effort. Handgrip strength is a reliable indicator of overall muscle strength and correlates with outcomes like physical performance, disability, and mortality (Bohannon, 2019). Its ease of administration, low cost, and ability to provide quick results make it a popular choice in geriatric studies. It has also been validated across different populations, including older adults, making it a consistent marker of sarcopenia and frailty. One of the main advantages is that it requires minimal equipment and training, which is useful in large-scale or community-based studies. However, it primarily reflects upper body strength and may not fully capture lower limb or core muscle strength.

In contrast, knee extension strength provides a more direct assessment of lower limb strength, which is crucial for activities like walking, standing up, and climbing stairs. Although more specific, it requires equipment like isokinetic devices or leg press machines and is more resource-intensive. Studies have shown that lower limb strength may be a better predictor of functional independence in older adults compared to upper limb strength (Nabuco et al., 2019; Michel et al., 2022). For this study, handgrip strength was selected due to its practicality, ease of use, and relevance as a general marker of muscular strength and functional status in older adults.

2.6 Physical Performance Assessment

The ability to assess physical performance is critical in frailty evaluation, as it helps determine the functional capabilities of older adults. The Short Physical Performance Battery (SPPB) is a widely used tool that includes three components: gait speed, the chair stand test, and balance tests. It provides a score ranging from 0 to 12, with higher scores indicating better physical performance. The SPPB is comprehensive, as it evaluates multiple aspects of lower extremity function and has been linked to disability, falls, and mortality (Guralnik et al., 1994; Pavasini et al., 2016).

Recent literature has continued to emphasize the importance of comprehensive tools like the Short Physical Performance Battery (SPPB), which remains a gold standard in geriatric studies. The SPPB evaluates gait speed, balance, and lower body strength, and is predictive of disability, hospitalization, and mortality in older adults (Pavasini et al., 2016). Recent studies support its ability to capture changes in physical function following nutritional and physical interventions, underscoring its applicability in clinical and research settings (Tieland et al., 2012; Park et al., 2019). It is well-validated and has been used extensively in clinical trials and observational studies. However, it may require more time to administer compared to simpler tests like the Timed Up and Go (TUG). Despite this, its ability to provide a detailed assessment of physical function makes it suitable for this research, where improvements in physical performance due to protein supplementation are being monitored.

The Timed Up and Go (TUG) test is another common tool that measures the time taken for a person to stand up from a chair, walk a few meters, turn, and return. It is a quick and practical test that focuses on speed, balance, and coordination (Browne & Nair,

2019). While it is useful for a rapid assessment of mobility, it does not provide as much information about other aspects of physical performance as the SPPB. Therefore, the SPPB was chosen for this study due to its more comprehensive nature, which allows for a more detailed understanding of the participants' physical performance.

2.7 Dietary Intake Assessment

In this study, the 3-day diet record method was used to assess participants' dietary intake. This approach involves participants recording all food and drink consumed over three non-consecutive days, including two weekdays and one weekend day. This method is often preferred over a single-day recall because it provides a more accurate representation of habitual dietary intake, especially for nutrients like protein that might vary from day to day. Compared to a 24-hour recall, the 3-day diet record reduces the impact of daily variability and is less prone to recall bias, as participants write down their intake in real-time. However, it requires active participation and may lead to underreporting if participants are not diligent (Rollo et al., 2016). Despite these challenges, the 3-day diet record is a validated tool and provides comprehensive data on nutrient intake, making it suitable for studies focusing on detailed dietary assessments, including protein consumption (Murai et al., 2023).

Another popular method is the Food Frequency Questionnaire (FFQ), which asks participants how often they consume specific foods over a defined period (e.g., weeks or months). The FFQ is less detailed than the 24-hour recall but can provide insights into habitual dietary patterns and nutrient intake. It is easy to administer, less time-consuming, and can be more reflective of regular intake. However, it may lack the precision needed to assess specific nutrient intakes accurately (Rollo et al., 2016).

CHAPTER 3

METHODOLOGY

3.1 Study Design

This study was a 12 weeks, double-blind, parallel randomized placebo-controlled clinical trial. Participants were randomly allocated into two groups to receive either whey protein supplement or placebo, and outcomes were measured at baseline (week 0), at the middle of the trial (6th week), and upon completion of 12 weeks of the trial. The study protocol complied with the guidelines of Standard Protocol Items: Recommendation for Clinical Trial (SPIRIT).

3.2 Study Location

This study was conducted at the Family Medicine Clinics and Diet Clinic of Hospital Sultan Abdul Aziz Shah (HSAAS), Selangor, Malaysia.

3.3 Study Population

The study population was men and women elderly FMC outpatients who were 60 years or older and classified (according to Fried criteria) as pre-frail older adults, which means they have one or two of most of the frailty phenotypes.

3.4 Sample Size Calculation

The sample size was calculated using OpenEpi Software (version 3.01), based on the findings of (Tieland, van de Rest, et al., 2012), according to Short Physical Performance Battery test score change in the 24th week in the placebo group 0.1(0.84) and whey protein group 1.1(0.84), with a power of 80% and an α level (2-tailed) of

5%. This gave a sample size of 12 participants/group and a total of 24 participants. With an expected dropout rate of 30%, a sample size of 16 participants/group and 32 as a total.

3.5 Eligibility and Recruitment

Patients were recruited from Family Medicine Clinic (FMC) and Dietetics clinics patients. Flyers were given to patients (in appendices) to explain the whole study program, then patients who agreed to participate were screened in HSAAS hospital using Fried Criteria Screen, and demographic and health-related information check.

Patients were screened according to the criteria listed below; the medical background screening was carried out to ensure that the older adults do not have the medical condition in the exclusion criteria. An information sheets was given and discussed following the invitation procedure. Patients who agreed to participate, were asked to sign written informed consent after their eligibility status screened to participate. Then, the patients were invited to perform the baseline assessment.

Inclusion criteria: Pre-frail men or women (1 or 2 of Fried frailty phenotypes) aged 60 years and above, willing to give informed consent to be randomized to either the protein supplement or control group, able to receive nutrients orally, and willing to follow the study protocol.

Exclusion criteria: Non or Frail older adults (Fried criteria), Patients scored by Age-adjusted Charlson Co-morbidity index by more than 3 points (considering the age points), participants who consume protein supplements or any other nutritional supplements, older adults with cognitive impairment, already involved or still

participating in any health interventional studies, any sustained fracture (hip, vertebrata) in past two month, any surgery (hip, abdominal area) in past two month.

ACCI Scale:

The Age-adjusted Charlson Co-morbidity Index (ACCI) is a modification of the Charlson Comorbidity Index that includes age as a factor in the scoring system. The ACCI score ranges from 0 to 37, with higher scores indicating a higher burden of comorbidities. The score is calculated by assigning weights to different comorbidities based on their impact on mortality, and then summing these weights to get a total score. ACCI scoring table for the important comorbidities is described below (Talley & Williams, 2015):

Table 3.1: The Age-adjusted Charlson Co-morbidity Index (ACCI) Scores

Score	Comorbid Condition
1	Myocardial infarction (MI) Congestive heart failure (CI-IF) Cerebral vascular disease Peripheral vascular disease Dementia Chronic obstructive pulmonary disease (COPD) Connective tissue disease Peptic ulcer disease (PUD) Uncomplicated Diabetes Mild liver disease Age*
2	Hemiplegia Moderate/severe renal disease Diabetes with end-organ damage Any solid tumor Leukemia Lymphoma
3	Moderate/severe liver disease
6	Metastatic solid tumor Acquired immunodeficiency syndrome (AIDS)

* For each decade after 40 years, a point is added (1 point for age group1-50, 2 points for age group 51-60, 3 points for 61-70, 4 points for 71 or oIder).

3.6 Sampling Design

3.6.1 Randomization and Allocation

After testing the eligibility, randomization took place by an independent third party (the enumerator, whose role was to translate interview questions into Malay for participants. Using a computer-generated random sequence number (Random.Org), A random sequence was generated for the participants' numbers, which results in a sequence of random numbers divided into two groups. The allocation ratio was 1:1. To guarantee allocation concealment, the randomization was carried out by the independent third party. The envelopes were opened on the spot, deciding which group the participant would be allocated to either Group 1 (PSG):

Intervention Group or Group 2 (CG): Control Group. The research assistant assigned a subject ID to each participant. The study participants, and researchers were blinded to the identity codes during the trial. The identity of the participants and the supplements they had taken were given to the researchers after they finished the study or withdrew from it.

Baseline Data Collection

Before trial commencement, demographic data of participants, anthropometric measurements, practice function/performance, muscle strength activities, and dietary intake (3-day food record) were done one week prior to the intervention period in order to get the baseline data as shown in table 2.

Face-to-face interviews

The participants were interviewed using a structured questionnaire (in appendices) including participant details and demographics, their names, hospital registration numbers, addresses, contact no., date of birth, age, gender, ethnic group, education level, job status, income level, and marital status. The medical history, including the type of disease, surgery, duration, treatment obtained, and medication received, was also asked. To overcome the language barrier during communication with the participants, the enumerator was tasked with conducting interviews and asking study questions in Malay, ensuring effective communication and understanding between the participants and the research team.

The measurements and interviews were conducted in the Dietetics Clinic in HSAAS Hospital. The outcome measures were obtained three times (at the beginning of the trial week 0, in the follow-up session 6th week, and at the end of the trial 12th week) as shown in Table (3.3). Each participant took a place for the assessments by around 30 minutes.

Table 3.2: The assessment procedures based on trial time points

The assessment procedure	Visit		
	Baseline Week 0	**6th week Assessment**	**Post-intervention 12th week**
Questionnaire			
Social demographicbackground	√		
Frailty status, scores, phenotypes	√	√	√
Dietary assessment			
3-day dietary record	√		√
Anthropometric measurements			
Body weight	√	√	√
Height	√		
Mid arm circumference	√	√	√
Total body fat	√	√	√
Skeletal Muscle mass	√	√	√
Calf circumference	√	√	√
Leg Lean Mass	√	√	√
Physical performance:			
SPPB score	√	√	√
Balance	√	√	√
Gait speed	√	√	√
Chair-stand time	√	√	√
Muscle strength			
Handgrip strength	√	√	√

3.7 Intervention

Intervention Group:

Protein Supplement group PSG:

Participants received 30g of *SUSTINEX* Hydrolyzed Whey Protein. The participants were asked to add it to their food or drinks to reach the goal of consuming 30g daily.

Control Group (CG):

Participants in CG received 30g of Maltodextrin (*Carbomax*). The participants were asked to mix the powder with their foods or drinks.

SUSTINEX Whey Protein Plus and Carbomax are authorized supplements which are manufactured and made in Malaysia by *Nova Laboratories Sdn. Bhd. (179832-D).* *SUSTINEX Whey Protein Plus* consists of whey protein isolate and whey protein hydrolyze, 30 g of WPS contains 27.39 g protein, and 115.8 kcal, while *Carbomax* consists corn starch with 28g CHO and 114 kcal per serving (30 g) as shown in table 4.4 and 4.5.

Table 3.3: Nutritional Value of Whey Protein Plus Supplement

Nutritional Information	Unit	Per (100g)	Per serving (30g)
Energy	Kcal	386	115.8
Protein	g	91.3	27.39
Fat	g	0.96	0.288
Saturated fat	g	0.52	0.156
Polyunsaturated fat	g	0.12	0.036
Monounsaturated fat	g	0.31	0.093
Trans fat	g	0.025	0.0075
Cholesterol	mg	79	23.7
Carbohydrate	g	0.56	0.168
Lactose	g	0.56	0.168
Minerals			
Calcium	mg	83	24.9
Sodium	mg	500	150
Potassium	mg	64	19.2
Magnesium	mg	65	19.5
Phosphorus	mg	48	14.4
BCCAs	g	26.00	7.8
Leucine	g	14.20	4.26
Isoleucine	g	6.20	1.86
Valine	g	5.60	1.68

Table 3.4: Nutritional Value of Carbomax Supplement

Nutritional Information	Unit	Per (100g)	Per serving (30g)
Energy	Kcal	380	114
Protein	g	0	0
Fat	g	0	0
Carbohydrate	g	94.5	28.35
Minerals			
Calcium	mg	25	7.5
Sodium	mg	70	21
Iron	mg	0	0
Potassium	mg	1	0.3
Chloride	mg	150	45
Phosphorus	mg	8	2.4

Supplements Supplying & Follow-up Process:

All participants were given scoops measured 15g and instructed on how to fill and level it up and mix supplements with their foods or drinks. They were instructed how to record their intake in the provided compliance diaries. The both supplements were in powder form in identical containers labeled by A,B as supplements ID and subjects were asked to mix 2 scoops daily in their soft foods or beverages (1 scoop in the morning & 1 scoop in the evening). Supplements were handed to the participants in the dietetic clinic at the beginning of the trial and in during the follow-up (6th week) of the trial and they were asked to return the previous containers in order to check the remaining amounts and compliance status. Participants were also advised not to change their habitual diet including dietary protein intake during the trial.

Compliance diaries were handed out to participants with instructions provided on how to complete them (in appendices); specifically, they were asked to record the timing and number of scoops consumed per day. The researcher checked the compliance with

the supplement's prescription on a daily- basis message reminder sent to each participant and they were asked to take a photo for the containers weekly. According to the remaining balance and the dairies, if the study team identified that a participant's compliance to the planned number of the supplement's scoops, is less than 70%, the researcher respectively called the participant to assess any difficulties and identify strategies that could facilitate compliance.

There is no any proven drug interaction with the study product, however, participants were asked not take any over-the-counter medications without informing the researcher and can continue taking the prescribed medications by their respective physicians.

The participants were asked on a daily basis (as a part of the taking supplement message reminder) if they felt any discomfort or unusual pain while taking the supplement; in case one existed, the researcher referred the medical doctor (Dr. Hakimah) to assess and monitor the case individually. The medical care was provided for the participant to monitor any side effects or discomfort during each visit.

3.8 Study Instruments

Table 3.5 shows the Study Variables and the applied tools.

Table 3.5: Study Variables and the applied tools

Variable	Instruments
Frailty phenotypes - Weight loss/ shrinking - Exhaustion - Weakness - Slowness - Low activity	- Report sheet according to Fried criteria (2001).
Body composition - Bogy weight, height, BMI - Body part circumference including mid-upper arm circumference (MUAC) and calf circumference (CC) - Total body fat (TBF) - Skeletal muscle mass - Leg lean mass	- A portable body composition analyzer (InBody 770). - A flexible non-stretchable measuring tape - A portable body composition analyzer (InBody 770).
Physical performance (PPB) - Gait speed - Balance test - Chair stand test	- Short physical performance battery protocol and score sheet (appendix)(Guralnik et al., 1994)
Muscle strength - Handgrip strength	-Hand dynamometer (Jamar Smart Digital Hand Dynamometer).
Dietary Intake	-3-days Diet Record

3.9 Outcomes Measurements

3.9.1 Frailty Status

Non-frailty (Normal)

According to Fried and colleagues' (Fried et al., 2001) five physical phenotypes (unintentional weight loss, tiredness, slowness, weakness, and physical inactivity), aged adults who match no criterion are classed as non-frailty (normal) (Fried et al., 2001).

Pre-frailty

Elderly persons who fulfil one or two of the five physical phenotypes (Fried criteria) will be classed as pre-frailty (Fried et al., 2001).

Frailty

Elderly adults who meet three or more of the five physical phenotypes (Fried criteria) are classed as frail (Fried et al., 2001).

3.9.2 Frailty Phenotypes

1) Weight loss/ shrinking

Body mass index (BMI: weight/height^2 <18.5 kg/m^2 or self-reported unintentional weight loss ≥10 pounds (4.5 kg) in the last 6 months (Feng et al., 2014).

2) Exhaustion

The participants were asked to read two statements from the Centre for Epidemiologic Studies-Depression Scale (Radolff, 1977):

"I felt that everything I did was an effort" and "I could not get going".

The participant will then be asked how often in the last week he/she felt this way.

- 0=rarely or none of the time
- 1=some or a little of the time (1–2 days)
- 2=moderate amount of the time (3–4 days)
- 3=most of the time

A score of 2 or 3 is defined as exhaustion.

3) Weakness

Grip strength was measured using a hand-held dynamometer (*Jamar Smart Digital Hand Dynamometer*). The average of two attempts on the dominant hand was used. Male respondents who scored <26 kg were classified as having weak grip strength, while female participants with a score of <18 kg were classified as having weak grip strength according to the modified cut-off point suggested by Asian Working for Sarcopenia (AWGS) (Chen et al., 2020).

4) Slowness

The time to walk four metres was measured without a walking aid. Those respondents with a walking time of ≥ 0.8 m/seconds were classified as having slow walking speed (Fairhall et al., 2008).

5) Low activity

Participants meet the criterion for physical inactivity if, in the past three months, they did not perform weight-bearing physical activity (any activity that forces to work against gravity, e.g.: weight training, jogging, climbing stairs, tai chi, yoga, racquet sports, hiking etc.), spent >4 hours per day sitting, and went for a short walk ≤1 per month (Cesari et al., 2009; Fairhall et al., 2008).

3.9.3 Physical Performance

Physical performance was assessed by the short physical performance battery (SPPB) (Guralnik et al., 1994), which consists of three components: balance, gait speed, and chair-stand ability. Scores of 1 to 4 are based on categories of performance in the

balance tests, on the time necessary to complete the walk, and on the time needed to perform the chair-rise test. A summary performance score of 0 to 12 was calculated by summing the scores of the tests (Tieland, van de Rest, et al., 2012).

A. Balance test

This test worth 4 out of 12 points. The participants were asked to stand unsupported in three different positions (feet together, semi-tandem, and full tandem) as shown in figure 3.1 for 10 seconds in each. Side-by-side stand and semi-tandem stand are worth 1 point and 2 points for the full tandem stand if done successfully.

Figure 3.1: The three positions of the balance test.

B. Gait Speed test

The participants were asked to walk to the line on the floor (3 and 4 meters) at a normal speed; then the time was recorded. The shorter time in both tests was chosen in both tests. The points are described as below:

Table 3.6: The points of the gait speed test according to the time in the 3 and 4-meter walking test.

Points	3 Meters	4 Meters
1	More than 6.52 sec	More than 8.70 sec
2	4.66 to 6.52 sec	6.21 to 8.70 sec
3	3.62 to 4.65 sec	4.82 to 6.20 sec
4	Less than 3.62 sec	Less than 4.82 sec

C. Chair Stand Test

Participants were asked to do a single chair stand (one time) to check their ability to stand unsupported (without using arms) as shown in Figure (3.2). If the attempt was successful, the repeated chair-stand test was performed. The time was recorded for full five-stand times and assessed according to the below table:

Table 3.7: The points of repeated chair-stand test recorded times

Points	Time
1	16.70 sec or more
2	13.70 to 16.69 sec
3	11.20 to 13.69 sec
4	11.19 sec or less

Figure 3.2: Chair-stand test technique.

3.9.4 Muscle Strength

The 1-RM grip strength was used for strength assessment since they are associated with physical disability, morbidity, and mortality in older persons (Nilsson et al., 2020). Patients were asked to sit with their elbows by their sides and flexed to right angles and neutral wrist position. Subjects were asked to hold the hand dynamometer (*Jamar Smart Digital Hand Dynamometer*) as shown in Figure (3.3) and press two times by their dominant hand. The mean of the two attempts was recorded in the assessment logbook.

Figure 3.3: The hand dynamometer (*Jamar Smart Digital Hand Dynamometer*) used during the trial

3.9.5 Body Composition

Body weight, total body fat (TBF), and skeletal muscle mass (SMM) for protein supplemented group (PSG) and control group (CG) were measured in the morning to the nearest 0.1 kg using bioelectrical impedance analysis (BIA) (InBody 770, from *InBody Asia, Kualalumpur- Malaysia*) as shown in Figure (3.4). Prior to the measurement, respondents need to remove all metal accessories that might interfere with the reading. Information like age, gender and height will be set into the analyzer. Respondents were required to stand on a weighing platform with bare feet in contact

with the metal parts while both hands holding tightly the grip electrodes with outstretched arms.

Figure 3.4: The in-body analyzer used during the trial (InBody 770).

Mid-upper arm circumference (MUAC) and calf circumference (CC) were assessed using the Lufkin Measuring Tape (W606PM-375 Model). MUAC was determined as the circumference of the midpoint between the acromion and olecranon process at the arm area, with the body in an upright position and the elbow flexed at a 90º angle (Figure 3.5). Meanwhile, to measure CC, the measuring tape was comfortably wrapped around the widest part of the calf while the participants were seated (Figure 3.6). Both measurements were recorded to the nearest 0.1cm.

A Mid-Upper Arm Circumference (MUAC) of less than 23.0 cm for men and 22.0 cm for women is indicative of reduced peripheral muscle mass, as established by Shahar Suzana and Siti Saifa (2007). Similarly, Calf Circumference (CC) values are used to

assess muscle loss in the lower limbs, with a threshold based on a local classification developed by Sakinah et al. (2004) for the Malaysian elderly population. According to this criterion, a CC measurement of less than 30.1 cm for men and 27.3 cm for women is suggestive of muscle loss.

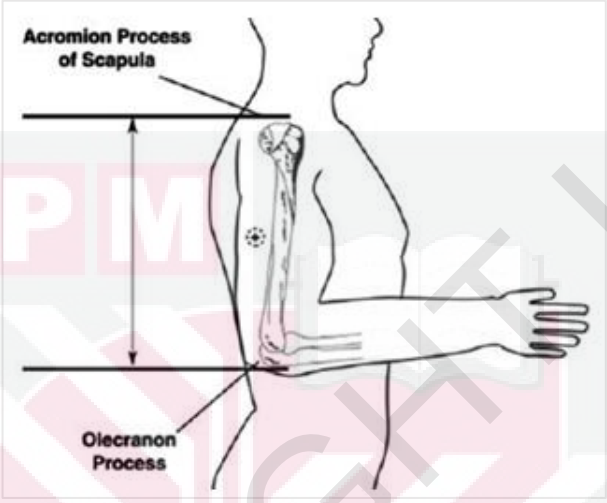


Figure 3.5: MUAC measurement technique.

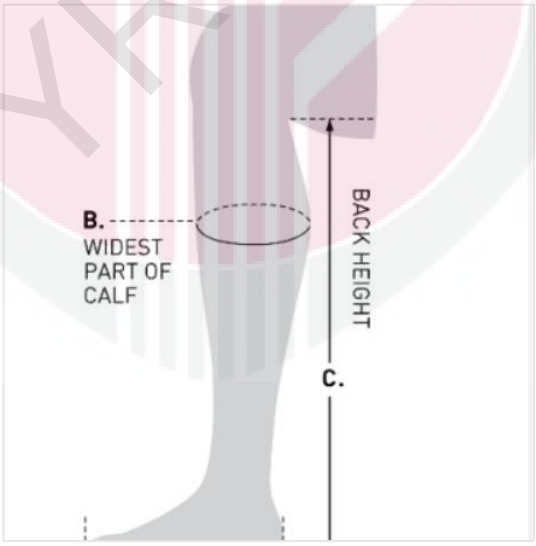


Figure 3.6: Calf circumference measurement technique

3.9.6 Dietary Assessment

Dietary intake was measured through a 3-day dietary record at baseline and 3 days prior final assessment to obtain total energy, carbohydrate (CHO), protein, and fat intake. The choice of a 3-day dietary record was based on its ability to provide a comprehensive yet feasible assessment of habitual intake. Research suggests that a 3-day record, including one weekend day, strikes a balance between accuracy and participant burden, offering good reliability without leading to participant fatigue (Thompson & Subar, 2017). Longer records may result in lower compliance and greater reporting fatigue, whereas shorter records (e.g., one day) may not adequately capture habitual intake.

The participants were asked to keep record of all food and beverage intakes during 2 weekdays and one weekend of the allocation week (prior supplement consumption) and at the 12th week of the trial. The selection of two weekdays and one weekend day was made to capture variations in dietary patterns between workdays and non-workdays. Research has shown that dietary intake can differ significantly between weekdays and weekends, with higher caloric, and fat consumption often observed on weekends (An, 2016; Gherasim et al., 2020). The participants were instructed to record their intake before the visit. Details of food and drinks information and descriptions were also recorded. In situations where foods and beverages were consumed away from home, the participants were encouraged to describe the quantities of foods and beverages consumed using the household measures as well (e.g., glass, cup, Chinese rice bowl, plate, tablespoon, and teaspoon). Both groups were provided an explanation by the researcher on how to record their 3-day intake in their food diaries. They were

also given a detailed set of instructions on how to measure, record their food intake together with the diet record.

Follow-up calls or messages were conducted after the 3-day records were submitted. These interviews were essential to verify the accuracy of the entries, particularly regarding portion sizes and forgotten items, which are common sources of error in dietary self-reporting. During the interviews, participants were asked to provide additional details on ambiguous or incomplete entries (e.g., the specific type of food item, cooking methods, or whether leftovers were consumed). For example, if a participant recorded "chicken," the follow-up would ensure the correct portion (breast, thigh) and preparation method (fried, grilled) were noted. If discrepancies were identified, such as implausibly low caloric intake for a reported day, participants were prompted to recall any items they may have forgotten, like snacks or beverages consumed between meals.

A computerized local dietary analysis program, Nutritionist Pro version 2.0 (2013) (First Data Bank, The Hearst Corp. United States of America [USA]) was used to analyze the macro nutrient intakes of the patients (carbohydrate, protein and fat intake). The serving size and the nutrition composition for each food items recorded were coded by type, and analyzed for nutrient content primarily based on the Malaysian Food Composition Table (Tee et al., 1997) and Atlas of Food Exchange and Portion Size (Abdul Manaf et al., 2015).

Overall, to ensure interrater reliability and minimize inter-researcher bias, all measurement records were analyzed by the researcher after intense training following standardized guidelines. A random subset of records was independently reviewed by

the supervisor to assess consistency. As part of quality control, periodic checks were conducted to verify that data entry was accurate, for instance, coding food items and quantities. Any discrepancies in coding were flagged and resolved through reanalysis by a second researcher (statistician). The entire analysis was conducted in a blinded manner, with researchers unaware of the participants' group allocation to minimize bias in data interpretation.

3.10 Pre-test of the Study Measurements

In order to ascertain the reliability of the variables and refine the appropriateness of the questionnaire items, as well as to ensure that enumerators were proficient in conducting the assessment procedures, a comprehensive pre-test of the study measures was conducted prior to the commencement of the main study in March 2023. This pre-test involved a subset of participants, comprising more than 10% of the intended sample size, to adequately represent the target population.

During the pre-test phase, participants underwent the assessment process, obtaining valuable insights on the questionnaire structure, clarity of questions, and overall study procedures. Enumerators were also thoroughly trained and familiarized with the assessment protocols to ensure consistency and accuracy in data collection.

Feedback obtained from the pre-test, including comments, suggestions, and any observed challenges, was meticulously reported to refine the study instruments and procedures. Modifications were made to address any identified issues and enhance the overall effectiveness of the data collection process.

3.11 Ethical Consideration(S)

3.11.1 Ethical Approval

The clinical study was carried out in accordance with the Helsinki Declaration and Good Clinical Practice Standards (GCP). Prior to the start of the trial, all research participants were asked to provide written informed permission. The permission to implement the trial was asked from the Ethics Committee for Research with Human Subjects at Universiti Putra Malaysia (JKEUPM-2022-907) and from Clinical Research Unit (CRU) of Hospital of Sultan Abdul Aziz Shah (HSAAS). All participants were requested to sign a permission form consenting to take part in the study. The trial also is registered at clinicaltrials.gov with the reference number (NCT05648617). All of these documents are attached in the appendices section.

3.11.2 Consent Process

Potential participants who were interested in participating were instructed to contact Principal investigator Dr. Siti Nur'Asyura Binti Adznam or Alaa Al-Rawhani for additional information (phone or email). If they were found to be eligible following the initial screening, potential participants were invited to an information session. Details of the study were given and written down in the form of a Patient Information Sheet at the information session. Prior to participating in any research activity, participants were requested to sign a permission form consenting to take part in the study. In the study, a Patient Information Sheet and a Consent Form in English and Malay were used. A summary of the findings upon completion of the study was provided to the participants upon request.

3.11.3 Anticipated Benefits

The originality of this research contributes significantly to the literature that aims to improve frailty outcomes for frail elderly, especially in Asian countries.

3.11.4 The Potential Risks and Side Effects of the Study

The risks connected with this study were expected to be modest and all the possible contradictions were well explained during the consent process. However, during the experiment, some individuals felt mild discomfort or stomach upset. They were asked to mix the supplement with plenty of water or soluble food and consume the supplement between meals to avoid any interference. No other side effects were observed until the end of the trial.

3.11.5 Protection of Privacy and Preservation of Confidentiality

Personal information obtained or created during the study was kept in a locked office and used solely in line with the study's goals. Data files for each participant were controlled using unique identifying numbers, and no personal information will be made available to other researchers or third parties. The study's findings may be published in peer-reviewed scientific publications, but the participants will not be identifiable in any manner. The data were enrolled into the password-locked computer for analysis purposes. Data from the study were archived and will be stored for a minimum of three years after the study completion, but the subjects' identities will not be revealed at any time.

3.11.6 Publication Policy

No personal data was disclosed, and participants were not identified during any stage of publication.

3.11.7 Declaration of Conflict of Interest

The principal investigator declares that there is no conflict of interest.

3.12 Data analysis

The research design is structured on three assessment points, which involved the baseline, the 6th week, and after the intervention, the 12th week. The analysis process was performed based on the intention-to-treat (ITT) principle, where data from all patients who consented to participate in the study were included in the analysis. Missing data were handled by Multiple Imputation way (5 imputations) as the most accurate way to handle missing data (Li et al., 2016). The imputed data were generated and then pooled to get the ITT population using SPSS (version 26).

Categorical variables were summarised by frequencies and percentages, and continuous variables were summarised by means and standard deviation. The chi-square test and Cochrane`s Q test were used to investigate the difference between and within groups with categorical data.

The continuous data were further examined for conformity to normality distribution assumptions; the continuous data with non-normality characteristics were summarized by the median (IQR). Analysis of variance (ANOVA) of General Linear Model (GLM) repeated measures for the second factor was used to assess variability in the numerical

measures between the baseline, middle, and post-intervention. Independent t-test was used for bivariate analysis (ex: Dietary Intake). Non-parametric statistical tests such as Mann Whitney and Wilcoxon signed rank test, among other statistical methodologies, were adopted accordingly for non-normal continuous variables and the Paired t-test or Wilcoxon Signed Rank test was used to compare each pair of time points within the group. All the statistical analyses were conducted using SPSS version 26 and the significant value is if the P value is less than 0.05.

CHAPTER 4

RESULTS

4.1 Recruitment, Enrolment, and Subjects Follow-up

The screening has been done for 116 patients; only 40 participants who meet the inclusion criteria of the study agreed and signed the consent form to participate in the study. Another 55 patients were excluded because of their medical history, 7 participants were frail, 10 were in normal status, and 4 pre-frail patients declined to join the study. The patients were recruited and randomly allocated to PSG (20 participants) and CG (20 participants), 36 participants who completed the study and 4 withdrawals, as shown in the CONSORT flow diagram Figure (4.1).

The eligible patients were interviewed to assess their frailty status, and took the required measurements by the researcher and her assistant. The researcher assistant (an independent party) has the codes of the participant's allocation, and all the study researchers were blinded to these allocations until the end of the study.

Participants were monitored daily throughout the study to ensure their well-being and compliance with the intervention. Each day, they were contacted either in person or by phone to check if they had experienced any discomfort, unusual symptoms, or side effects. If a participant reported any concerns, the case was referred to the medical supervisor, who evaluated whether the issue was related to the supplement and provided appropriate advice or treatment if necessary. This regular follow-up also played a key role in promoting adherence to the study protocol. Participants were reminded of their supplement intake, dietary records, and other instructions to maintain

consistency throughout the trial. The daily contact helped build rapport and encouraged participants to remain committed to the study.

It’s important to note that no adverse effects related to the supplements were reported during the study. This suggests the supplements were well tolerated, and there were no significant issues that required intervention or changes to the study protocol.

The daily follow-up was crucial for monitoring compliance, with participants required to bring both their compliance forms and supplement containers to each assessment visit. Compliance was tracked using two main methods: the participants' self-reported compliance on the forms, and the physical count of remaining supplements in the containers. When there was a discrepancy between the two, the container balance was given priority as it provided a more objective measure of actual consumption.

Compliance was classified as a percentage based on the amount of supplement consumed compared to the prescribed amount. Participants were considered compliant if their consumption rate was 75% or higher. Those with consumption below this threshold were flagged as non-compliant. For instance, two participants in the PSG (protein supplement group) showed compliance below 75%, indicating poor adherence to the supplementation regimen. Their levels were classified as low compliance based on their reported rates of less than 75%.

On the other hand, the majority of participants demonstrated high levels of compliance. The overall compliance rate was 94.37% across both groups, with a near-perfect rate of 98.6% in the control group (CG) and a slightly lower but still strong compliance rate of 90.13% in the protein supplement group (PSG). Three participants in the PSG

had compliance percentages just above the 75% threshold, with values of 78%, 81.5%, and 78%, respectively. These were considered moderate compliance, where participants adhered to the regimen but with minor deviations.

By monitoring compliance daily and using the container balance as the primary measure, the study ensured a high level of adherence, which is important for the validity of the intervention outcomes. Any deviations were closely documented, and participants with lower compliance were noted as potential outliers in the analysis, particularly in the PSG group where compliance issues were slightly more frequent.

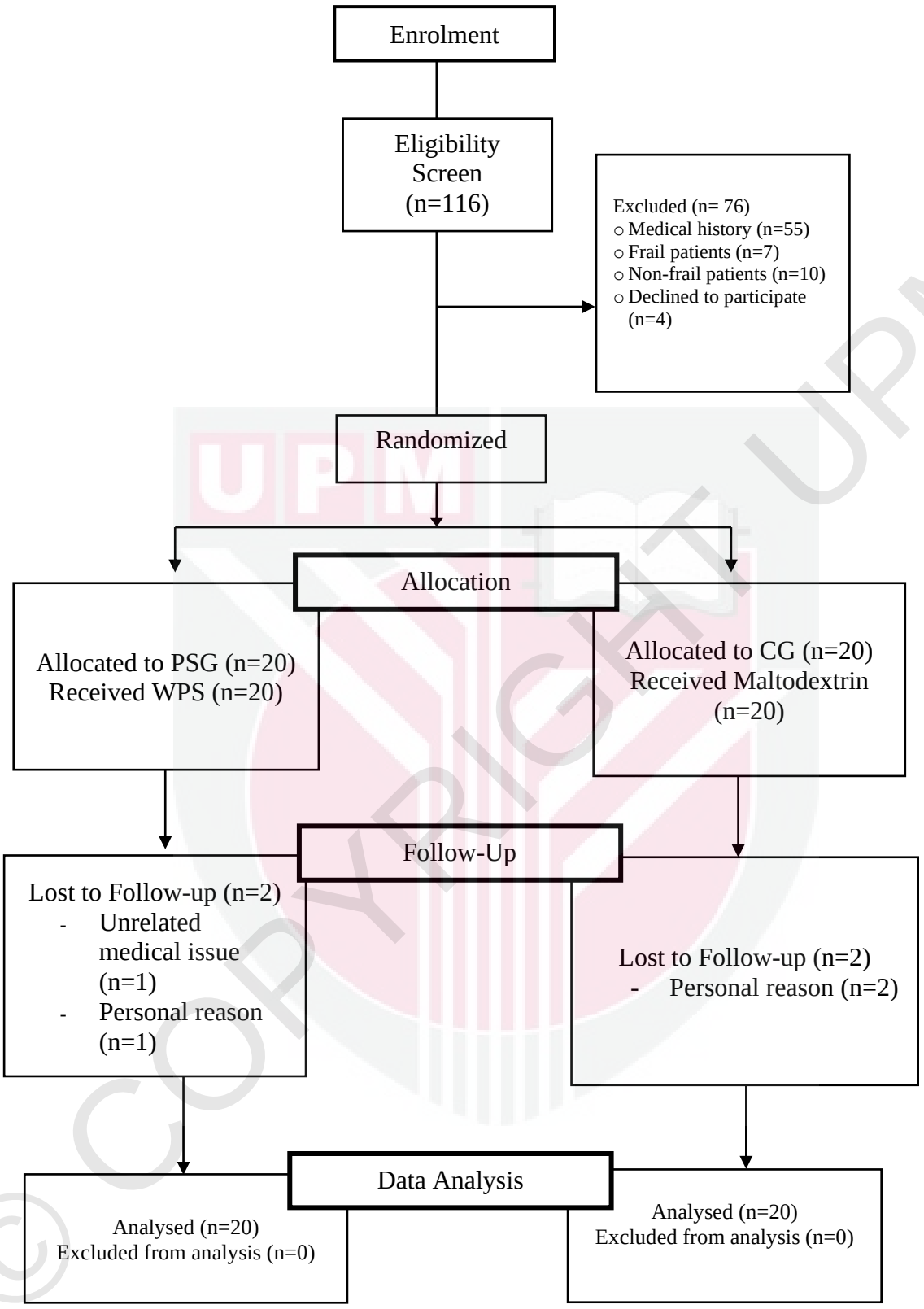


Figure 4.1: CONSORT flow diagram of the study

4.2 Baseline Comparison Between Groups

4.2.1 Socio-demographic Characteristics of Groups Participants

The socio-demographic characteristics of the participants are presented in Table (4.1). The mean age of the participants was 66.42±3.78, with the majority of females (n=25) compared to males (n=15), with no statistical difference between the two groups. Besides, the majority of the participants were Malay (39), and 1 participant was Chinese. The marital status has the same ratio as well; 39 participants were married, and another one was divorced. Most of the participants had a university education (72.5%), and 27.5% had a tertiary or primary school. The majority of the patients were retirees (87.5%) and relied on pensions for their income (65%) or other sources (9%) or from their family (12.5%), as shown in the table 4.1. In general, there was no statistical difference in socio-demographic characteristics between the groups.

Table 4.1: Socio-demographic data of the pre-frail older adults in the interventional group (PSG) and control group (CG) at Baseline

Characteristic	Overall (n=40)	PSG (n=20)	CG (n=20)	p-value[b]
Age, years[a]	66.42±3.78	66.85±3.34	66±4.21	0.484
Gender (%)				
- Male	15 (37.5)	8 (40)	7 (35)	0.744
- Female	25 (62.5)	12 (60)	13 (65)	
Race (%)				
- Malay	39 (97.5)	19 (95)	20 (100)	0.5
- Chinese	1 (2.5)	1 (5)		
Marital Status (%)				
- Married	39 (97.5)	20(100)	19 (95)	0.5
- Divorced	1 (2.5)	-	1 (5)	
Education (%)				
- School	11 (27.5)	6 (30)	5 (25)	0.93
- University	29 (72.5)	14 (70)	15 (75)	
Work Status (%)				
- Housewives/still work	5 (12.5)	- 3 (15)	2 (10)	0.081
- Retiree	35 (87.5)	- 17(85)	18 (90)	
Financial Resources:				
- Pension	26 (65)	14 (70)	12 (60)	0.329
- Family	5 (12.5)	2 (10)	3 (15)	
- Other	9 (22.5)	4 (20)	5 (25)	

* (mean ±SD)

[a]Independent t-test, α was set at 0.05, [b]Chi-square test, α was set at 0.05

4.2.2 Anthropometric Measurements of Groups Participants

The anthropometric characteristics of the participants at baseline were analyzed, including height, weight, BMI, mid-upper arm circumference (MUAC), calf circumference (CC), total body fat (TBF) percentage, skeletal muscle mass (SMM), and leg lean mass (LLM). There was no significant difference between groups in these measurements at baseline. Generally, height, weight, and BMI were almost similar between the groups. The mean weight in both groups was 65±8.8 kg. The majority of the study participants were classified as overweight (72.5%) with an average BMI of 26.49±3.13 kg/m2. MUAC and CC were consistent across the groups with no significant difference, with a mean of MUAC 27.85±2.78cm and a median of CC 35 cm. Total body fat percentage was almost similar in both groups and higher than the normal range, with a median of 39.6 (13.08)%, 37% of body weight in PSG and CG. SMM was 21.62±4.78kg in PSG and 22.17±4.34kg in CG. Additionally, LLM was comparable as well in both groups with a mean range of 12.42±2.85kg. the anthropometric data of study participants are shown in Table 4.2.

Table 4.2: Anthropometric data of the pre-frail older adults in the interventional group (PSG) and control group (CG) at Baseline.

Measurement	Overall (n=40)	PSG (n=20)	CG (n=20)	p-value
Height (m)	1.57±0.07	1.56±0.06	1.57±0.09	0.903
Weight (kg)	65.29±8.8	64.29±9.32	66.29±8.55	0.483
BMI (kg/m^2)	26.49±3.13	26.14±3.33	26.84±2.96	0.482
- Normal n(%)	- 11(27.5)	- 6(30)	- 5(25)	
- Overweight n(%)	- 29(72.5)	- 14(70)	- 15(75)	
MUAC (cm)	27.85±2.78	27.9±2.9	27.8±2.7	0.911
- Muscle wasting n(%)	1(2.5)	1(2.5)	-	
CC (cm)	35 (3.88) *	35.37±3.08	36.05±3.41	0.516
- Muscle wasting n(%)	-	-	-	
TBF (%)	39.6 (13.08) *	37.34±8.4	37.48±7.95	0.956
SMM (kg)	21.89±4.52	21.62±4.78	22.17±4.34	0.706
LLM (kg)	12.42±2.85	12.14±2.81	12.7±2.94	0.535

*Non-normally distributed data presented as median (IQR).
[a]Independent t-test, α was set at 0.05.
BMI: Body Mass Index; MUAC: Midd-upper Arm Circumference; CC: Calf Circumference; TBF: Total Body Fat; SMM: Skeletal Muscle Mass; LLM: Leg Lean Mass.

4.2.3 Frailty Status, Scores and Phenotypes of the Participants

While the study population were pre-frail elderly people, all the study participants had pre-frailty status at baseline. However, 65% of the study population had pre-frailty status with 2 scores, 70% in PSG and 60% in CG. In addition, one-score- pre-frailty had the lesser percentage (35%) in both groups, 30% and 40% in PSG and CG, respectively. When it comes to the frailty phenotypes, most of the participants were low-active at baseline (95% in PSG, and 70% in CG). The slow-walking (≤ 0.8 m/s) was the second most prevalent frailty criterion among the participants (30% in PSG and 60% in CG). Finally, 40% of PSG subjects had muscle weakness, and 20% in CG. There was no participant suffering from fatigue at baseline. Overall, there was no significant difference in frailty scores or criteria between the groups at baseline, as shown in Table 4.3.

Table 4.3: Frailty Scores and Phenotypes of the pre-frail older adults in the interventional group (PSG) and control group (CG) at Baseline.

Measurement	Overall (n=40)	PSG (n=20)	CG (n=20)	p-value[a]
Frailty status n(%)				
Pre-frail (1 point)	14 (35)	6(30)	8(40)	0.507
Pre-frail (2 points)	26 (65)	14(70)	12(60)	
Frailty phenotypes n(%):	3 (7.5)	1(5)	2(10)	1.0
Weight loss	-	-	-	-
Fatigue	12 (30)	8 (40)	4(20)	0.168
Muscle Weakness	18 (45)	6 (30)	12(60)	0.057
Slow walking	33 (82.5)	19(95)	14(70)	0.091
Low activity				

[a]Chi-square test, α was set at 0.05

4.2.4 Muscle Strength and Physical Performance of Groups Participants

Handgrip strength (HGS) was comparable in both groups (23.58±7.29 kg in PSG, and 23.53±5.71 kg in CG) with no significant difference between groups at baseline. The mean range of gait speed (GS) in both groups was 0.9±1.67 m/s with similar values in each group. Chair-stand test (CST), Short Physical Performance Battery tests (SPPBT), and 4-meters walking test (4MWT) were not normally distributed at baseline, however, these measurements values were similar in both groups, the median of CST was 14.7(5.6) seconds. Furthermore, participants in both groups had the median of 10 scores out of 12 in SPPBT. Finally, 4MWT was 4.46 (1.14) at baseline in both groups. There was no significant difference in muscle strength and physical performance measurements at baseline between PSG and CG as shown in table 4.4.

Table 4.4: Muscle Strength and Physical Performance of the pre-frail older adults in the interventional group (PSG) and control group (CG) at Baseline.

Measurement	Overall (n=40)	PSG (n=20)	CG (n=20)	p-value
HGS (kg)	23.58±7.29	23.63±8.74	23.53±5.71	0.967
GS (m/s)	0.9±1.67	0.93 ±0.17	0.87±0.158	0.285
CST (s) [b]	14.7(5.6) [*]	14.25 (4) [*]	15.77±4.97	0.565
SPPBT[b]	10(2) [*]	10 (1.75) [*]	9.8±1.23	0.968
4MWT (S) [b]	4.46 (1.14) [*]	4.28 (0.78) [*]	4.61±0.76	0.183

*Non-normally distributed data presented as median (IQR).
[a]Independent t-test, α was set at 0.05, [b]Mann-Whitney test, α was set at 0.05
HGS: Handgrip Strength; GS: Gait Speed; CST: Chair-stand test; SPPB: Short Physical Performance Battery test; 4MWT: 4-meters Walking test

4.2.5 Dietary Intake of Groups Participants

The average energy intake of the 40 participants in both groups was approximately 1780 kcal/day, which converts to roughly 27 kcal/kg/day based on the average weight of participants (65kg). According to the Recommended Nutrient Intakes for Malaysia, older adults require between 1780kcal/day for males and 1550 kcal/day for females, suggesting that participants were within the recommended range according to their physical activity. CG consumed slightly more (200 kcal) than PSG, with an average of 1882 kcal/day. The habitual protein intake in both groups was in between the RDA limits, which is 1.03g/kg of body weight per day and approximately 66g/day and within the acceptable range of intake (10-35% of total calories) according to RNI of Malaysia elderly.

The participants of the interventional group consume 0.99g/kg/d at baseline. However, subjects in the control group have more carbohydrate and fat intake than the interventional group (260g/d and 68g/d), respectively. However, all the dietary baseline data reveals no significant difference between groups, as shown in Table 4.5.

Table 4.5: Dietary Intake data of the pre-frail older adults in the interventional group (PSG) and control group (CG) at Baseline.

Macronutrient	Overall (n=40)	PSG (n=20)	CG (n=20)	p-value[a]
Energy (kcal/d)	1779±496	1676±495	1882±488	0.194
Protein				
(g/d)	66.12±20.12	63.37±22.8	68.86±17.16	0.341
(g/kg/d)	1.036±0.32	0.99±0.34	1.07±0.3	0.441
(%)	15.03±3.11	15.08±3.34	14.97±2.95	0.915
Carbohydrate				
(g/d)	242.59±74.4	224.23±73.88	260.95±72.09	0.06
(%)	54.65±6	53.75±7.14	55.55±4.64	0.758
Fat				
(g/d)	63.25±32.64	58.4±20.69	68.1±41.35	0.445
(%)	31.19±8.15	31±4.5	31.35±10.77	0.659

[a]Independent t-test, α was set at 0.05.

4.3 Effect of Whey Protein Supplements on Frailty Improvement

4.3.1 Frailty Status

There was a significant improvement in frailty status over time across both groups, as indicated by the P value of 0.00. In the PSG group, 70% of participants were classified as pre-frail (level 2) at baseline, and by Week 12, this reduced significantly to only 15%, with 45% transitioning to a normal frailty status. Similarly, the CG group also experienced improvements, though more modestly. At baseline, 60% of participants were pre-frail; by Week 12, this dropped to 25%, with 40% reaching normal frailty status, as shown in Table (4.6) and Figure (4.2).

The intervention in PSG and placebo effect in CG can justify these improvements in frailty status. In the PSG group, which likely received protein supplementation, this can be attributed to the beneficial effects of increased protein intake, which is known to support muscle mass and overall physical function. Although the changes were less pronounced in the CG group, the improvements may be linked to the placebo effect or lifestyle changes during the study, such as better adherence to healthier habits. No significant interaction between time and group effects (P value 0.73) suggests that both groups improved similarly over time, likely due to a combination of intervention and external factors such as motivation and engagement in the study.

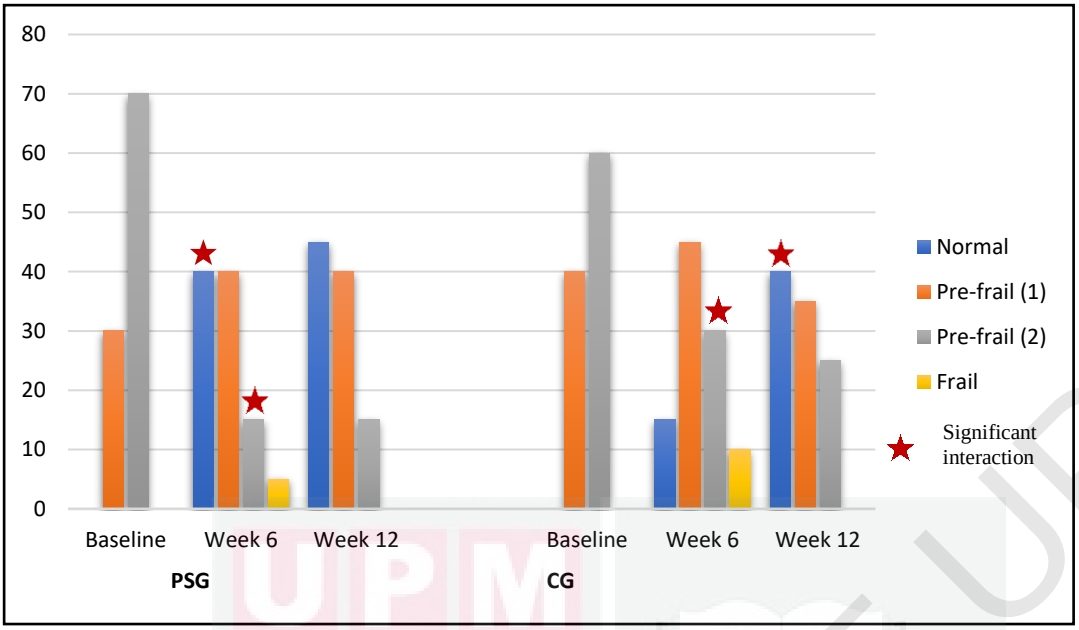


Figure 4.2: The prevalence of frailty status changes during pre, middle and post-intervention of the pre-frail older adults in the interventional group (PSG) and control group (CG).

4.3.2 Frailty Scores

Frailty scores also showed significant improvements over time in both groups, with a P value of 0.00. In the PSG group, the median frailty score decreased from 2 (IQR = 1) at baseline to 1 (IQR = 1) by Week 12. Similarly, in the CG group, the frailty score reduced from 2 (IQR = 1) to 1.75 (IQR = 1). These reductions in frailty scores reflect the positive impact of the interventions over time, particularly in helping participants maintain or improve muscle strength, mobility, and physical function.

The significant improvements in frailty scores in the PSG group are likely due to the targeted protein supplementation, which helps preserve muscle mass and strength, key components of frailty. In the CG group, although slightly less, the reduction in frailty scores can be explained by the placebo effect or improvements in daily activity and nutrition awareness brought about by the study. Since no significant time-by-group interaction was found (P value 0.60), the data suggest that both groups experienced

similar declines in frailty scores, reinforcing the idea that both the intervention and the control conditions facilitated improvements.

4.3.3 Frailty Phenotypes

Moving on to frailty phenotypes, the analysis of specific components such as weight loss, muscle weakness, slow walking, and low activity revealed varying degrees of prevalence across the groups. Notably, most of the phenotypes except weight loss and fatigue exhibited a significant time difference with a p-value of less than 0.050. However, all of the frailty phenotypes, including weight loss, muscle weakness, slow walking, and low activity, did not show significant differences between the groups at the specified time points.

The predominant characteristics among the participants as shown in figure (4.3) were muscle weakness and low activity. Although the changes observed in these aspects among participants in the Protein Supplementation Group (PSG) from baseline to week 12 were not statistically significant, there was a notable improvement. Specifically, the incidence of muscle weakness decreased from 40% (n=8) to 20% (n=4), while the prevalence of low activity decreased from 95% (n=19) to 30% (n=6).

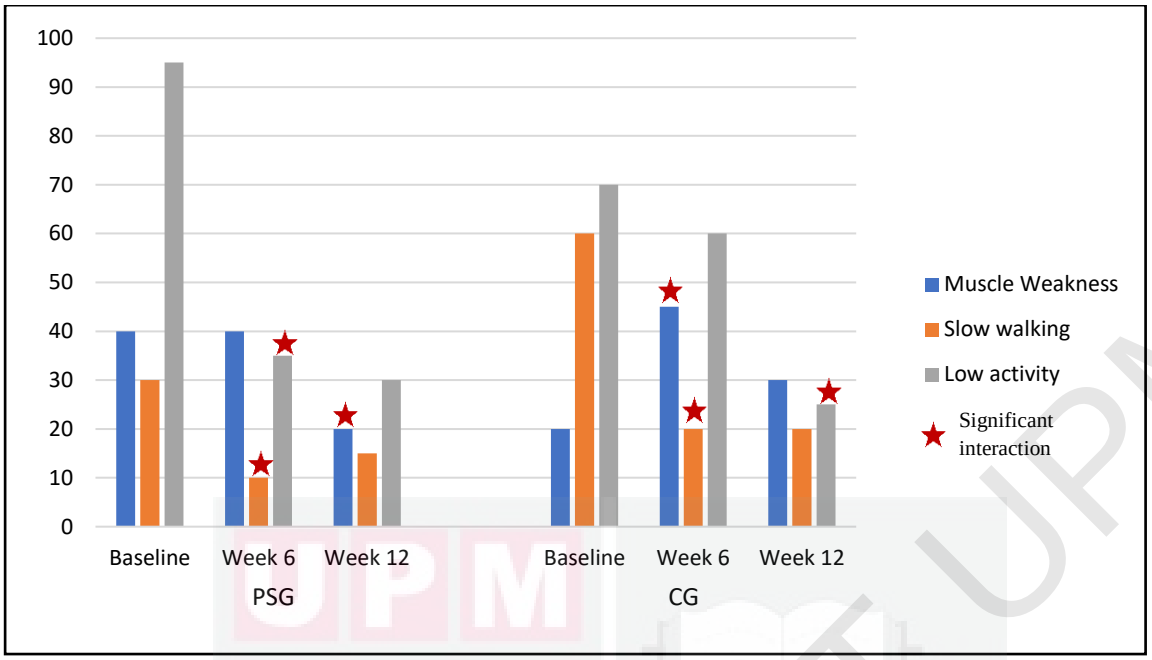


Figure 4.3: The changes of the prevalence of frailty phenotypes during pre, middle and post-intervention of the pre-frail older adults in the interventional group (PSG) and control group (CG).

4.3.3.1 Muscle Weakness

The presence of muscle weakness decreased over time in both groups, with a P value of 0.05 for the time effect. In the PSG group, muscle weakness was reported by 40% of participants at baseline, and by Week 12, this dropped to 20%. In the CG group, muscle weakness was initially present in 45% of participants, which slightly reduced to 30% by Week 12.

The reduction in muscle weakness within the PSG group can be explained by the benefits of whey protein supplementation, which provides essential amino acids that help prevent muscle catabolism and promote muscle protein synthesis. This, in turn, supports muscle strength and function. Although smaller for the CG group when compared to PSG, the reduction in muscle weakness might be due to the placebo effect. The lack of a significant time-by-group interaction (P = 0.465) suggests that the

improvements in muscle weakness occurred similarly in both groups. However, the PSG group showed more improvement due to the targeted supplementation.

4.3.3.2 Slow Walking

Significant improvements were observed in the proportion of participants with slow walking over time, as indicated by a P value of 0.00 for the time effect. In the PSG group, the proportion of participants with slow walking dropped from 30% at baseline to 15% at Week 12. The CG group also saw a less pronounced reduction, with slow walking decreasing from 60% at baseline to 20% at Week 12.

The marked reduction in slow walking in the PSG group is likely due to the positive effects of the protein supplementation, which may have improved overall muscle strength and mobility, contributing to better walking speed. For the CG group, the reduction in slow walking could be due to enhanced awareness of physical health and adherence to the study protocol, which may have encouraged better physical activity practices. Since the time-by-group interaction was insignificant (P = 0.677), the data suggest that both groups experienced improvements in walking speed. However, the PSG group showed slightly more benefit from the supplementation.

4.3.3.3 Low Activity

Low physical activity significantly improved over time, with a P value of 0.00. In the PSG group, 95% of participants reported low activity at baseline, but this decreased significantly to 30% by Week 12. Similarly, the CG group reduced from 70% at baseline to 25% at Week 12.

The significant reduction in low activity in the PSG group is likely attributable to the intervention, which may have boosted energy levels and muscle mass, facilitating higher physical activity levels. For the CG group, the reduction in low activity could be attributed to the placebo effect, lifestyle changes, or a combination of both. The non-significant time-by-group interaction (P = 0.723) indicates that both groups saw reductions in low activity levels at similar rates, though the PSG group may have experienced a slightly greater benefit from the targeted intervention.

Overall, significant improvements were observed in frailty status, frailty scores, and several frailty phenotypes (muscle weakness, slow walking, and low activity) over time in both the PSG and CG groups. The PSG group, receiving protein supplementation, tended to show slightly greater improvements in these outcomes, but the lack of significant time-by-group interactions suggests that both groups improved similarly. This implies that both the supplementation and control conditions and external factors like participant motivation and increased health awareness played important roles in enhancing these frailty-related outcomes.

Table 4.6: Frailty status, scores and phenotypes changes during baseline, middle, and post-intervention of the pre-frail older adults in the interventional group (PSG) and control group (CG).

Measurement	PSG (n=20)			CG (n=20)			Time		Time*Groups	
	Baseline	Week 6	Week 12	Baseline	Week 6	Week 12	df	P value	df	P value
Frailty status n(%)							2	**0.00**[d]		0.731[b]
- Normal	-	8 (40) **	9(45)	-	3(15)	8(40) **				
- Pre-frail (1)	6(30)	8(40)	8(40)	8(40)	9(45)	7(35)				
- Pre-frail (2)	14(70)	3(15) **	3(15)	12(60)	6(30) **	5(25)				
- Frail	-	1(5)	-	-	2(10)	-				
Frailty Scores*	2(1)	1(1)[c**]	1(1)	2(1)	1(1)	1(1.75) [c**]	2	**0.00**[d]		0.602[e]
Frailty phenotypes n (%):										
Weight loss	1(5)	1(5)	1(5)	2(10)	2(10)	2(10)	2	1.0	1	0.548[b]
Fatigue	-	-	-	-	-	-	-	-	-	-
Muscle Weakness	8(40)	8(40)	4(20)[a**]	4(20)	9(45) [a**]	6(30)	2	**0.050**[a]	1	0.465[b]
Slow walking	6(30)	2(10)[a**]	3(15)	12(60)	4(20) [a**]	4(20)	2	**0.00**[a]	1	0.677[b]
Low activity	19(95)	7(35) [a**]	6(30)	14(70)	12(60)	5(25) [a**]	2	**0.00**[a]	1	0.723[b]

*Non-normally distributed data presented as median (IQR), ** significant time interaction $p<0.05$.
[a]Cochrane's Q test; [b]Chi-square test; [c]Wilcoxon Signed Rank test; [d]Friedman test; [e]Mann-Whitney U test, α was set at 0.05.

4.4 The Effect of Whey Protein Supplements on Body Composition

The study investigated changes in various anthropometric measures over a 12-week intervention period in pre-frail elderly individuals. Starting with weight, both groups experienced a slight increase from baseline to week 12, with the PSG group going from 64.29±9.32 kg to 65.23±9.59 kg, while the CG group moved from 66.06±7.91 kg to 67.21±8.78 kg. This increase was more pronounced in the CG group. However, neither the time effect nor the interaction between time and group was significant (p value 0.62), which suggests that although there were changes in weight over time, these changes were consistent across both groups and not a direct result of the intervention.

For BMI, both groups saw minor fluctuations over time, with the PSG group showing a slight increase from 26.14±3.33 at baseline to 26.59±3.37 at week 12, while the CG group increased from 26.62±2.96 to 27.01±2.55. As with weight, no significant time or interaction effects were detected (p value 0.48), indicating that these changes were not meaningfully different between groups, despite the observed trend.

Regarding MUAC (mid-upper arm circumference), a significant time effect (p value <0.001) was noted, with both groups experiencing an increase over time. The PSG group had a slight increase from 27.9±2.9 cm at baseline to 28.51±2.6 cm at week 12. The CG group showed a slightly larger increase, going from 28.11±2.26 cm to 28.93±2.31 cm by week 12. The interaction between time and group was not significant, but the trend suggests that the intervention group might have experienced a gradual increase in muscle mass in the arm, even if this was not statistically verified.

In terms of CC (calf circumference), there was a significant time effect (p value 0.029), with both groups experiencing an increase over time. The PSG group's calf circumference increased from 35.37±3.08 cm at baseline to 36.37±2.96 cm at week 12, while the CG group increased from 36.05±3.02 cm to 36.49±2.94 cm over the same period. The increase in calf circumference was more pronounced in the PSG group, suggesting a possible intervention-related effect, though the interaction effect was not significant.

For total body fat (TBF%), there was a significant time effect (p value < 0.001), with an increase in both groups, particularly in the CG group. The PSG group increased from 37.34±8.41% at baseline to 38.87±7.33% at week 12, while the CG group increased more substantially from 37.48±7.95% to 39.26±8.53% by week 12. The more substantial rise in TBF% in the CG group could imply that the intervention may have helped the PSG group manage fat gain to some extent, though again, the lack of a significant interaction (p value of 0.87) makes this conclusion speculative.

Finally, skeletal muscle mass (SMM) and leg lean mass (LLM) showed relatively stable trends across the intervention period. For SMM, there was no significant change over time (p value of 0.05), with the PSG group showing a slight decrease from 21.62±4.78 kg at baseline to 21.45±4.56 kg at week 12, while the CG group decreased from 22.17±4.34 kg to 21.94±4.54 kg. Similarly, for LLM, both groups remained relatively stable over time, with the PSG group slightly decreasing from 12.14±2.81 kg to 12.22±2.84 kg and the CG group showing a minimal change from 12.71±2.84 kg to 12.73±2.73 kg. Neither measure showed significant time or interaction effects.

The changes in skeletal muscle mass (SMM) between the pre-frail elderly receiving protein supplementation (PSG) and the control group (CG) are illustrated across the three-time points in Figure (4.4). At baseline, the PSG group had an estimated marginal mean SMM of 21.62 kg, which slightly increased to 21.87 kg by week 6, likely due to the protein supplementation, reflecting an initial positive response. However, by week 12, the SMM decreased to 21.46 kg, suggesting that the initial gains in muscle mass were not sustained over time, potentially due to factors such as aging or reduced compliance with the intervention. In contrast, the CG started with a slightly higher baseline SMM of 22.17 kg, which increased modestly to 22.28 kg at week 6, potentially reflecting the effects of normal dietary intake or incidental physical activity. By week 12, the CG also experienced a decline in SMM, dropping to 21.94 kg. While this decline was less pronounced than the PSG group, it still indicates the natural progression of muscle loss in aging populations without targeted interventions.

The pattern observed in both groups, where SMM peaked at week 6 and then declined by week 12, suggests that while protein supplementation may provide short-term benefits in preserving muscle mass, these effects may not be sustained over a longer period without additional strategies, such as increased supplementation or resistance exercise. Furthermore, although the CG maintained slightly higher SMM values throughout the study, the differences between the groups were not substantial enough to result in significant changes in frailty status without further intervention.

Figure (4.5) shows the trends in total body fat (TBF) across the intervention period for both the protein supplementation group (PSG) and the control group (CG). At baseline, the PSG group had an estimated marginal mean TBF of 37.34%, while the CG group had a slightly higher mean of 37.49%. Both groups exhibited negligible changes by

week 6, with the PSG group at 37.36% and the CG group at 37.42%. This suggests that, initially, the intervention did not significantly impact body fat in either group.

However, by week 12, a notable increase in TBF was observed in both groups, with the PSG reaching 38.88% and the CG increasing sharply to 39.26%. Since caloric intake remained stable throughout the study period, the increase in body fat can likely be attributed to other factors such as decreased physical activity, potential changes in metabolism, or muscle-fat redistribution due to aging. It's also possible that despite protein supplementation and consistent calorie intake, the body composition changes (particularly in frail or elderly individuals) reflect a reduction in energy expenditure or a shift toward fat storage.

This rise in TBF, particularly in the CG, highlights the natural tendency toward fat accumulation over time without any muscle-preserving interventions. In contrast, the PSG group showed a smaller increase in TBF, which may suggest that the protein supplementation, while beneficial for maintaining muscle mass, may not have been sufficient to fully prevent fat gain.

In Figure 4.6, the changes in leg lean mass (LLM) for both the protein supplementation group (PSG) and control group (CG) are shown over 12 weeks. The PSG group saw an increase in LLM from 12.14 kg at baseline to 12.31 kg at week 6, but this dropped slightly to 12.22 kg by week 12, suggesting an initial benefit from protein supplementation that was not sustained. The CG group, starting at 12.71 kg, experienced minimal inconsistency, with a slight drop at week 6 (12.64 kg) and returning to baseline by week 12. These results imply that while protein supplementation may lead to modest LLM gains, its effects may plateau without

further interventions. The CG's stable LLM indicates no significant muscle mass change without supplementation.

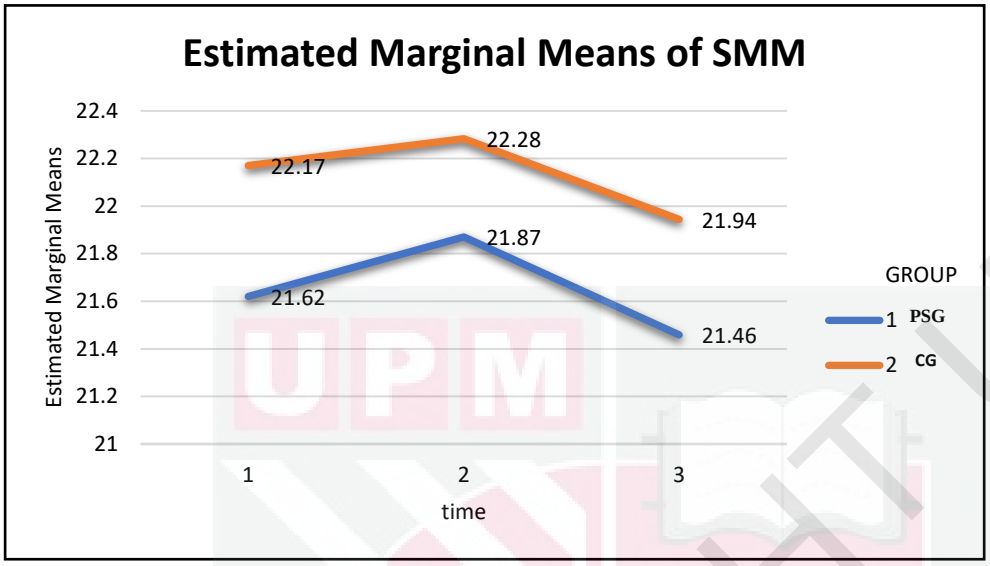


Figure 4.4: Mean changes of skeletal muscle mass during week 0, week 6, and week 12 between PSG and CG.

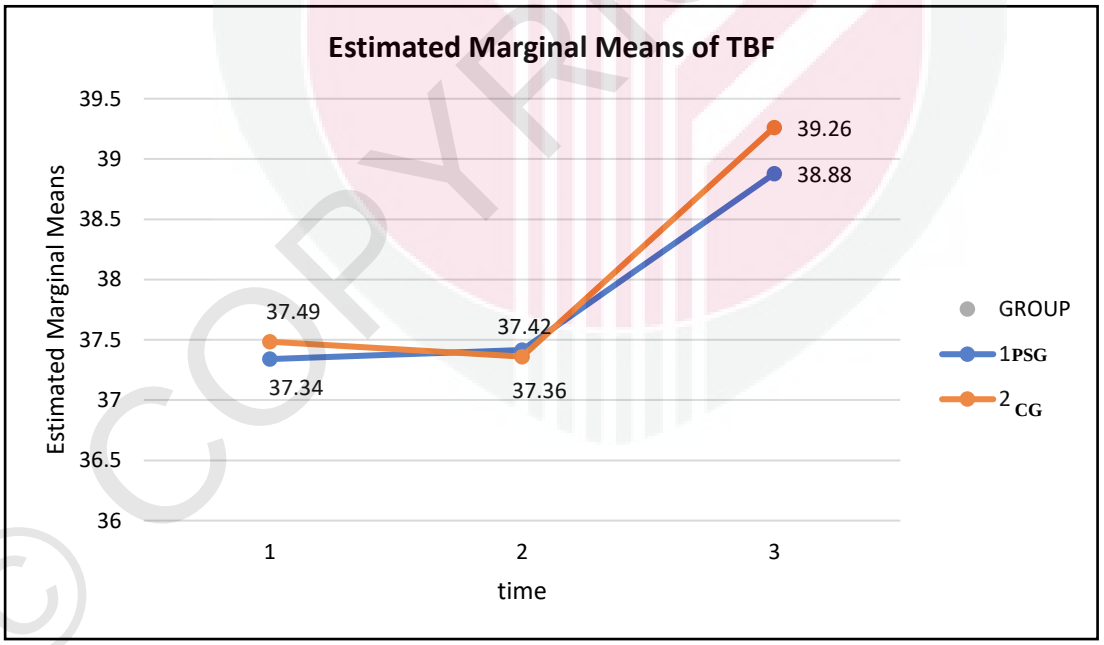


Figure 4.5: Mean changes of total body fat during week 0, week 6, and week 12 between PSG and CG.

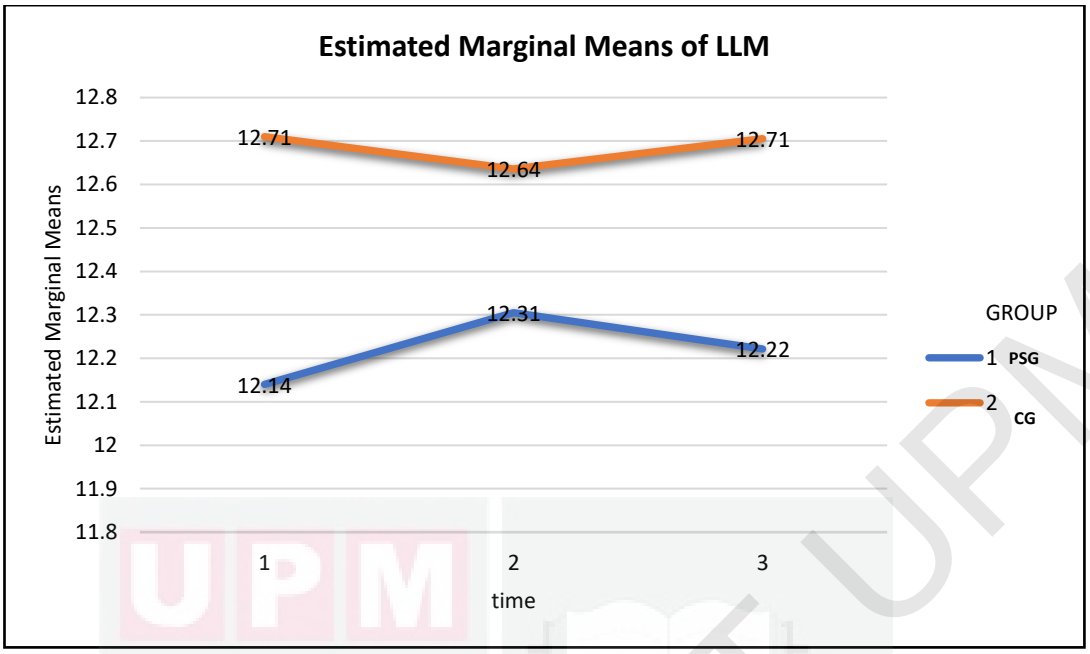


Figure 4.6: Mean changes of leg lean mass during week 0, week 6, and week 12 between PSG and CG.

Table 4.7: Anthropometric changes during baseline, middle, and post-intervention on the pre-frail older adults in the interventional group (PSG) and control group (CG).

Measurement	PSG (n=20)			CG (n=20)			Time[a]		Time*Group[a]	
	Baseline	Week 6	Week 12	Baseline	Week 6	Week 12	F	P value	F	P value
Weight (kg)	64.29±9.32	64.75±9.62	65.23±9.59	66.29±8.55	66.06±7.91	67.21±8.78	3.07	0.052	0.466	0.629
BMI (kg/m^2)	26.14±3.33	26.34±3.57	26.59±3.37	26.84±2.96	26.62±2.24	27.01±2.35	1.845	0.165	0.652	0.485
MUAC (cm)	27.9±2.9	28±2.57	28.51±2.6 [b**]	27.8±2.72	28.11±2.26	28.93±2.31[b**]	10.46	**0.00**	0.869	0.423
CC (cm)	35.37±3.08	35.95±3.04	36.37±2.96 [b**]	36.05±3.41	36.38±3.02 [b**]	36.49±2.9	4.049	**0.029**	0.587	0.529
TBF (%)	37.34±8.41	37.41±7.54	38.87±7.33 [b**]	37.48±7.95	37.36±6.99	39.26±6.53 [b**]	10.11	**0.00**	0.131	0.878
SMM (kg)	21.62±4.78	21.87±4.51	21.45±4.56	22.17±4.34	22.28±4.38	21.94±4.4	3.11	0.050	0.104	0.901
LLM	12.14±2.81	12.3±2.83	12.22±2.84	12.71±2.94	12.63±2.84	12.7±3.02	0.157	0.85	0.968	0.379

[a]General Linear Model (repeated measures ANOVA test); [b]Paired t-test, α was set at 0.05.
**p<0.05 indicate significant difference
BMI: Body Mass Index; MUAC: Midd-upper Arm Circumference; CC: Calf Circumference; TBF: Total Body Fat; SMM: Skeletal Muscle Mass; LLM: Leg Lean Mass.

4.5 The Effect of Whey Protein Supplements on Muscle Strength and Physical Performance

The study investigated various physical performance measures, including handgrip strength (HGS), gait speed (GS), chair stand test (CST), Short Physical Performance Battery Test (SPPBT), and 4-meter walk test (4MWT), in the intervention (PSG) and control (CG) groups over three time points.

For handgrip strength (HGS), there were significant time effects [F(2,76) = 3.284, p value 0.04, partial eta squared= 0.08)]and a trend towards a time*group interaction [F(2,76) = 2.428, p value 0.09, partial eta squared= 0.06], indicating changes in HGS over the assessment period, with a potential differential effect between groups as shown in Figure (4.7).

Gait speed (GS) exhibited significant time effects (F = 6.877, p = 0.002), indicating changes over time. There were no significant time*group interactions (F = 0.845, p = 0.432), suggesting that the changes in GS were similar between groups. The trend of slightly higher LLM in the PSG group could potentially support the hypothesis that maintaining or slightly increasing muscle mass in the legs aids mobility.

The chair stand test (CST) showed significant time effects (F = 5.49, p value 0.005), indicating changes in CST performance over time. There was no significant time*group interaction (F = 0.12, p value 0.88), suggesting that the changes in CST were not different between groups. For the Short Physical Performance Battery Test (SPPBT), there were significant time effects (F = 14.83, p value 0.00), indicating changes over time. There was no significant time*group interaction (F = 0.032, p value 0.96), suggesting that the changes in SPPBT were similar between groups.

The 4-meter walk test (4MWT) exhibited significant time effects (F = 11.9, p value 0.00), indicating changes over time. There was no significant time*group interaction (F = 0.181, p value 0.83), suggesting that the changes in 4MWT were not different between groups.

These findings suggest that WPS had no significant effects on various physical performance measures, with some measures showing potential group-specific responses.

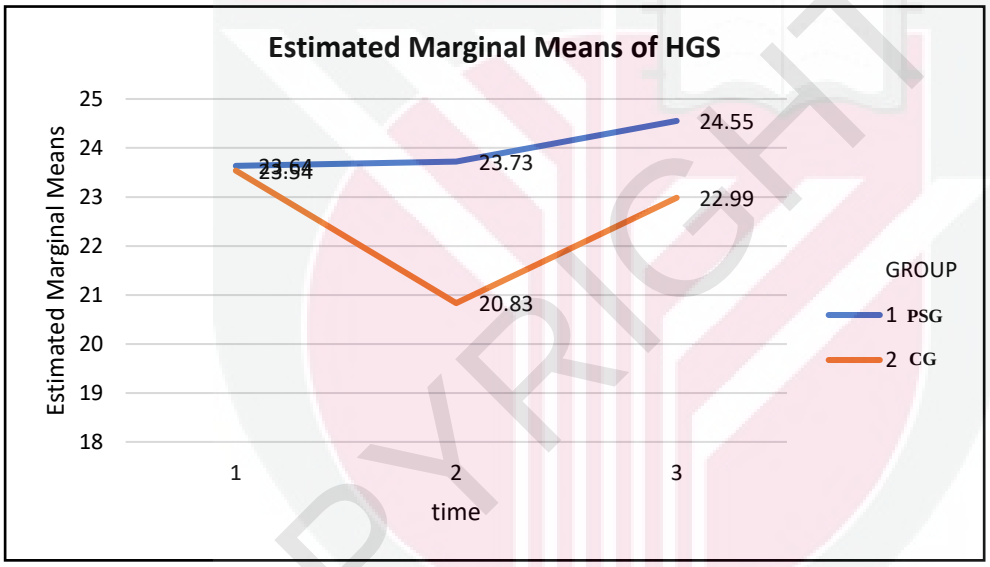


Figure 4.7: Mean changes of handgrip strength during week 0, week 6, and week 12 between PSG and CG.

Table 4.8: Handgrip strength and physical performance changes during baseline, middle, and post-intervention of the pre-frail older adults in the interventional group (PSG) and control group (CG).

Measurement	PSG (n=20)			CG (n=20)			Time		Time*Group	
	Baseline	Week 6	Week 12	Baseline	Week 6	Week 12	F	P value	F	P value
HGS (kg)[a]	23.63±8.74	23.72±8.13	24.55±7.85	23.53±5.72	20.83±4.8[e**]	22.98±5.8[e**]	3.284	**0.043**	2.428	0.095
GS (m/s) [*b]	0.92(0.17)	0.98(0.23)[d**]	1.05(0.26)	0.81(0.24)	1.01(0.26)[d**]	1.09(0.23)[d**]	6.877	**0.002[c]**	0.845	0.432
CST (s) [*b]	14.25(4)	12.06(5)[d**]	11.11(2.35)	14.75(6.8)	12.68(5.63)	12.09(3.81)	5.49	**0.005[c]**	0.12	0.887
SPPBT[*b]	10(1.75)	11(2)[d**]	11(1) [d**]	10(2)	11(2)[d**]	11(1.75)	14.83	**0.00[c]**	0.032	0.969
4MWT (S)[*b]	4.28(0.78)	4.07(0.88)[d**]	3.8(0.84)	4.8(1.16)	3.95(0.65)[d**]	3.77(0.75)[d**]	11.9	**0.00[c]**	0.181	0.83

*Non-normally distributed data presented as median (IQR).
**p<0.05 indicate significant difference
[a]General Linear Model (repeated measures ANOVA test), α was set at 0.05; [b]Mann-Whiteny U test; [c]Friedman test; [d]Wilcoxon Signed Rank test; [e]Paired t-test, α was set at 0.05.
HGS: Handgrip Strength; GS: Gait Speed; CST: Chair-stand test; SPPB: Short Physical Performance Battery test; 4MWT: 4-meters Walking test.

4.6 Effect of Whey Protein Supplements on Dietary Intake

In comparing macronutrient intake between the PSG and CG at baseline and week 12 as shown in Table (4.9), we observed several noteworthy findings. First, regarding energy intake (kcal/d), there was no significant difference between the two groups at both time points, with p-values greater than 0.05. This suggests that energy consumption did not vary significantly between the PSG and CG.

Moving on to protein intake, measured in grams per day (g/d), grams per kilogram per day (g/kg/d), and percentage of total intake (%), we found significant differences between the two groups. Specifically, the PSG exhibited a substantial increase in protein intake compared to the CG at week 12, as indicated by the t-test results with p-values of 0.001, 0.00, and 0.00 for g/d, g/kg/d, and %, respectively.

In terms of carbohydrate intake (g/d and %), the PSG demonstrated a significant decrease compared to the CG at week 12, as evidenced by t-test results with p-values of 0.001 and 0.00. These findings suggest that carbohydrate consumption underwent significant alterations in the PSG relative to the CG during the intervention.

Contrarily, fat intake, both in grams per day (g/d) and as a percentage of total intake (%), did not exhibit significant differences between the two groups at week 12. The t-test results yielded p-values of 0.48 and 0.56, indicating that fat consumption remained relatively consistent between the PSG and CG.

The analysis of energy and fat intake over the 12-week intervention period revealed no significant differences between the intervention group (PSG) and the control group (CG). This finding suggests that both groups maintained similar dietary habits

throughout the study, particularly with respect to their total caloric and fat intake. The lack of significant differences could indicate that the dietary intervention did not result in major changes in energy consumption or fat intake between the groups, possibly due to consistent dietary behaviors or guidelines followed by participants in both groups.

This result also implies that any observed changes in body composition, muscle mass, or physical performance (as discussed in previous sections) are unlikely to be attributed to differences in energy or fat intake. Instead, these changes might be more closely linked to the specific effects of the intervention (e.g., exercise, protein supplementation) or other factors such as muscle function, rather than dietary modifications

Table 4.9: Dietary Intake changes during baseline, middle, and post-intervention of the pre-frail older adults in the interventional group (PSG) and control group (CG).

Macronutrient	PSG (n=20)		CG (n=20)		t-value	F	p-value[a]
	Baseline	Week 12*	Baseline	Week 12*			
Energy (kcal/d)	1676±495	1826±440	1882±488	2020±318	-1.6	3.76	0.118
Protein							
(g/d)	63.37±22.8	97.21±25.11	68.86±17.16	72.72±15.54	-	-	**0.001**
(g/kg/d)	0.995±0.34	1.5±0.36	1.07±0.3	1.08±0.238	4.214	1.243	**0.00**
(%)	15.08±3.34	21.77±4.68	14.97±2.95	14.35±2.4	6.302	5.238	**0.00**
Carbohydrate							
(g/d)	224.23±73.88	222.88±64.57	260.95±72.09	288.78±53.7	-3.5	2.32	**0.001**
(%)	53.75±7.14	48.72±5.82	55.55±4.64	57.1±4.83	-4.96	0.534	**0.00**
Fat							
(g/d)	58.4±20.69	60.54±18.72	68.11±41.35	64.11±12.97	-0.7	3.174	0.48
(%)	31.03±4.5	29.36±3.95	31.35±10.77	28.64±3.94	0.58	0.009	0.56

[a]Independent t-test, α was set at 0.0

CHAPTER 5

DISCUSSION

To our knowledge, this is the first trial that has studied the effect of taking 30g/d of leucine-enriched WPS on pre-frail individuals, which is equivalent to 1.5g/kg/d. The trial was conducted using a double-blinded, randomized, placebo-controlled design. The study aimed to assess the impact of WPS on frailty status, muscle strength, physical performance, and body composition. The results showed no significant effect on frailty status, muscle strength, or physical performance after 12 weeks of WPS supplementation. Additionally, there was no significant change in body composition, particularly in lean body mass (LBM) and total body fat (TBF). However, there was a slight improvement, although not significant, in handgrip strength (HGS).

In our study, we employed a leucine-enriched whey protein supplement since research has shown its efficiency in preventing muscle loss when taken at rest or after exercises in older adults and rapid digestion compared to other types of protein (Burd et al., 2012; Devries & Phillips, 2015; Tang et al., 2009). Previous studies showed that 30g (L. Kang et al., 2019; Tieland, Dirks, et al., 2012; Tieland, van de Rest, et al., 2012) or 1.5g/kg/d (Park et al., 2018) has a positive effect on muscle strength and physical performance. Conversely, our findings oppose this hypothesis as the dietary protein intake (1.5g/kg/d) did not significantly affect the aforementioned measurements.

5.1 Frailty Status, Scores, and its Phenotypes

Frailty Status and its phenotypes were the primary outcome of this study. There was no significant effect of WPS on frailty status or its phenotypes. However, the count of

pre-frail individuals with 2 was higher in the Protein Supplementation Group (PSG) (n=14, 70%), notably decreasing to (n=3, 15%) by week 12. Muscle weakness and low activity were the prevailing criteria among the study participants. It's noteworthy that there was an improvement, although statistically insignificant, observed in these criteria among PSG participants, with muscle weakness decreasing from (n=8, 40%) to (n=4, 20%) and low activity from (n=19, 95%) to (n=6, 30%) from baseline to week 12, respectively.

Considering the limited number of studies that tested the effect of protein supplementation on frailty status (Amasene et al., 2022; Biesek et al., 2021; Park et al., 2018), our findings align with the above-cited reference, which shows no significant improvement in frailty status. However, no available studies mentioned the effect of WPS on frailty criteria except (Biesek et al., 2021), who highlighted a great reduction in fatigue after utilizing WPS in pre-frail women. A plausible explanation for these results might be that the most prevalent criterion among study participants was low activity (n=33,82.5%), which is self-reported and lacks measurement, potentially influencing the findings' accuracy.

5.2 Muscle Strength and Physical Performance

For muscle strength, the results show an increase but insignificant in HGS in PSG compared to CG (p=0.095) and no significant difference in physical performance measurements such as GS and CST. One explanation of these results is that the study supplement was not combined with resistance training as in some other previous trials proved the Efficacy of protein supplement on muscle strength (Karelis et al., 2015; Mori & Takudo, 2018; Reule et al., 2017; Yoshimura et al., 2019) and physical

function (Griffen et al., 2022; Tieland, Dirks, et al., 2012; Trabal et al., 2015) when combined with RT.

However, our findings are in line with some previous studies that tested the effect of protein supplementation on physical performance and showed no significant improvement in these measures, whether combined with training (Amasene et al., 2019; Björkman et al., 2020; Gade et al., 2019; Neske & Azevedo, 2022) or consuming the supplement alone (Jadczak et al., 2021; Murphy et al., 2021; Zhu et al., 2015). In addition, our findings align with two previous meta-analyses tested the effect of PS and amino-acids supplements without training in elderly people (Tieland et al., 2016) or pre-frail and frail older adults (Oktaviana et al., 2020). They found no significant effect of PS on the aforementioned measurements. Worthy to mention, among the RT-included studies, there are numerous trials that included groups without RT and revealed a negative effect of WPS when consumed alone on muscle strength and physical performance (Biesek et al., 2021; Griffen et al., 2022; Krause et al., 2019; Mori & Takudo, 2018).

In contrast, two trials reported significant improvement in gait speed (Niccoli et al., 2017; Park et al., 2018) and knee extension (Niccoli et al., 2017) in older adults after WPS consumption. Besides, a meta-analysis done by (Kirwan et al., 2022) studied the effect of PS or high-protein on lean mass and muscle strength using twenty-eight study's findings, which showed that PS can augment HGS in the presence of RT only. This could be another argument for the lack of PS impact on muscle strength and physical performance (Liao et al., 2018). Another explanation is the dosage applied by some studies and reported significant improvement in muscle strength at level of 60g/d

(Bell et al., 2017), 40g/d (Atherton et al., 2020), and 10g of leucine per day (Trabal et al., 2015).

5.3 Body Composition

This study tested the hypothesis that taking leucine-rich WPS twice daily would improve body composition, especially SMM and TBF. Despite the significant time effect observed, no significant interaction effects were observed in body composition measurements. These findings are consistent with majority of older adults' previous trials (Amasene et al., 2019; Arnarson et al., 2013; Biesek et al., 2021; Bouillanne et al., 2013; Holwerda et al., 2018; Kirk et al., 2020; Leenders et al., 2011; Ottestad et al., 2017; Villanueva et al., 2014; Zhu et al., 2015). Studies have shown that the higher increase in muscle protein synthesis can be attributed to whey protein because, when taken at rest or after exercise, whey causes a larger MPS response than casein (Burd et al., 2012; Pennings et al., 2011; Tang et al., 2009).

Additionally, it has been reported that whey protein increased muscle mass when consumed 1.3-1.5g/kg/d compared to 1.0g/kg/d in elderly people (Kerstetter et al., 2015; Moore et al., 2014; Park et al., 2018). However, most studies that observed synergetic effects of protein supplement on lean body mass, fat mass, or muscle mass, recruited sarcopenic (Nabuco, Tomeleri, Fernandes, et al., 2019), frail (L. Kang et al., 2019; Niccoli et al., 2017; Tieland, Dirks, et al., 2012), malnourished older adults (Bouillanne et al., 2013; Park et al., 2018), or consumed insufficient protein intake at baseline (Ten Haaf et al., 2020) while our study population status was pre-frail with high habitual protein intake (1.03g/kg/d) which can be considered as another justification of the study findings. Another point to be in consider, is the overall status

of the study participants, with a percentage of 82.5% low-active pre-frail participants (19 of 20 subjects of PSG). Volbi et al. stated that RDA0.8g/kg daily could be sufficient for sedentary or low-active older adults to avoid protein inadequacy(Volpi et al., 2013). However, some trials observed a positive PS intervention effect on body composition measurements and strength loss of sedentary (Buckinx et al., 2019; Gryson et al., 2014; Krause et al., 2019), or low-active (Reule et al., 2017) older participants.

5.4 Dietary Intake

The habitual protein intake of this study participants (1.03g/kg of body weight) is comparable with the average intake of physically active older adults in a cross-sectional study done by (Ten Haaf et al., 2018) and community-dwelling older adults in an RCT done by (Kirk et al., 2020). Consuming WPS daily in the morning and evening significantly improved the intake of protein to 1.5g/kg/d. However, this amount of protein did not yield a positive effect on the study outcome measurements. This may be justified by the sufficient amount of their daily protein consumption at baseline 1.03g/k of body weight. Moreover, resistance training was not included in our protein intervention. The current study's goal was to find out how pre-frail older adults' status was affected by a protein intervention alone.

In general, our study results indicate that whey protein supplementation does not significantly enhance muscle strength or physical performance which aligns with many cases (Björkman et al., 2011, 2020; Gade et al., 2019; Jadczak et al., 2021; Park et al., 2018; Zhu et al., 2015) except one study (Niccoli et al., 2017). These results coincide with two meta-analyses that tested the effect of increased protein intake

beyond RDA, which found that high consumption of protein intake has limited effects on muscle strength when not associated with physical training (Hengeveld et al., 2020; Nunes et al., 2022).

Furthermore, the impact of whey protein appears to be more evident in terms of body composition improvements, including enhancements in muscle mass (Björkman et al., 2011; Park et al., 2018) and fat-free mass (Kerstetter et al., 2015). Nevertheless, our study in addition to previous literature remains a certain degree of contradiction concerning the effects of whey protein supplementation on body composition (Gade et al., 2019; Jadczak et al., 2021; Oikawa et al., 2020; Park et al., 2018; Zhu et al., 2015).

Intriguingly, studies have highlighted that milk and leucine-rich protein supplements have demonstrated superior effects compared to whey protein supplementation over a relatively short duration (ranging from 3 to 6 months) on the body composition of older adults (Bouillanne et al., 2013; Ispoglou et al., 2016; Y. Kang et al., 2020; Norton et al., 2016; ten Haaf et al., 2019; Yoshimura et al., 2019).

However, even studies with a 12-week intervention period have reported contradictory results regarding the effectiveness of whey protein. Some trials have shown improvements in muscle mass and strength (Tieland et al., 2012; Rondanelli et al., 2016), while others did not find significant changes, suggesting that factors such as baseline nutrition status, overall protein intake, physical activity levels, and the presence of "anabolic resistance" in older adults might play a role (Reidy & Rasmussen, 2016; Devries & Phillips, 2015). These discrepancies underscore the need

for further research to clarify the most effective duration and type of protein supplementation for muscle health in older populations.

In addition, our study applied single domain intervention, which is 30 g of whey protein daily. However, when combined with supervised RT, it appears to exhibit positive effects on muscle strength (Atherton et al., 2020; Bell et al., 2017; Mori & Takudo, 2018) and body composition (Junior et al., 2017; Krause et al., 2019; Mori & Takudo, 2018; Nabuco, Tomeleri, Fernandes, et al., 2019), particularly in terms of lean body mass enhancements(Bell et al., 2017; Fernandes et al., 2018; Nabuco, Tomeleri, Fernandes, et al., 2019; Nabuco, Tomeleri, Junior, et al., 2019). However, the consistency of these results is not definitive, as several studies present contradictory findings. Specifically, certain investigations indicate no significant difference in muscle strength improvements when incorporating whey protein into RT programs (Amasene et al., 2019; Gade et al., 2019; Gerontol et al., 2012; Griffen et al., 2022; Holwerda et al., 2018; Mojtahedi et al., 2011), nor in terms of body composition changes (Arnarson et al., 2013; Biesek et al., 2021; Gade et al., 2019; Gerontol et al., 2012; Michel et al., 2022; Mojtahedi et al., 2011; Neske & Azevedo, 2022; Villanueva et al., 2014).

The addition of whey protein to RT does not seem to significantly enhance physical performance, except for two studies that reported improved gait speed when whey protein supplementation was combined with RT compared to RT alone (Griffen et al., 2022; Mori & Takudo, 2018). These outcomes contrast with the findings of a systematic review and meta-analysis of whey protein supplementation, which highlighted a notable impact of whey protein supplement on lean mass and gait speed

among individuals with sarcopenia (Nasimi et al., 2023). Creatine supplementation occupies the second position among the types of supplements utilized alongside resistance training (RT) in studies involving older adults (10 trials). It predominantly demonstrates a positive impact on body composition enhancements (Aguiar et al., 2013; Bernat et al., 2012; D. Candow et al., 2015; D. G. Candow et al., 2014; Gualano et al., 2014; Johannsmeyer et al., 2016), followed by improvements in muscle strength (Aguiar et al., 2013; Bernat et al., 2012; D. Candow et al., 2015; D. G. Candow et al., 2014; Gualano et al., 2014) within a supervised training context.

Interestingly, milk protein exhibits a superior effect in terms of body composition improvement compared to whey protein (Francis et al., 2017; Gryson et al., 2014; Nakayama et al., 2021; Tieland, Dirks, et al., 2012). This observation aligns with the findings of a systematic review and meta-analysis that compared the effects of milk protein versus whey protein on resistance training-induced lean body mass (LBM) or fat-free mass (FFM) gains among older adults (Huang et al., 2021).

Moreover, the effect of protein supplementation on the physical performance of older adults remains uncertain, as suggested by the findings from the encompassed trials, mainly owing to the scarcity of studies that have directly investigated this aspect. Another meta-analysis conducted on a distinct cohort of middle-aged to older adults, with or without existing sarcopenia, also yielded inconclusive results regarding the impact of dairy protein supplementation (Hanach et al., 2019). Soy and leucine supplements appear to exhibit a potentially beneficial impact on muscle strength and physical performance in conjunction with training programs. Nevertheless, findings from two comprehensive reviews/meta-analysis suggests some beneficial effects of

dietary protein on physical function measures of older people (Cawood et al., 2012; Milne et al, 2009). However, it is important to acknowledge that further research is required to substantiate this assertion, given the limited number of conducted trials exploring this area.

In our study, the population was not malnourished and generally maintained a normal range of calorie and protein intake. This differentiates the study cohort from other groups where protein supplementation might be particularly beneficial. Previous research has shown that protein supplements can be especially effective in older adults with specific conditions that may compromise their muscle health or nutritional status. For instance, supplementation has been found to aid those with arthritis (Ikeda et al., 2018; Neves et al., 2011), osteopenia or osteoporosis (Gualano et al., 2014; Shenoy et al., 2013), as well as inpatient older adults, who often experience acute or chronic malnutrition (Bouillanne et al., 2013; Niccoli et al., 2017). Additionally, protein supplementation has been helpful for post-stroke sarcopenic individuals, aiding in the recovery of muscle strength and mass (Yoshimura et al., 2019), and for malnourished older adults, where it has contributed to improved nutritional status and functional outcomes (Park et al., 2019). These findings highlight the diverse applications of protein supplementation, but our study uniquely focuses on older adults who are not experiencing these severe deficits, thereby allowing us to assess the potential benefits of protein intake within a healthier, but still at-risk, aging population.

Furthermore, previous literature did not reveal any discernible correlation between the timing or duration of supplement intake and its impact on outcome measures in older adults. This observation aligns with the findings of a systematic review, which

similarly indicated the absence of a favorable effect from specific timing of protein intake on metrics such as lean body mass (LBM), handgrip strength, and leg press strength (Wirth et al., 2020).

To sum up, while whey protein is widely utilized in clinical trials, the outcomes from our study reflect the varied and sometimes conflicting evidence regarding its efficacy in enhancing frailty status and phenotypes, muscle strength, physical performance, and body composition, among the elderly. In line with previous findings, our results underscore the nuanced effects of protein supplementation on different aspects of frailty. Studies have shown that, when combined with resistance training, protein supplements such as whey, creatine, and milk protein generally lead to improved muscle strength and favorable changes in body composition, with whey protein consistently demonstrating positive outcomes in older adults (Devries & Phillips, 2015; Rondanelli et al., 2016). Importantly, these improvements can also affect frailty indicators like grip strength, physical endurance, and overall physical performance.

However, our findings, along with other research, suggest that without concurrent resistance training, the improvements seen in body composition do not always translate into significant gains in muscle strength or improvements in frailty status. While some studies have reported enhanced muscle function with leucine-rich, milk, and whey proteins, the impact on specific frailty phenotypes, such as exhaustion, low physical activity, and slow walking speed, remains less clear (Tieland et al., 2012; Bauer et al., 2015). Moreover, the hierarchy of benefits can vary, with some studies highlighting the role of leucine and milk proteins in improving body composition without significant changes in muscle strength or frailty measures.

Our study specifically contributes to understanding how protein supplementation may affect overall frailty status by targeting phenotypes such as muscle weakness, slowness, and low physical activity. The effects of protein supplementation on these frailty phenotypes and overall frailty reduction remain inconclusive across studies, indicating that further comprehensive trials are needed to clarify the role of different protein sources in modifying frailty status and phenotypes among older adults (Reidy & Rasmussen, 2016; Moore et al., 2015). Overall, our study adds to the growing body of research highlighting the complex and multifaceted nature of protein supplementation outcomes in aging populations, emphasizing the importance of considering both physical performance and frailty indicators when evaluating the benefits of dietary interventions.

CHAPTER 6

CONCLUSION, LIMITATIONS AND RECOMMENDATION

6.1 Conclusion

In conclusion, this study investigated the effects of whey protein supplementation on frailty status, anthropometric measures, and physical performance in pre-frail older adults over a 12-week intervention period.

The main focus of this research was to evaluate the influence of Whey Protein Supplementation (WPS) on frailty status and its characteristics. There was a noticeable decline in the number of pre-frail individuals with a score of 2 within the Protein Supplementation Group (PSG), decreasing from 70% (n=14) to 15% (n=3) by week 12. However, it did not have a significant impact on either frailty status or its characteristics. Muscle weakness and low activity were prevalent features among the participants, showing some improvement in the PSG, although the changes were not statistically significant. Specifically, muscle weakness decreased from 40% (n=8) to 20% (n=4), while low activity decreased from 95% (n=19) to 30% (n=6) from baseline to week 12.

Anthropometric measures WPS did not lead to notable changes in certain anthropometric measures, including mid-upper arm circumference, calf circumference, total body fat percentage, skeletal muscle mass, and lower limb muscle mass, although significant time effects were observed.

In terms of physical performance, whey protein supplementation did not significantly increase handgrip strength, gait speed, chair stand test performance, short physical performance battery test scores, or 4-meter walk test outcomes. However, significant time effects were observed for most physical performance measures, indicating changes over time.

Furthermore, whey protein supplementation led to significant alterations in macronutrient intake, particularly an increase in protein intake and a decrease in carbohydrate intake compared to the control group. These findings underscore the complex interplay between whey protein supplementation, frailty status, anthropometric measures, physical performance, and macronutrient intake in pre-frail older adults.

In summary, while 30g of whey protein supplementation led to alterations in macronutrient intake, it did not exert significant effects on frailty status or physical performance measures in pre-frail older adults compared to the control group. Combining the results of our study with those of other studies, it appears that participants' nutritional state and regular protein consumption will determine how beneficial a protein intervention is for maintaining the health of their muscles in older individuals. The study's comprehensive approach contributes valuable insights to the existing literature on nutritional interventions for older adults, highlighting the need for further research to elucidate the complexities of individual responses in such interventions.

6.2 Strengths and Limitations

This study represents an effort to investigate the impact of Whey Protein Supplementation (WPS) on the frailty status of pre-frail older adults, marking a significant contribution to the existing literature on nutritional interventions for frailty, particularly within Asian populations. Notably, our research stands out as the first trial of its kind to focus on this particular stage of frailty, providing light on an important field of research that has received little attention in earlier studies.

Moreover, we employed a placebo-controlled, double-blinded approach. By adopting such a robust methodology, we aimed to enhance the credibility of our findings while avoiding the potential influence of biases inherent in the research process.

Furthermore, we used a range of validated assessment and measuring techniques to evaluate frailty, muscle strength, and physical performance. We enhanced the validity of our research findings by ensuring the precision and reliability of our measures through the use of known and standardized instruments.

Additionally, it is noteworthy that our research represents the first Double-Blinded Randomized Controlled Trial (DB-RCT) conducted in Malaysia to specifically target pre-frailty and assess its status and phenotypes following protein supplementation intervention.

This study had some limitations as well. Firstly, the method of administering supplements using 15g scoops instead of pre-packed sachets for daily consumption may have introduced variability in daily intake accuracy among participants. This

variability could impact result consistency and reliability, as precise dosage control is crucial in nutritional intervention studies.

Secondly, participant compliance levels were lower than anticipated, with four individuals in the Protein Supplementation Group (PSG) exhibiting a compliance level of less than 80%. This suboptimal compliance raises concerns about intervention's potential impact on study outcomes.

6.3 Future Recommendations

Given our primary focus on evaluating the impact of whey protein supplementation (WPS) on outcome measurements, it is advisable for future studies to consider combining the supplement with resistance training (RT). Research suggests that RT may enhance the beneficial effects of protein supplements on muscle strength and physical performance, thereby potentially improving frailty status.

Furthermore, our study findings suggest a potential positive impact of WPS on handgrip strength (HGS). To obtain more reliable and conclusive findings, it is recommended to conduct long-term intervention studies.

Moreover, given the encouraging outcomes regarding frailty status and handgrip strength, we suggest exploring the potential effects of WPS on the outcome measures through higher dosage in a more extensive sample size.

Additionally, considering the variations observed in our findings between males and females, we recommend conducting further studies with a focus on sex differences.

Understanding these differences could provide valuable insights into the Efficacy of WPS across different demographic groups.

Finally, to ensure the accuracy of supplement consumption by participants, we recommend utilizing pre-measured and identically packed sachets for the supplements in future studies. This approach would help standardize intake and minimize variability in dosage administration.

REFERENCES

Aas, S. N., Seynnes, O., Benestad, H. B., & Raastad, T. (2020). Strength training and protein supplementation improve muscle mass, strength, and function in mobility-limited older adults: a randomized controlled trial. *Aging Clinical and Experimental Research*, *32*(4), 605–616. https://doi.org/10.1007/s40520-019-01234-2

Aguiar, A. F., Balvedi, M. C. W., Buzzachera, C. F., Altimari, L. R., Lozovoy, M. A. B., Bigliassi, M., Januário, R. S. B., Pereira, R. M., Sanches, V. C., da Silva, D. K., & Muraoka, G. A. (2016). l-Arginine supplementation does not enhance blood flow and muscle performance in healthy and physically active older women. *European Journal of Nutrition*, *55*(6), 2053–2062. https://doi.org/10.1007/s00394-015-1019-6

Aguiar, A. F., Januário, R. S. B., Junior, R. P., Gerage, A. M., Pina, F. L. C., Do Nascimento, M. A., Padovani, C. R., & Cyrino, E. S. (2013). Long-term creatine supplementation improves muscular performance during resistance training in older women. *European Journal of Applied Physiology*, *113*(4), 987–996. https://doi.org/10.1007/s00421-012-2514-6

Amasene, M., Cadenas-Sanchez, C., Echeverria, I., Sanz, B., Alonso, C., Tobalina, I., ... & Besga, A. (2021). Effects of resistance training intervention along with leucine-enriched whey protein supplementation on sarcopenia and frailty in post-hospitalized older adults: preliminary findings of a randomized controlled trial. *Journal of Clinical Medicine*, *11*(1), 97.

Amasene, M., Besga, A., Echeverria, I., Urquiza, M., Ruiz, J. R., Rodriguez-Larrad, A., ... & Labayen, I. (2019). Effects of Leucine-enriched whey protein supplementation on physical function in post-hospitalized older adults participating in 12-weeks of resistance training program: A randomized controlled trial. *Nutrients*, *11*(10), 2337.

An, R. (2016). Weekend-weekday differences in diet among U.S. adults, 2003–2012. *Annals of Epidemiology*, *26*(1), 57–65. https://doi.org/10.1016/j.annepidem.2015.10.010

Arnarson, A., Geirsdottir, O. G., Ramel, A., Briem, K., Jonsson, P. V, & Thorsdottir, I. (2013). Effects of whey proteins and carbohydrates on the efficacy of resistance training in elderly people : double blind , randomised controlled trial. *European Journal of Clinical Nutrition*, October 2012, 1–6. https://doi.org/10.1038/ejcn.2013.40

Atherton, C., McNaughton, L. R., Close, G. L., & Sparks, A. (2020). Post-exercise provision of 40 g of protein during whole body resistance training further augments strength adaptations in elderly males. *Research in Sports Medicine*, *28*(4), 469–483. https://doi.org/10.1080/15438627.2020.1770251

Badrasawi, M., Shahar, S., Zahara, A. M., Nor Fadilah, R., & Singh, D. K. A. (2016). Efficacy of L-carnitine supplementation on frailty status and its biomarkers,nutritional status,and physical and cognitive function among prefrail older adults: A double-blind,randomized,placebo-controlled clinical trial. *Clinical Interventions in Aging*, *11*, 1675–1686. https://doi.org/10.2147/CIA.S113287

Bailey, C. H., Signorile, J. F., Perry, A. C., Jacobs, K. A., & Myers, N. D. (2018). Beta-Alanine Does Not Enhance the Effects of Resistance Training in Older Adults. *Journal of Dietary Supplements*, *15*(6), 860–870. https://doi.org/10.1080/19390211.2017.1406422

Baker, T. P., Candow, D. G., & Farthing, J. P. (2016). Effect of preexercise creatine ingestion on muscle performance in healthy aging males. *Journal OfStrength and Conditioning Research*, *30*(6), 1763–1766.

Bauer, J., Biolo, G., Cederholm, T., Cesari, M., Cruz-Jentoft, A. J., Morley, J. E., Phillips, S., Sieber, C., Stehle, P., Teta, D., Visvanathan, R., Volpi, E., & Boirie, Y. (2013). Evidence-based recommendations for optimal dietary protein intake in older people: A position paper from the prot-age study group. *Journal of the American Medical Directors Association*, *14*(8), 542–559. https://doi.org/10.1016/j.jamda.2013.05.021

Bell, K. E., Snijders, T., Zulyniak, M., Kumbhare, D., Parise, G., Chabowski, A., & Phillips, S. M. (2017). A whey protein-based multi-ingredient nutritional supplement stimulates gains in lean body mass and strength in healthy older men : A randomized controlled trial. *PLoS One*, *12*(7), 1–18.

Bemben, M. G., Witten, M. S., Carter, J. M., Eliot, K. A., Knehans, A. W., & Bemben, D. A. (2010). The effects of supplementation with creatine and protein on muscle strength following a traditional resistance training program in middle-aged and older men. *Journal of Nutrition, Health and Aging*, *14*(2), 155–159. https://doi.org/10.1007/s12603-009-0124-8

Bernat, P., Candow, D. G., Geryzb, K., Butchart, S., Schoenfeld, B. J., & Bruno, P. (2012). Effects of high-velocity resistance training and creatine supplementation in untrained healthy aging males. *AIDS Research and Human Retroviruses*, *2016*(133), 1–28.

Biesek, S., Vojciechowski, A. S., Filho, J. M., Carolina, A., Menezes, R. De, Borba, C., & Rabito, E. I. (2021). Effects of Exergames and Protein Supplementation on Body Composition and Musculoskeletal Function of Prefrail Community-Dwelling Older Women : A Randomized , Controlled Clinical Trial. *Int. J. Environ. Res. Public Health*, *18*(9324), 1–19.

Björkman, M. P., Pilvi, T. K., Kekkonen, R. A., Korpela, R., & Tilvis, R. S. (2011). Similar effects of leucine rich and regular dairy products on muscle mass and functions of older polymyalgia rheumatica patients: A randomized crossover trial. *Journal of Nutrition, Health and Aging*, *15*(6), 462–467. https://doi.org/10.1007/s12603-010-0276-6

Björkman, M. P., Suominen, M. H., Kautiainen, H., Jyväkorpi, S. K., Finne-Soveri, H. U., Strandberg, T. E., Pitkälä, K. H., & Tilvis, R. S. (2020). Effect of Protein Supplementation on Physical Performance in Older People With Sarcopenia–A Randomized Controlled Trial. *Journal of the American Medical Directors Association, 21*(2), 226-232. https://doi.org/10.1016/j.jamda.2019.09.006

Bohannon, R. W. (2019). Grip strength: An indispensable biomarker for older adults. *Clinical Interventions in Aging, 14*, 1681–1691. https://doi.org/10.2147/CIA.S194543

Bonnefoy, M., Boutitie, F., Mercier, C., Gueyffier, F., Carre, C., Guetemme, G., ... & Cornu, C. (2012). Efficacy of a home-based intervention programme on the physical activity level and functional ability of older people using domestic services: a randomised study. *The Journal of nutrition, health and aging*, *16*(4), 370-377.

Bouillanne, O., Curis, E., Hamon-Vilcot, B., Nicolis, I., Chrétien, P., Schauer, N., Vincent, J. P., Cynober, L., & Aussel, C. (2013). Impact of protein pulse feeding on lean mass in malnourished and at-risk hospitalized elderly patients: A randomized controlled trial. *Clinical Nutrition, 32*(2), 186–192. https://doi.org/10.1016/j.clnu.2012.08.015

Browne, W., & Nair, B. K. R. (2019). The timed up and go test. *Medical Journal of Australia*, *210*(1), 13–15. https://doi.org/10.5694/mja2.12045

Buckinx, F., Marcangeli, V., Carvalho, L. P., Dulac, M., Boutros, G. H., Gouspillou, G., Gaudreau, P., Morais, J., Noirez, P., & Aubertin-Leheudre, M. (2019). Initial dietary protein intake influence muscle function adaptations in older men and women following high-intensity interval training combined with citrulline. *Nutrients*, *11*(7). https://doi.org/10.3390/nu11071685

Burd, N. A., Yang, Y., Moore, D. R., Tang, J. E., Tarnopolsky, M. A., & Phillips, S. M. (2012). Greater stimulation of myofibrillar protein synthesis with ingestion of whey protein isolate v. micellar casein at rest and after resistance exercise in elderly men. *British Journal of Nutrition, 108*(6), 958–962. https://doi.org/10.1017/S0007114511006271

Caballero-García, A., Pascual-Fernández, J., Noriega-González, D. C., Bello, H. J., Pons-Biescas, A., Roche, E., & Córdova-Martínez, A. (2021). L-citrulline supplementation and exercise in the management of sarcopenia. *Nutrients*, *13*(9), 3133.https://doi.org/10.3390/nu13093133

Candow, D. G., Chilibeck, P. D., Gordon, J., Vogt, E., Landeryou, T., Kaviani, M., & Paus-Jensen, L. (2021). Effect of 12 months of creatine supplementation and whole-body resistance training on measures of bone, muscle and strength in older males. *Nutrition and Health*, 27(2), 151-159. https://doi.org/10.1177/0260106020975247

Candow, D. G., Zello, G. A., Ling, B., Farthing, J. P., Chilibeck, P. D., McLeod, K., Harris, J., & Johnson, S. (2014). Comparison of creatine supplementation

before versus after supervised resistance training in healthy older adults. *Research in Sports Medicine, 22*(1), 61-74. https://doi.org/10.1080/15438627.2013.852088

Candow, D. G., Vogt, E., Johannsmeyer, S., Forbes, S. C., & Farthing, J. P. (2015). Strategic creatine supplementation and resistance training in healthy older adults. *Applied Physiology, Nutrition, and Metabolism, 40*(7), 689-694.

Carlsson, M., & Haglin, H. L. Y. G. L. L.-O. N. L. E. R. L. (2011). Effect of high-intensity exercise and protein supplementation on muscle mass in adl dependent older people with and without malnutrition-A randomized controlled trial. *Journal of Nutrition, Health and Aging, 16*(8), 736. https://doi.org/10.1007/s12603-012-0392-6

Cawood, A. L., Elia, M., & Stratton, R. J. (2012). Systematic review and meta-analysis of the effects of high protein oral nutritional supplements. *Ageing Research Reviews, 11*(2), 278–296. https://doi.org/10.1016/j.arr.2011.12.008

Cesari, M., Landi, F., Vellas, B., Bernabei, R., & Marzetti, E. (2014). Sarcopenia and physical frailty: Two sides of the same coin. *Frontiers in Aging Neuroscience, 6*(7), 192. https://doi.org/10.3389/fnagi.2014.00192

Cesari, M., Leeuwenburgh, C., Lauretani, F., Onder, G., Bandinelli, S., Maraldi, C., ... & Ferrucci, L. (2006). Frailty syndrome and skeletal muscle: results from the Invecchiare in Chianti study. *The American journal of clinical nutrition, 83*(5), 1142-1148.

Chen, L. K., Woo, J., Assantachai, P., Auyeung, T. W., Chou, M. Y., Iijima, K., Jang, H. C., Kang, L., Kim, M., Kim, S., Kojima, T., Kuzuya, M., Lee, J. S. W., Lee, S. Y., Lee, W. J., Lee, Y., Liang, C. K., Lim, J. Y., Lim, W. S., … Arai, H. (2020). Asian Working Group for Sarcopenia: 2019 Consensus Update on Sarcopenia Diagnosis and Treatment. *Journal of the American Medical Directors Association, 21*(3), 300-307.e2. https://doi.org/10.1016/j.jamda.2019.12.012

Christie, D. R., Grant, J., Darnell, B. E., Chapman, V. R., Gastaldelli, A., & Sites, C. K. (2010). Metabolic effects of soy supplementation in postmenopausal Caucasian and African American women: A randomized, placebo-controlled trial. *American Journal of Obstetrics and Gynecology, 203*(2), 153.e1-153.e9. https://doi.org/10.1016/j.ajog.2010.02.058

Coelho-Júnior, H. J., Rodrigues, B., Uchida, M., & Marzetti, E. (2018). Low protein intake is associated with frailty in older adults: A systematic review and meta-analysis of observational studies. *Nutrients, 10*(9), 1–14. https://doi.org/10.3390/nu10091334

Collard, R. M., Boter, H., Schoevers, R. A., & Oude Voshaar, R. C. (2012). Prevalence of frailty in community-dwelling older persons: A systematic review. *Journal of the American Geriatrics Society, 60*(8), 1487–1492. https://doi.org/10.1111/j.1532-5415.2012.04054.x

Corral-Pérez, J., Ávila-Cabeza-de-Vaca, L., González-Mariscal, A., Espinar-Toledo, M., Ponce-González, J. G., Casals, C., & Vázquez-Sánchez, M. Á. (2023). Risk and Protective Factors for Frailty in Pre-Frail and Frail Older Adults. *International Journal of Environmental Research and Public Health*, *20*(4). https://doi.org/10.3390/ijerph20043123

Deutz, N. E. P., Bauer, J. M., Barazzoni, R., Biolo, G., Boirie, Y., Bosy-Westphal, A., Cederholm, T., Cruz-Jentoft, A., Krznariç, Z., Nair, K. S., Singer, P., Teta, D., Tipton, K., & Calder, P. C. (2014). Protein intake and exercise for optimal muscle function with aging: Recommendations from the ESPEN Expert Group. *Clinical Nutrition*, *33*(6), 929–936. https://doi.org/10.1016/j.clnu.2014.04.007

Devries, M. C., & Phillips, S. M. (2015). Supplemental protein in support of muscle mass and health: Advantage whey. *Journal of Food Science*, *80*(1), 8-15. https://doi.org/10.1111/1750-3841.12802

Dodds, R., & Sayer, A. A. (2016). Sarcopenia and frailty: new challenges for clinical practice. *Clinical Medicine*, *16*(5), 455-458.

Doody, P., Asamane, E. A., Aunger, J. A., Swales, B., Lord, J. M., Greig, C. A., & Whittaker, A. C. (2022). The prevalence of frailty and pre-frailty among geriatric hospital inpatients and its association with economic prosperity and healthcare expenditure: A systematic review and meta-analysis of 467,779 geriatric hospital inpatients. *Ageing research reviews*, *80*, 101666.

Embong, J., Amir, K., Nawawi, A., Razali, R. M., & Justine, M. (2021). Prevalence, risk factors and measures of frailty in Malaysia: A scoping review. *Malaysian Journal of Medicine and Health Sciences*, *17*(14), 308–319.

Fairhall, N., Aggar, C., Kurrle, S. E., Sherrington, C., Lord, S., Lockwood, K., ... & Cameron, I. D. (2008). Frailty intervention trial (FIT). *BMC geriatrics*, *8*, 1-10. https://doi.org/10.1186/1471-2318-8-27

Feng, L., Nyunt, M. S. Z., Feng, L., Yap, K. B., & Ng, T. P. (2014). Frailty predicts new and persistent depressive symptoms among community-dwelling older adults: Findings from singapore longitudinal aging study. *Journal of the American Medical Directors Association*, *15(*1), 76.e7-76.e12. https://doi.org/10.1016/j.jamda.2013.10.001

Fernandes, R. R., Nabuco, H. C. G., Sugihara, P., Cavalcanti, E. F., Tomeleri, C. M., Ribeiro, A. S., Barbosa, D. S., Venturini, D., Schoenfeld, B. J., & Cyrino, E. S. (2018). E ff ect of protein intake beyond habitual intakes following resistance training on cardiometabolic risk disease parameters in pre-conditioned older women. *Experimental Gerontology*, *110*(March), 9-14. https://doi.org/10.1016/j.exger.2018.05.003

Francis, P., Mc Cormack, W., Toomey, C., Norton, C., Saunders, J., Kerin, E., Lyons, M., & Jakeman, P. (2017). Twelve weeks' progressive resistance training combined with protein supplementation beyond habitual intakes increases

upper leg lean tissue mass, muscle strength and extended gait speed in healthy older women. *Biogerontology, 18*(6), 881-891. https://doi.org/10.1007/s10522-016-9671-7

Fried, L. P., Tangen, C. M., Walston, J., Newman, A. B., Hirsch, C., Gottdiener, J., ... & McBurnie, M. A. (2001). Frailty in older adults: evidence for a phenotype. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *56*(3), 146-157.

Frost, R., Belk, C., Jovicic, A., Ricciardi, F., Kharicha, K., Gardner, B., ... & Walters, K. (2017). Health promotion interventions for community-dwelling older people with mild or pre-frailty: a systematic review and meta-analysis. *BMC geriatrics*, *17*, 1-13. https://doi.org/10.1186/s12877-017-0547-8

Gade, J., Beck, A. M., Andersen, H. E., Christensen, B., Ronholt, F., Klausen, T. W., Vinther, A., & Astrup, A. (2019). Protein supplementation combined with low-intensity resistance training in geriatric medical patients during and after hospitalisation: A randomised, double-blind, multicentre trial. *British Journal of Nutrition, 122*(9), 1006–1020. https://doi.org/10.1017/S0007114519001831

Gerontol, J., Sci, A. B., Sci, M., Chalé, A., Cloutier, G. J., Hau, C., Phillips, E. M., Dallal, G. E., & Fielding, R. A. (2012). Efficacy of Whey Protein Supplementation on Resistance Exercise – Induced Changes in Lean Mass , Muscle Strength , and Physical Function in Mobility-Limited Older Adults. *J Gerontol A Biol Sci Med Sci.*, 1–9. https://doi.org/10.1093/gerona/gls221

Gherasim, A., Arhire, L. I., Niță, O., Popa, A. D., Graur, M., & Mihalache, L. (2020). The relationship between lifestyle components and dietary patterns. *Proceedings of the Nutrition Society, 79*(3), 311–323. https://doi.org/10.1017/S0029665120006898

Gill, T. M., Gahbauer, E. A., Allore, H. G., & Han, L. (2006). Transitions between frailty states among community-living older persons. *Archives of internal medicine*, *166*(4), 418-423.

Griffen, C., Duncan, M., Hattersley, J., Weickert, M. O., Dallaway, A., & Renshaw, D. (2022). Effects of resistance exercise and whey protein supplementation on skeletal muscle strength , mass , physical function , and hormonal and inflammatory biomarkers in healthy active older men : a randomised , double-blind , placebo-controlled trial. *Experimental Gerontology, 158*(December 2021), 111651. https://doi.org/10.1016/j.exger.2021.111651

Gryson, C., Ratel, S., Rance, M., Penando, S., Bonhomme, C., Le Ruyet, P., Duclos, M., Boirie, Y., & Walrand, S. (2014). Four-Month Course of Soluble Milk Proteins Interacts With Exercise to Improve Muscle Strength and Delay Fatigue in Elderly Participants. *Journal of the American Medical Directors Association, 15*(12), 958.e1-958.e9. https://doi.org/10.1016/j.jamda.2014.09.011

Gualano, B., Macedo, A. R., Alves, C. R. R., Roschel, H., Benatti, F. B., Takayama, L., de Sá Pinto, A. L., Lima, F. R., & Pereira, R. M. R. (2014). Creatine supplementation and resistance training in vulnerable older women: A randomized double-blind placebo-controlled clinical trial. *Experimental Gerontology, 53*, 7-15. https://doi.org/10.1016/j.exger.2014.02.003

Guralnik, J. M., Simonsick, E. M., Ferrucci, L., Glynn, R. J., Berkman, L. F., Blazer, D. G., Scherr, P. A., & Wallace, R. B. (1994). A Short Physical Performance Battery Assessing Lower Extremity Function : Association With Self-Reported Disability and Prediction of Mortality and Nursing Home Admission. Journal of Gerontology: *Medical Sciences, 49*(2), 85–94.

Hanach, N. I., Mccullough, F., & Avery, A. (2019). The Impact of Dairy Protein Intake on Muscle Mass, Muscle Strength, and Physical Performance in Middle-Aged to Older Adults with or without Existing Sarcopenia : A Systematic Review and Meta-Analysis. *6*, 59–69.

Hengeveld, L. M., Boer, J. M., Gaudreau, P., Heymans, M. W., Jagger, C., Mendonça, N., ... & Visser, M. (2020). Prevalence of protein intake below recommended in community-dwelling older adults: a meta-analysis across cohorts from the PROMISS consortium. *Journal of cachexia, sarcopenia and muscle*, *11*(5), 1212-1222. https://doi.org/10.1002/jcsm.12580

Holwerda, A. M., Overkamp, M., Paulussen, K. J. M., Smeets, J. S. J., Van Kranenburg, J., Backx, E. M. P., Gijsen, A. P., Goessens, J. P. B., Verdijk, L. B., & Van Loon, L. J. C. (2018). Protein Supplementation after Exercise and before Sleep Does Not Further Augment Muscle Mass and Strength Gains during Resistance Exercise Training in Active Older Men. *Journal of Nutrition, 148*(11), 1723–1732. https://doi.org/10.1093/jn/nxy169

Houston, D. K., Nicklas, B. J., Ding, J., Harris, T. B., Tylavsky, F. A., & Newman, A. B. (2018). Dietary protein intake is associated with lean mass change in older , community-dwelling adults : the Health , Aging , and Body Composition (Health ABC) Study 1 – 3. February, 150–155.

Huang, L.-P., Condello, G., & Kuo, C.-H. (2021). Effects of Milk Protein in Resistance Training-Induced Lean Mass Gains for Older Adults Aged ≥ 60 y : A Systematic Review and Meta-Analysis. *Neutrients, 13*(8), 2815.

Ikeda, T., Jinno, T., Masuda, T., Aizawa, J., Ninomiya, K., Suzuki, K., & Hirakawa, K. (2018). Effect of exercise therapy combined with branched-chain amino acid supplementation on muscle strengthening in persons with osteoarthritis. *Hong Kong Physiotherapy Journal*, *38*(1), 23–31. https://doi.org/10.1142/S1013702518500038

Ispoglou, T., White, H., Preston, T., McElhone, S., McKenna, J., & Hind, K. (2016). Double-blind, placebo-controlled pilot trial of L-Leucine-enriched amino-acid mixtures on body composition and physical performance in men and women aged 65-75 years. *European Journal of Clinical Nutrition, 70*(2), 182–188. https://doi.org/10.1038/ejcn.2015.91

Jadczak, A. D., Visvanathan, R., Barnard, R., & Luscombe-Marsh, N. (2021). A Randomized Controlled Pilot Exercise and Protein Effectiveness Supplementation Study (EXPRESS) on Reducing Frailty Risk in Community-Dwelling Older People. *Journal of Nutrition in Gerontology and Geriatrics, 40*(1), 26–45. https://doi.org/10.1080/21551197.2021.1886222

Johannsmeyer, S., Candow, D. G., Brahms, C. M., Michel, D., & Zello, G. A. (2016). Effect of creatine supplementation and drop-set resistance training in untrained aging adults. *Experimental Gerontology, 83*, 112–119. https://doi.org/10.1016/j.exger.2016.08.005

Junior, P. S., Ribeiro, S. A., Nabuco, C. G. H., & Rodrigo R. Fernandes1, Crisieli M. Tomeleri1, Paolo M. Cunha1, Danielle Venturini3, Décio S. Barbosa3, Brad J. Schoenfeld4, and E. S. C. (2017). Effects of Whey Protein Supplementation Associated With Resistance Training on Muscular Strength, Hypertrophy and Muscle Quality in Pre-Conditioned Older Women. *International Journal of Sport Nutrition and Exercise Metabolism*.

Kang, L., Gao, Y., Liu, X., Liang, Y., Chen, Y., Liang, Y., Zhang, L., Chen, W., Pang, H., & Peng, L. N. (2019). Effects of whey protein nutritional supplement on muscle function among community-dwelling frail older people: A multicenter study in China. *Archives of Gerontology and Geriatrics, 83*, 7–12. https://doi.org/10.1016/j.archger.2019.03.012

Kang, Y., Kim, N., Choi, Y. J., Lee, Y., Yun, J., Park, S. J., Park, H. S., Chung, Y., & Park, Y. K. (2020). Leucine-Enriched Protein Supplementation Increases Lean Body Mass in Healthy Korean Adults Aged 50 Years and Older: A Randomized, Double-Blind, Placebo-Controlled Trial Yeji. *Neutrients*, 1–19.

Karelis, A. D., Messier, V., Suppère, C., & Briand, P. (2015). Effect of cysteine-rich whey protein (÷mmunocal ®) supplementation in combination with resistance training on muscle strength and lean body mass in non-frail elderly subjects : a randomized , double-blind controlled study. *J Nutr Health Aging, 19*(5), 531–536.

Kawada, S., Okamoto, Y., Ogasahara, K., Yanagisawa, S., Ohtani, M., & Kobayashi, K. (2013). Resistance exercise combined with essential amino acid supplementation improved walking ability in elderly people. *Acta Physiologica Hungarica, 100*(3), 329–339. https://doi.org/10.1556/APhysiol.100.2013.008

Kerstetter, J. E., Bihuniak, J. D., Brindisi, J., Sullivan, R. R., Mangano, K. M., Larocque, S., Kotler, B. M., Simpson, C. A., Cusano, A. M., Gaffney-Stomberg, E., Kleppinger, A., Reynolds, J., Dziura, J., Kenny, A. M., & Insogna, K. L. (2015). The effect of a whey protein supplement on bone mass in older Caucasian adults. Journal of Clinical Endocrinology and Metabolism, 100(6), 2214–2222. https://doi.org/10.1210/jc.2014-3792

Kim, H. K., Suzuki, T., Saito, K., Yoshida, H., Kobayashi, H., Kato, H., & Katayama, M. (2012). Effects of exercise and amino acid supplementation on body composition and physical function in community-dwelling elderly Japanese

sarcopenic women: A randomized controlled trial. Journal of the American Geriatrics Society, 60(1), 16–23. https://doi.org/10.1111/j.1532-5415.2011.03776.x

Kirk, B., Mooney, K., Amirabdollahian, F., & Khaiyat, O. (2019). Exercise and Dietary-Protein as a Countermeasure to Skeletal Muscle Weakness : Liverpool Hope University – Sarcopenia Aging Trial. Front. Physiol, 10(April), 1–11. https://doi.org/10.3389/fphys.2019.00445

Kirk, B., Mooney, K., Cousins, R., Angell, P., Jackson, M., Pugh, J. N., Coyles, G., Amirabdollahian, F., & Khaiyat, O. (2020). Effects of exercise and whey protein on muscle mass , fat mass , myoelectrical muscle fatigue and health related quality of life in older adults : a secondary analysis of the Liverpool Hope University — Sarcopenia Ageing Trial (LHU SAT). European Journal of Applied Physiology, 120(0123456789), 493_503. https://doi.org/10.1007/s00421-019-04293-5

Kirwan, R. P., Mazidi, M., García, C. R., Lane, K. E., Jafari, A., & Butler, T. (2022). Protein interventions augment the effect of resistance exercise on appendicular lean mass and handgrip strength in older adults : a systematic review and meta-analysis of randomized controlled trials. The American Journal of Clinical Nutrition, 115(3), 897–913. https://doi.org/10.1093/ajcn/nqab355

Kojima, G. (2015). Prevalence of Frailty in Nursing Homes: A Systematic Review and Meta-Analysis. In Journal of the American Medical Directors Association (Vol. 16, Issue 11, pp. 940–945). Elsevier Inc. https://doi.org/10.1016/j.jamda.2015.06.025

Krause, M., Crognale, D., Cogan, K., Contarelli, S., Egan, B., Newsholme, P., & De Vito, G. (2019). The effects of a combined bodyweight-based and elastic bands resistance training, with or without protein supplementation, on muscle mass, signaling and heat shock response in healthy older people. Experimental Gerontology, 115(September 2018), 104–113. https://doi.org/10.1016/j.exger.2018.12.004

Landi, F., Calvani, R., Tosato, M., Martone, A. M., Ortolani, E., Savera, G., Angelo, E. D., Sisto, A., & Marzetti, E. (2016). Protein Intake and Muscle Health in Old Age : From Biological Plausibility to Clinical Evidence. Nutrients, 8, 1–12. https://doi.org/10.3390/nu8050295

Landi, F., Marzetti, E., & Bernabei, R. (2013). Perspective: Protein: What Kind, How Much, When? In Journal of the American Medical Directors Association (Vol. 14, Issue 1, pp. 66–67). Elsevier Inc. https://doi.org/10.1016/j.jamda.2012.09.020

Leenders, M., Verdijk, L. B., van der Hoeven, L., van Kranenburg, J., Hartgens, F., Wodzig, W. K. W. H., Saris, W. H. M., & van Loon, L. J. C. (2011). Prolonged leucine supplementation does not augment muscle mass or affect glycemic control in elderly type 2 diabetic men. Journal of Nutrition, 141(6), 1070–1076. https://doi.org/10.3945/jn.111.138495

Leng, S., Chen, X., & Mao, G. (2014). Frailty syndrome: an overview. Clinical Interventions in Aging, 433. https://doi.org/10.2147/cia.s45300

Li, P., Stuart, E., & Allison, D. (2016). Multiple Imputation: A Flexible Tool for Handling Missing Data. Physiology & Behavior, 176(3), 139–148. https://doi.org/10.1159/000444169.Carotid

Liao, C. De, Lee, P. H., Hsiao, D. J., Huang, S. W., Tsauo, J. Y., Chen, H. C., & Liou, T. H. (2018). Effects of protein supplementation combined with exercise intervention on frailty indices, body composition, and physical function in frail older adults. In Nutrients (Vol. 10, Issue 12). https://doi.org/10.3390/nu10121916

Lobo, D. M., Tritto, A. C., da Silva, L. R., de Oliveira, P. B., Benatti, F. B., Roschel, H., Nieß, B., Gualano, B., & Pereira, R. M. R. (2015). Effects of long-term low-dose dietary creatine supplementation in older women. Experimental Gerontology, 70, 97–104. https://doi.org/10.1016/j.exger.2015.07.012

Markofski, M. M., Jennings, K., Timmerman, K. L., Dickinson, J. M., Fry, C. S., Borack, M. S., Reidy, P. T., Deer, R. R., Randolph, A., Rasmussen, B. B., & Volpi, E. (2019). Effect of aerobic exercise training and essential amino acid supplementation for 24 weeks on physical function, body composition, and muscle metabolism in healthy, independent older adults: A randomized clinical trial. Journals of Gerontology - Series A Biological Sciences and Medical Sciences, 74(10), 1598–1604. https://doi.org/10.1093/gerona/gly109

Mertz, K. H., Reitelseder, S., Bechshoeft, R., Bulow, J., Højfeldt, G., Jensen, M., Schacht, S. R., Lind, M. V., Rasmussen, M. A., Mikkelsen, U. R., Tetens, I., Engelsen, S. B., Nielsen, D. S., Jespersen, A. P., & Holm, L. (2021a). The effect of daily protein supplementation, with or without resistance training for 1 year, on muscle size, strength, and function in healthy older adults: A randomized controlled trial. American Journal of Clinical Nutrition, 113(4), 790–800. https://doi.org/10.1093/ajcn/nqaa372

Mertz, K. H., Reitelseder, S., Bechshoeft, R., Bulow, J., Højfeldt, G., Jensen, M., Schacht, S. R., Lind, M. V., Rasmussen, M. A., Mikkelsen, U. R., Tetens, I., Engelsen, S. B., Nielsen, D. S., Jespersen, A. P., & Holm, L. (2021b). The effect of daily protein supplementation, with or without resistance training for 1 year, on muscle size, strength, and function in healthy older adults: A randomized controlled trial. American Journal of Clinical Nutrition, 113(4), 790–800. https://doi.org/10.1093/ajcn/nqaa372

Michel, J. M., Lievense, K. K., Norton, S. C., Costa, J. V., Alphin, K. H., Bailey, L. A., & Miller, G. D. (2022). The Effects of Graded Protein Intake in Conjunction with Progressive Resistance Training on Skeletal Muscle Outcomes in Older Adults: A Preliminary Trial. Nutrients, 14(13). https://doi.org/10.3390/nu14132739

Milne et al. (2009). Protein and energy supplementation in elderly people at risk frommalnutrition (Review). 2.

Mirnalini, K., Zalilah, M. S., Safiah, M. Y., Tahir, A., Siti, H. M. D., Siti, R. D., Khairul, Z. M. Y., Mohd, H. S., & Normah, H. (2008). Energy and nutrient intakes: Findings from the Malaysian Adult Nutrition Survey (MANS). Malaysian Journal of Nutrition, 14(1), 1–24.

Mojtahedi, M. C., Thorpe, M. P., Karampinos, D. C., Johnson, C. L., Layman, D. K., Georgiadis, J. G., & Evans, E. M. (2011). The Effects of a Higher Protein Intake During Energy Restriction on Changes in Body Composition and Physical Function in Older Women. J Gerontol A Biol Sci Med Sci., 66A(11), 1218–1225. https://doi.org/10.1093/gerona/glr120

Moore, D. R., Churchward-venne, T. A., Witard, O., Breen, L., Burd, N. A., Tipton, K. D., & Phillips, S. M. (2014). Protein Ingestion to Stimulate Myofibrillar Protein Synthesis Requires Greater Relative Protein Intakes in Healthy Older Versus Younger Men. Journals of Gerontology: MEDICAL SCIENCES, 9, 5–8. https://doi.org/10.1093/gerona/glu103

Mori, H., & Takudo, Y. (2018). Effect of whey protein supplementation after resistance exercise on the muscle mass and physical function of healthy older women : A randomized controlled trial. Geriatr. Gerontol. Int., 18, 1398–1404. https://doi.org/10.1111/ggi.13499

Morton, R. W., Murphy, K. T., Mckellar, S. R., Schoenfeld, B. J., Henselmans, M., Helms, E., Aragon, A. A., Devries, M. C., Banfield, L., Krieger, J. W., & Phillips, S. M. (2017). A systematic review , meta-analysis and meta-regression of the effect of protein supplementation on resistance training-induced gains in muscle mass and strength in healthy adults. 1–10. https://doi.org/10.1136/bjsports-2017-097608

Murai, U., Tajima, R., Matsumoto, M., Sato, Y., Horie, S., Fujiwara, A., Koshida, E., Okada, E., Sumikura, T., Yokoyama, T., Ishikawa, M., Kurotani, K., & Takimoto, H. (2023). Validation of Dietary Intake Estimated by Web-Based Dietary Assessment Methods and Usability Using Dietary Records or 24-h Dietary Recalls: A Scoping Review. Nutrients, 15(8). https://doi.org/10.3390/nu15081816

Murphy, C. H., Flanagan, E. M., De Vito, G., Susta, D., Mitchelson, K. A. J., De Marco Castro, E., Senden, J. M. G., Goessens, J. P. B., Mikłosz, A., Chabowski, A., Segurado, R., Corish, C. A., Mccarthy, S. N., Egan, B., Van Loon, L. J. C., & Roche, H. M. (2021). Does supplementation with leucine-enriched protein alone and in combination with fish-oil-derived n-3 PUFA affect muscle mass, strength, physical performance, and muscle protein synthesis in well-nourished older adults? A randomized, double-blind, placebo. American Journal of Clinical Nutrition, 113(6), 1411–1427. https://doi.org/10.1093/ajcn/nqaa449

Murukesu, R. R., Kaur, D., Singh, A., & Shahar, S. (2020). A Multi-Domain Intervention Protocol for the Potential Reversal of Cognitive Frailty : " WE-RISE " Randomized Controlled Trial. 8(September), 1–11. https://doi.org/10.3389/fpubh.2020.00471

Nabuco, H. C. G., Tomeleri, C. M., Fernandes, R. R., Sugihara, P., Cavalcante, E. F., Cunha, P. M., Antunes, M., Barbosa, S., Carlos, R., & Venturini, D. (2019). Clinical Nutrition ESPEN Effect of whey protein supplementation combined with resistance training on body composition , muscular strength , functional capacity , and plasma-metabolism biomarkers in older women with sarcopenic obesity : A randomized , doub. Clinical Nutrition ESPEN, 32(2019), 88–95. https://doi.org/10.1016/j.clnesp.2019.04.007

Nabuco, H. C. G., Tomeleri, C. M., Junior, P. S., & Fernandes, R. R. (2019). Nutrition , Metabolism & Cardiovascular Diseases Effects of pre- or post-exercise whey protein supplementation on body fat and metabolic and in fl ammatory pro fi le in pre-conditioned older women : A randomized , double-blind , placebo-controlled trial. Nutrition, Metabolism and Cardiovascular Diseases, 29(3), 290–300. https://doi.org/10.1016/j.numecd.2018.11.007

Nakayama, K., Saito, Y., Sanbongi, C., Murata, K., & Urashima, T. (2021). Effects of low-dose milk protein supplementation following low-to-moderate intensity exercise training on muscle mass in healthy older adults: a randomized placebo-controlled trial. European Journal of Nutrition, 60(2), 917–928. https://doi.org/10.1007/s00394-020-02302-4

Nasimi, N., Sohrabi, Z., Nunes, E. A., Sadeghi, E., Jamshidi, S., Gholami, Z., Akbarzadeh, M., Faghih, S., Akhlaghi, M., & Phillips, S. M. (2023). Whey Protein Supplementation with or without Vitamin D on Sarcopenia-Related Measures : A Systematic Review and Meta-Analysis. Advances in Nutrition, 14(4), 762–773. https://doi.org/10.1016/j.advnut.2023.05.011

Neske, R., & Azevedo, S. De. (2022). Can supplemental protein to low-protein containing meals superimpose on resistance-training muscle adaptations in older adults ? A randomized clinical trial. Experimental Gerontology, 162(March). https://doi.org/10.1016/j.exger.2022.111760

Neves, M., Gualano, B., Roschel, H., Fuller, R., Benatti, F. B., De Sá Pinto, A. L., Lima, F. R., Pereira, R. M., Lancha, A. H., & Bonfá, E. (2011). Beneficial effect of creatine supplementation in knee osteoarthritis. Medicine and Science in Sports and Exercise, 43(8), 1538–1543. https://doi.org/10.1249/MSS.0b013e3182118592

Niccoli, S., Kolobov, A., Bon, T., Rafilovich, S., Munro, H., Tanner, K., Pearson, T., & Lees, S. J. (2017). Whey Protein Supplementation Improves Rehabilitation Outcomes in Hospitalized Geriatric Patients: A Double Blinded, Randomized Controlled Trial. Journal of Nutrition in Gerontology and Geriatrics, 36(4), 149–165. https://doi.org/10.1080/21551197.2017.1391732

Nilsson, M. I., Mikhail, A., Lan, L., Carlo, A. di, Hamilton, B., Barnard, K., Hettinga, B. P., Hatcher, E., Tarnopolsky, M. G., Nederveen, J. P., Bujak, A. L., May, L., & Tarnopolsky, M. A. (2020). A five-ingredient nutritional supplement and home-based resistance exercise improve lean mass and strength in free-living elderly. Nutrients, 12(8), 1–28. https://doi.org/10.3390/nu12082391

Nohan, A. F., Adznam, S. N. A., Jamaluddin, R., & Norazman, C. W. (2020). Diet Quality and its Associated Factors among Community Dwelling Older Adults in Urban District in Kuala Lumpur, Malaysia. *Malaysian Journal of Medicine & Health Sciences, 16*(6).

Norton, C., Toomey, C., McCormack, W. G., Francis, P., Saunders, J., Kerin, E., & Jakeman, P. (2016). Protein supplementation at breakfast and lunch for 24 weeks beyond habitual intakes increases whole-body lean tissue mass in healthy older adults. *Journal of Nutrition, 146*(1), 65–69. https://doi.org/10.3945/jn.115.219022

Nunes, E. A., Colenso-Semple, L., McKellar, S. R., Yau, T., Ali, M. U., Fitzpatrick-Lewis, D., ... & Phillips, S. M. (2022). Systematic review and meta-analysis of protein intake to support muscle mass and function in healthy adults. *Journal of cachexia, sarcopenia and muscle*, *13*(2), 795-810.

Oikawa, S. Y., Kamal, M. J., Webb, E. K., McGlory, C., Baker, S. K., & Phillips, S. M. (2020). Whey protein but not collagen peptides stimulate acute and longer-term muscle protein synthesis with and without resistance exercise in healthy older women: a randomized controlled trial. *The American Journal of Clinical Nutrition*, *111*(3), 708-718.

Oktaviana, J., Zanker, J., Vogrin, S., & Duque, G. (2020). The effect of protein supplements on functional frailty in older persons: A systematic review and meta-analysis. *Archives of gerontology and geriatrics*, *86*, 103938.

Orsatti, F. L., Maestá, N., de Oliveira, E. P., Nahas Neto, J., Burini, R. C., Nunes, P. R., ... & Nahas, E. P. (2018). Adding soy protein to milk enhances the effect of resistance training on muscle strength in postmenopausal women. *Journal of dietary supplements*, *15*(2), 140-152.

Ottestad, I., Løvstad, A. T., Gjevestad, G. O., Hamarsland, H., Šaltytė Benth, J., Andersen, L. F., Bye, A., Biong, A. S., Retterstøl, K., Iversen, P. O., Raastad, T., Ulven, S. M., & Holven, K. B. (2017). Intake of a protein-enriched milk and effects on muscle mass and strength. A 12-week randomized placebo controlled trial among community-dwelling older adults. *Journal of Nutrition, Health and Aging, 21*(10), 1160–1169. https://doi.org/10.1007/s12603-016-0856-1

Park, Y., Choi, J. E., & Hwang, H. S. (2018). Protein supplementation improves muscle mass and physical performance in undernourished prefrail and frail elderly subjects: A randomized, double-blind, placebo-controlled trial. *American Journal of Clinical Nutrition, 108*(5), 1026–1033. https://doi.org/10.1093/ajcn/nqy214

Pavasini, R., Guralnik, J., Brown, J. C., di Bari, M., Cesari, M., Landi, F., Vaes, B., Legrand, D., Verghese, J., Wang, C., Stenholm, S., Ferrucci, L., Lai, J. C., Bartes, A. A., Espaulella, J., Ferrer, M., Lim, J. Y., Ensrud, K. E., Cawthon, P., … Campo, G. (2016). Short Physical Performance Battery and all-cause

mortality: Systematic review and meta-analysis. *BMC Medicine, 14*(1), 1–9. https://doi.org/10.1186/s12916-016-0763-7

Pennings, B., Boirie, Y., Senden, J. M., Gijsen, A. P., Kuipers, H., & van Loon, L. J. (2011). Whey protein stimulates postprandial muscle protein accretion more effectively than do casein and casein hydrolysate in older men. *The American journal of clinical nutrition*, *93*(5), 997-1005.

Phillips, S. M. (2016). The impact of protein quality on the promotion of resistance exercise-induced changes in muscle mass. *Nutrition & Metabolism*, 1–9. https://doi.org/10.1186/s12986-016-0124-8

Pineda-Juárez, J. A., Sánchez-Ortiz, N. A., Castillo-Martínez, L., Orea-Tejeda, A., Cervantes-Gaytán, R., Keirns-Davis, C., Pérez-Ocampo, C., Quiroz-Bautista, K., Tenorio-Dupont, M., & Ronquillo-Martínez, A. (2016). Changes in body composition in heart failure patients after a resistance exercise program and branched chain amino acid supplementation. Clinical Nutrition, 35(1), 41–47. https://doi.org/10.1016/j.clnu.2015.02.004

Krok-Schoen, J. L., Price, A. A., Luo, M., Kelly, O. J., & Taylor, C. A. (2019). Low dietary protein intakes and associated dietary patterns and functional limitations in an aging population: a NHANES analysis. *The Journal of nutrition, health and aging*, *23*(4), 338-347.

Radolff, L. S. (1977). The CES-D scale: a self-report depression scale for research in the general population. *Applied Psychological Measurement, 1*(3), 385–401.

Reule, C. A., Scholz, C., Schoen, C., Brown, N., Siepelmeyer, A., & Alt, W. W. (2017). Reduced muscular fatigue after a 12-week leucine-rich amino acid supplementation combined with moderate training in elderly: a randomised, placebo-controlled, double-blind trial. *BMJ Open Sport & Exercise Medicine*, *2*(1), e000156.

Rockwood, K., & Mitnitski, A. (2007). Frailty in relation to the accumulation of deficits. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences*, *62*(7), 722–727. https://doi.org/10.1093/gerona/62.7.722

Rockwood, K., Song, X., MacKnight, C., Bergman, H., Hogan, D. B., McDowell, I., & Mitnitski, A. (2005). A global clinical measure of fitness and frailty in elderly people. CMAJ. *Canadian Medical Association Journal, 173*(5), 489–495. https://doi.org/10.1503/cmaj.050051

Rollo, M. E., Williams, R. L., Burrows, T., Kirkpatrick, S. I., Bucher, T., & Collins, C. E. (2016). What are they really eating? A review on new approaches to dietary intake assessment and validation. *Current Nutrition Reports*, *5*(4), 307–314. https://doi.org/10.1007/s13668-016-0182-6

Rondanelli, M., Guido, D., Perna, S., Faliva, M. A., Donelli, C., Peroni, G., Rollone, M., Ricevuti, G., Guerriero, F., Sgarlata, C., & Nichetti, M. (2013). Effects of proteins and essential amino acids supplementation on body composition in

sarcopenic elderly. *European Geriatric Medicine, 4*, S125–S126. https://doi.org/10.1016/j.eurger.2013.07.412

Sales, L. P., Pinto, A. J., Rodrigues, S. F., Alvarenga, J. C., Gonçalves, N., Sampaio-Barros, M. M., ... & Rodrigues Pereira, R. M. (2020). Creatine supplementation (3 g/d) and bone health in older women: a 2-year, randomized, placebo-controlled trial. *The Journals of Gerontology: Series A, 75*(5), 931-938.

Sawicka, A. K., Hartmane, D., Lipinska, P., Wojtowicz, E., Lysiak-Szydlowska, W., & Olek, R. A. (2018). L-carnitine supplementation in older women. A pilot study on aging skeletal muscle mass and function. *Nutrients, 10*(2). https://doi.org/10.3390/nu10020255

Shahar S., & Siti Saifa, H. (2007). Validation of nutritional screening tools against anthropometric and functional assessments among elderly people in Selangor. *Malaysian Journal of Nutrition, 13*(1), 29–44.

Shahar S., Rafidah G., Noor Aini, M. Y., Nik Shahnita, S., Zahara, A. M., & Shahrul, A. M. H. (2002). Atlas of food exchange & portion size. MDC Publisher Printers Sdn. Bhd. Malaysia Book Publishers Association.

Shahar, S., Kamaruddin, N. S., Badrasawi, M., Mohamed Sakian, N. I., Manaf, Z. A., Yassin, Z., & Joseph, L. (2013). Effectiveness of exercise and protein supplementation intervention on body composition, functional fitness, and oxidative stress among elderly Malays with sarcopenia. *Clinical Interventions in Aging, 8,* 1365–1375. https://doi.org/10.2147/CIA.S46826

Shenoy, S., Bedi, R., & Sandhu, J. S. (2013). Effect of Soy Isolate Protein and Resistance Exercises on Muscle Performance and Bone Health of Osteopenic/Osteoporotic Post-Menopausal Women. *Journal of Women and Aging, 25*(2), 183–198. https://doi.org/10.1080/08952841.2013.764252

Shikany, J. M., Barrett-Connor, E., Ensrud, K. E., Cawthon, P. M., Lewis, C. E., Dam, T. T. L., ... & Osteoporotic Fractures in Men (MrOS) Research Group. (2014). Macronutrients, diet quality, and frailty in older men. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences, 69*(6), 695-701.

Siriwardhana, D. D., Hardoon, S., Rait, G., Weerasinghe, M. C., & Walters, K. R. (2018). Prevalence of frailty and prefrailty among community-dwelling older adults in low-income and middle-income countries: A systematic review and meta-analysis. *BMJ Open, 8*(3), 1–17. https://doi.org/10.1136/bmjopen-2017-018195

Suffian, N. I. M., Adznam, S. N. A., Saad, H. A., Chan, Y. M., Ibrahim, Z., Omar, N., & Murat, M. F. (2020). Frailty intervention through nutrition education and exercise (Fine). a health promotion intervention to prevent frailty and improve frailty status among pre-frail elderly—a study protocol of a cluster randomized controlled trial. *Nutrients, 12*(9), 1–12. https://doi.org/10.3390/nu12092758

Talley, C. H., & Williams, K. P. (2015). Impact of Age and Comorbidity on Cervical and Breast Cancer Literacy of African Americans, Latina, and Arab Women. *Nursing Clinics of North America*, *50*(3), 545–563. https://doi.org/10.1016/j.cnur.2015.05.008

Tang, J. E., Moore, D. R., Kujbida, G. W., Tarnopolsky, M. A., & Phillips, S. M. (2009). Ingestion of whey hydrolysate, casein, or soy protein isolate: Effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. *Journal of Applied Physiology*, *107*(3), 987–992. https://doi.org/10.1152/japplphysiol.00076.2009

Ten Haaf, D. S. M., Bongers, C. C. W. G., Hulshof, H. G., Eijsvogels, T. M. H., & Hopman, M. T. E. (2020). The impact of protein supplementation on exercise-induced muscle damage, soreness and fatigue following prolonged walking exercise in vital older adults: A randomized double-blind placebo-controlled trial. *Nutrients*, *12*(6), 1–14. https://doi.org/10.3390/nu12061806

Ten Haaf, D. S. M., De Regt, M. F., Visser, M., Witteman, B. J. M., De Vries, J. H. M., Eijsvogels, T. M. H., & Hopman, M. T. (2018). Insufficient protein intake is highly prevalent among physically active elderly. *The Journal of nutrition, health and aging*, *22*(9), 1112-1114.

ten Haaf, D. S. M., Eijsvogels, T. M. H., Bongers, C. C. W. G., Horstman, A. M. H., Timmers, S., de Groot, L. C. P. G. M., & Hopman, M. T. E. (2019). Protein supplementation improves lean body mass in physically active older adults: a randomized placebo-controlled trial. *Journal of Cachexia, Sarcopenia and Muscle*, *10*(2), 298–310. https://doi.org/10.1002/jcsm.12394

Tessier, A. J., & Chevalier, S. (2018). An update on protein, leucine, omega-3 fatty acids, and vitamin D in the prevention and treatment of sarcopenia and functional decline. *Nutrients*, *10*(8), 1099.

Thompson, F. E., & Subar, A. F. (2017). Dietary assessment methodology. In Nutrition in the Prevention and Treatment of Disease (Fourth Edi). Elsevier Inc. https://doi.org/10.1016/B978-0-12-802928-2.00001-1

Tieland, M., Dirks, M. L., van der Zwaluw, N., Verdijk, L. B., van de Rest, O., de Groot, L. C. P. G. M., & van Loon, L. J. C. (2012). Protein Supplementation Increases Muscle Mass Gain During Prolonged Resistance-Type Exercise Training in Frail Elderly People: A Randomized, Double-Blind, Placebo-Controlled Trial. *Journal of the American Medical Directors Association*, *13*(8), 713–719. https://doi.org/10.1016/j.jamda.2012.05.020

Tieland, M., Franssen, R., Dullemeijer, C., van Dronkelaar, C., Kim, H. K., Ispoglou, T., ... & de Groot, L. C. (2017). The impact of dietary protein or amino acid supplementation on muscle mass and strength in elderly people: individual participant data and meta-analysis of RCT's. *The Journal of nutrition, health and aging*, *21*(9), 994-1001.

Tieland, M., van de Rest, O., Dirks, M. L., van der Zwaluw, N., Mensink, M., van Loon, L. J. C., & de Groot, L. C. P. G. M. (2012). Protein Supplementation Improves Physical Performance in Frail Elderly People: A Randomized, Double-Blind, Placebo-Controlled Trial. *Journal of the American Medical Directors Association, 13*(8), 720–726. https://doi.org/10.1016/j.jamda.2012.07.005

Trabal, J., Forga, M., Leyes, P., Torres, F., Rubio, J., Prieto, E., & Farran-Codina, A. (2015). Effects of free leucine supplementation and resistance training on muscle strength and functional status in older adults: A randomized controlled trial. *Clinical Interventions in Aging, 10*, 713–723. https://doi.org/10.2147/CIA.S75271

Traylor, D. A., Gorissen, S. H. M., & Phillips, S. M. (2018). Perspective: Protein requirements and optimal intakes in aging: Are we ready to recommend more than the recommended daily allowance? In Advances in Nutrition (Vol. 9, Issue 3, pp. 171–182). Oxford University Press. https://doi.org/10.1093/advances/nmy003

Villanueva, M. G., He, J., & Schroeder, E. T. (2014). Periodized resistance training with and without supplementation improve body composition and performance in older men. *European journal of applied physiology*, *114*, 891-905.

Volpi, E., Campbell, W. W., Dwyer, J. T., Johnson, M. A., Jensen, G. L., Morley, J. E., & Wolfe, R. R. (2013). Is the optimal level of protein intake for older adults greater than the recommended dietary allowance? *Journals of Gerontology - Series A Biological Sciences and Medical Sciences, 68*(6), 677–681. https://doi.org/10.1093/gerona/gls229

Weisgarber, K. D., Candow, D. G., & Farthing, J. P. (2015). Whey protein and high-volume resistance training in postmenopausal women. *The Journal of nutrition, health and aging*, *19*(5), 511-517.

Werner, C., Wolf-Belala, N., Nerz, C., Abel, B., Braun, T., Grüneberg, C., Thiel, C., Büchele, G., Muche, R., Hendlmeier, I., Schäufele, M., Dams, J., König, H. H., Bauer, J. M., Denkinger, M., & Rapp, K. (2022). A multifactorial interdisciplinary intervention to prevent functional and mobility decline for more participation in (pre-)frail community-dwelling older adults (PromeTheus): study protocol for a multicenter randomized controlled trial. *BMC Geriatrics, 22*(1), 1–18. https://doi.org/10.1186/s12877-022-02783-4

Wirth, J., Hillesheim, E., & Brennan, L. (2020). The Role of Protein Intake and its Timing on Body Composition and Muscle Function in Healthy Adults : A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Journal of Nutrition, 150*(6), 1443–1460. https://doi.org/10.1093/jn/nxaa049

Xue, Q. L. (2011). The Frailty Syndrome: Definition and Natural History. *Clinics in Geriatric Medicine, 27*(1), 1–15. https://doi.org/10.1016/j.cger.2010.08.009

Yamamoto, Y., Nagai, Y., Kawanabe, S., Hishida, Y., Hiraki, K., Sone, M., & Tanaka, Y. (2021). Effects of resistance training using elastic bands on muscle strength with or without a leucine supplement for 48 weeks in elderly patients with type 2 diabetes. *Endocrine Journal*, *68*(3), 291–298. https://doi.org/10.1507/endocrj.EJ20-0550

Yoshimura, Y., Bise, T., Shimazu, S., Tanoue, M., Tomioka, Y., Araki, M., Nishino, T., Kuzuhara, A., & Takatsuki, F. (2019). Effects of a leucine-enriched amino acid supplement on muscle mass, muscle strength, and physical function in post-stroke patients with sarcopenia: A randomized controlled trial. *Nutrition*, *58*, 1–6. https://doi.org/10.1016/j.nut.2018.05.028

Zdzieblik, D., Oesser, S., Baumstark, M. W., Gollhofer, A., & König, D. (2015). Collagen peptide supplementation in combination with resistance training improves body composition and increases muscle strength in elderly sarcopenic men: a randomised controlled trial. *British Journal of Nutrition*, *114*(8), 1237-1245. https://doi.org/10.1017/S0007114515002810

Zhu, K., Kerr, D. A., Meng, X., Devine, A., Solah, V., Binns, C. W., & Prince, R. L. (2015). Two-Year Whey Protein Supplementation Did Not Enhance Muscle Mass and Physical Function in Well-Nourished Healthy Older Postmenopausal Women 1 – 3. C, 1–7. https://doi.org/10.3945/jn.115.218297.1

Appendix C.1

The screening questionnaire

RESPONDENT CODE

Day: Date:

Screening Questionnaire:
INSTRUCTIONS: Please tick (/) the appropriate answer

Date of Birth:		
Sex:		
PART A: PAST MEDICAL HISTORY:		**Note**
1.	**Please circle each condition that you have been told you have (or had):** □Cancer □Diabetes □Kidney Disease □Liver Disease □Stroke □Heart Disease □COPD □Ulcers □ Fibromyalgia □Osteoporosis □Osteoarthritis □Rheumatoid Arthritis □Sexually Transmitted Disease □Dementia □ Lung disease □Other: ……	
2.	**SURGERIES:** Type of surgery and specific date or your age at surgery: _____	
3.	**Fractures:** Do you have any sustained fracture (hip, vertebrata) in past six months: ☐ YES ☐ NO if YES specify please: …… **Have you applied any surgery (hip, abdominal area) in past six months:** ☐ YES ☐ NO if YES specify please: ……	

RESPONDENT CODE

PART B: FRAILTY STATUS SCREENING:
Frailty phenotype, (Fried et al., 2001, Suffian N et al., 2020)
INSTRUCTIONS: Please tick / in the answer space provided

		FRAILTY CRITERIA
1.	**Weight loss / weight loss:** (a) During the past 6 months, was your BMI less than 18.5 kg/m2? 0.☐ No. 1.☐ Yes (b) During the past 6 months, have you experienced unplanned weight loss (not due to diet or exercise) of more than 10 pounds (4.5kg)? 0.☐ No. 1.☐ Yes **Subjects who chose the answer "1" for one of questions (a) or (b), had frailty criteria.** **If so, mark / in the frailty criterion space next to it.** **(Ng et al., 2015).**	☐
2.	**Fatigue:** **During the past week, how often did feel the following?** (a) Feeling of difficulty when wanting to do something 0.☐ Rarely/ none (1 day) 1.☐ Sometimes (1-2 days) 2.☐ Often (3-4 days) 3.☐ At all times (5-7 days) (b) Having trouble getting on with life 0.☐ Rarely/ none (1 day) 1.☐ Sometimes (1-2 days) 2.☐ Often (3-4 days) 3.☐ At all times (5-7 days) **Subjects who chose the answer "2" or "3" for one of questions (a) or (b), had frailty criteria (Fairhall et al., 2008; Radloff, 1977). If so, mark / in the frailty criterion space next to it.**	☐

RESPONDENT CODE

3.	**Muscle weakness:** **Hand grip strength (kg) on the dominant hand using Jamar Hand Dynamometer:** Determinants for grip strength (kg): **Men** / **Women** 0. ☐ ≥ 26kg / 0. ☐ ≥18kg 1. ☐ <26kg / 1. ☐ <18kg **Male or female subjects who had an answer of "1", had frailty criteria (Fairhall et al., 2008; Fried et al., 2001). If so, tick / in the frailty criterion space next to it.**	☐
4.	**Walking speed:** Time period (seconds) taken using a stopwatch to walk 4 meters (13.12 feet): _____ seconds **Subjects with a time period of ≤ 0.8 m/s to walk along 4 meters, had frailty criteria (Fairhall et al., 2008). If so, mark / in the frailty criterion space next to it.**	☐
5.	**Low Activity:** In the past three months, **I did not perform** weight-bearing physical activity (any activity that forces to work against gravity, e.g.: ☐ **Weight training, jogging, climbing stairs, tai chi, yoga, racquet sports, hiking etc.)** **OR** ☐ **Spent >4 hours per day sitting.** ☐ **Went for a short walk ≤1 hour per month.**	☐
	Total Score of Frailty Criteria	**/5**
	Frailty Criteria Score Scale: ≥ 3 *frailty Criteria* = *Frail* 1 – 2 *frailty Criteria* = *Pre-frail* 0 *frailty Criteria* = Normal	

Appendix C.2

Informed Consent Form for the eligible study participants

JAWATANKUASA ETIKA UNIVERSITI UNTUK PENYELIDIKAN MELIBATKAN MANUSIA (JKEUPM)
UNIVERSITI PUTRA MALAYSIA, 43400 UPM SERDANG, SELANGOR, MALAYSIA

BORANG 2.4: HELAIAN MAKLUMAT RESPONDEN DAN BORANG IZIN MAKLUM

Sila baca maklumat berikut dengan teliti dan jangan teragak-agak untuk berbincang dengan penyelidik sekiranya anda ada sebarang soalan.

Nama penyelidik: Ala'a Hussein Al-Rawhani

1. **TAJUK KAJIAN: Keberkesanan suplemen protein ke atas penambahbaikan kelemahan, kekuatan otot dan prestasi fizikal dalam kalangan orang tua pra-lemah di Selangor-Malaysia: sebuah kajian klinikal terawak**

2. PENGENALAN:

Penuaan populasi yang semakin meningkat di seluruh dunia, dengan 2 bilion individu dijangka melebihi umur 65 tahun menjelang 2050 mempunyai kesan yang cukup besar bagi kesihatan dan perancangan dan pengendalian penjagaan sosial (Clegg et al., 2013). Ekspresi penuaan populasi yang paling bermasalah ialah keadaan klinikal bagi kelemahan. Dengan penuaan, terdapat pengurangan tekal dalam rizab fisiologi badan, tetapi dalam kelemahan, penurunan ini dipercepat dan proses imbangan sel dan fungsi mulai gagal (Ferrucci et al.,2002). Bagi mengekang kerapuhan dalam kalangan orang dewasa yang lebih tua, anda terpilih untuk terlibat dalam penyelidikan ini yang bertujuan untuk mengkaji kesan suplemen protein ke atas penambahbaikan kerapuhan, kekuatan otot dan prestasi fizikal.
Perincian penyelidikan percubaan dijelaskan dalam dokumen ini. Adalah penting anda memahami tujuan penyelidikan in dijalankan dan perkara yang terlibat. Sila ambil masa anda untuk membaca maklumat ini dengan teliti sebelum anda berhasrat untuk terlibat. Bertanyalah kepada staf kajian sekiranya ada sebarang perkara yang tidak jelas atau sekiranya anda mahu lebih maklumat. Selepas anda berpuas hati dan memahami kajian ini, dan anda ingin terlibat, anda mesti tandatangan borang izin maklum. Bagi anda yang terlibat dalam kajian ini, anda perlu memberikan maklumat mengenai sejarah kesihatan anda; anda mungkin memudaratkan diri sendiri jika anda tidak jujur dengan maklumat yang diberikan.

Penglibatan anda adalah sukarela. Anda tidak perlu terlibat dalam kajian ini sekiranya anda tidak mahu. Anda juga boleh menolak untuk menjawab sebarang soalan sekiranya anda tidak mahu. Sekiranya anda sukarela untuk menyertai kajian ini, anda boleh menarik diri pada bila-bila masa. Sekiranya anda menarik diri, sebarang data yang dikumpulkan sehingga tempoh anda menarik diri masih akan digunakan bagi kajian ini. Penolakan anda untuk terlibat atau menarik diri tidak akan memberikan kesan pada sebarang manfaat kesihatan atau perubatan yang sepatutnya anda layak.

Kajian ini ditaja oleh Universiti Putra Malaysia (UPM) dan telah diluluskan oleh Jawatankuasa Etika dan Penyelidikan Perubatan, Kementerian Kesihatan Malaysia.

3. APAKAH TUJUAN KAJIAN INI?

Tujuan kajian ini adalah untuk mengkaji kesan suplemen protein ke atas penambahbaikan kerapuhan, kekuatan otot dan prestasi fizikal.

Sejumlah 40 subjek seperti anda dari pelbagai hospital di Selangor, Malaysia akan terlibat dalam kajian ini.

Kajian ini akan mengambil masa selama 3 bulan untuk tamat. Berdasarkan kepada kelayakan penyertaan, penilaian berikutakan dilaksanakan:

a. Sosiodemografik
b. Sejarah keluarga, latar belakang lampau dan keadaan perubatan
c. Karakteristik gaya hidup (merokok, kualiti tidur, aktiviti fizikal, sedentarisme)
d. Penilaian kerapuhan (kehilangan berat badan/ pengecutan, keletihan, kelemahan, kelambatan, kurang aktiviti)
e. Pengukuran antropometrik (ketinggian, berat, indeks jisim tubuh, lilitan trisep pertengahan lengan,ketebalan lilitan kulit, lilitan betis)
f. Analisis komposisi tubuh (jisim lemak, jisim otot, lemak viseral ...dsb.)
g. Prestasi fizikal (seperti ujian keseimbangan, ujian kelajuan gaya jalan).
h. Ujian kekuatan otot (seperti kekuatan genggaman).
i. Pengambilan dietari (3-hari ingat semula dietari).

4. APAKAH YANG PERLU ANDA LAKUKAN?

Anda akan dibahagikan sama ada kepada kumpulan intervensi atau kumpulan kawalan, menggunakan prosedur perawakan mudah. Perawakan tersebut akan berdasarkan kod pratentu yang dijana oleh komputer dan peluang untuk berada dalam kumpulan kawalan atau kumpulan intervensi ialah 50:50.

Sekiranya anda dibahagikan dalam kumpulan intervensi, anda akan menerima suplemen protein wei SUSTINEX secara bulanan selama 3 lawatan ke hospital. Tiada sebarang bayaran akan dikenakan daripada anda semasa percubaan. Suplemen protein ini ditaja dan akan disediakan oleh Syarikat Nova Laboratories. Tidak terdapat bahan lembu atau khinzir dalam suplemen dietari ini. Bagi melengkapi keperluan protein yang diperlukan, ia mengandungi wei protein isolat dan wei protein terhidrolisis. Suplemen ini diambil dua kali setiap hari (30 gram setiap hari) dicampur dengan makanan lembut atau minuman ringan anda dan ini akan mengambil masa selama tiga. Anda juga akan menerima nasihat dietari tentang cara untuk mematuhi diet sihat yang sesuai dengan umur dan keperluan anda. Kumpulan WhatsApp akan dibina bagi menyampaikan maklumat kepada anda mengenai maklumat diet kesihatan setiap minggu sepanjang tempoh kajian dan mengumpulkan rekod dietari.

Sekiranya anda dibahagikan dalam kumpulan kawalan, anda akan masih menerima perubatan standard rutin dan penjagaan nutrisi daripada pihak hospital. Anda tidak perlu mengubah pengambilan dietari anda atau tahap aktiviti fizikal sepanjang tempoh kajian. Anda juga akan menerima nasihat dietari tentang cara untuk mematuhi diet sihat yang sesuai dengan umur dan keperluan anda. Kumpulan WhatsApp akan dibina bagi menyampaikan maklumat mengenai pelbagai maklumat diet sihat setiap minggu sepanjang tempoh kajian dan kumpul rekod dietari.

Sepanjang tempoh kajian, anda dikehendaki menjalani penilaian berikut:
1- Penilaian Keburukan Kesihatan: Ini akan dilakukan menggunakan Fenotip Fried dan akan melibatkan pengukuran kelajuan berjalan, kekuatan cengkaman, kehilangan berat badan, dan tahap aktiviti fizikal anda. Penilaian ini akan mengambil masa kira-kira 10 minit.

2- Prestasi Fizikal: Ini akan dilakukan menggunakan Ujian Bateri Prestasi Fizikal Pendek dan akan melibatkan pengukuran keseimbangan, kelajuan berjalan, dan keupayaan untuk bangun dari kerusi. Penilaian ini akan mengambil masa kira-kira 15 minit.

3- Kekuatan Otot: Ini akan dilakukan menggunakan ujian kekuatan cengkaman tangan dan akan melibatkan pengukuran kekuatan cengkaman tangan anda. Penilaian ini akan mengambil masa kira-kira 5 minit.

4- Rekod Pemakanan: Anda dikehendaki menyimpan rekod pemakanan selama tiga hari, dan ini akan melibatkan mencatat segala yang anda makan dan minum dalam tempoh tersebut.

Adalah penting anda menjawab semua soalan yang diajukan oleh staf kajian sejujur dan selengkapnya. Ujian penilaian akan dilakukan tiga kali (pada awal minggu percubaan 0, pada pertengahan minggu ke-6, dan pada akhir percubaan minggu ke-12). Setiap peserta mengambil masa sekitar 30 minit untuk kesemua penilaian .Sekiranya kondisi dan keadaan berubah semasa kajian, anda mesti memberitahu doktor kajian. Mungkin terdapat ubat-ubat tertentu yang tidak boleh anda makan ketika terlibat dalam kajian ini. Doktor akan berbincang tentang ubat-ubat tersebut dengan anda. Anda tidak harus mengambil ubat-ubat lain tanpa berbincang dengan doktor kajian anda. Anda mesti memberitahu doktor kajian dengan segera sekiranya anda membuat sebarang perubahan kepada rawatan terkini anda, walaupun rawatan tersebut telah lama anda ikuti.

Adalah penting doktor kajian anda diberitahu dengan cepat akan sebarang perubahan pada kesihatan anda semasa penglibatan anda dalam kajian ini. Bagi keselamatan diri anda, adalah penting anda mematuhi arahan doktor kajian di sepanjang tempoh kajian.

Sekiranya terdapat sebarang perubahan, kemas kini atau maklumat baharu yang berkaitan dengan persetujuan ini, pasukan penyelidik akan memaklumkan kepada anda dengan segera.

5. SIAPAKAH YANG TIDAK PATUT TERLIBAT DALAM KAJIAN?

Orang dewasa yang lebih tua yang bukan rapuh, individu didiagnosis dengan penyakit kronik (penyakit berkaitan jantung, COPD, strok, kanser, asma, difungsi renal, sakit yang membawa maut), terlantar sakit di katil, pernah terlibat atau masih terlibat dalam sebarang kajian intervensional kesihatan, partisipan yang mengambil suplemen protein, sebarang keretakan berterusan (pinggul, vertebrata) dalam tempoh enam bulan yang lalu, sebarang pembedahan (pinggul, kawasan abdominal) dalam tempoh enam bulan yang lalu.

6. MANFAAT KAJIAN:

(a) KEPADA ANDA SEBAGAI SUBJEK?

Anda turut serta dalam kajian ini dapat membantu meningkatkan pemahaman mengenai kesan suplemen protein terhadap fungsi fizikal dalam golongan dewasa yang terdedah kepada kekurangan fizikal awal. Anda juga berpeluang untuk mempelajari mengenai diet sihat dan cara ia dapat membantu anda meningkatkan prestasi fizikal dan kekuatan otot anda. Dapatan penyelidikan ini juga akan dikongsi sekiranya anda memerlukannya.

(b) KEPADA PENYELIDIK?

Objektif kajian ini adalah untuk menentukan keberkesanan suplemen protein ke atas pencegahan kerapuhan dalam kalangan orang dewasa yang lebih tua. Dapatan kajian ini amat berguna bagi pencegahan kerapuhan masa hadapan dan bagi profesional penjagaan kesihatan untuk memformulasi pencegahan yang sesuai bagi orang dewasa yang lebih tua.

BORANG IZIN MAKLUM

1. Tajuk Kajian: Keberkesanan suplemen protein ke atas penambahbaikan kerapuhan, kekuatan otot dan prestasi fizikal dalam kalangan orang tua prarapuh di Selangor- Malaysia: sebuah kajian klinikal terawak

Dengan menandatangani di bawah saya mengesahkan perkara berikut:

- Saya telah diberikan maklumat secara oral dan bertulis bagi kajian di atas dan telah membaca dan memahami maklumat yang diberikan.
- Saya mempunyai masa yang mencukupi untuk menimbang penglibatan dalam kajian ini dan mempunyai peluang untuk bertanya soalan dan semua soalan saya telah dijawab dengan memuaskan.
- Saya memahami penglibatan saya adalah sukarela dan pada bila-bila masa saya boleh menarik diri dari kajian ini tanpa memberi sebab dan ini tidak akan memberi kesan pada rawatan masa hadapan saya. Saya tidak mengambil bahagian dalam sebarang kajian lain pada masa ini. Saya memahami risiko dan manfaat, dan saya dengan rela hati memberikan izin maklum bagi terlibat di bawah syarat yang telah dinyatakan. Saya memahami bahawa saya mesti mematuhi arahan doktor kajian (penyelidik) berkaitan dengan penglibatan saya dalam kajian ini.
- Saya memahami bahawa staf kajian, monitor dan auditor yang berkelayakan, penaja atau ahli gabungannya, dan kerajaan atau autoriti kawal selia, mempunyai akses langsung kepada rekod perubatan saya bagi memastikan kajian ini dijalankan dengan sewajarnya dan data direkod dengan betul. Semua maklumat peribadi akan diperlakukan sebagai SANGAT SULIT
- Saya akan menerima salinan maklumat subjek/borang izin maklum yang ditandatangani dan bertarikh untuk simpanan.

Subjek:

Tandatangan: Nombor Kad Pengenalan:

Nama: Tarikh:

Izin maklum pelaksana penyelidik:

Tandatangan: Nombor Kad Pengenalan:

Nama: Tarikh:

Saksi Saksama :

Tandatangan: Nombor Kad Pengenalan:

Nama: Tarikh:

Appendix C.3

The Assessment Booklet for the Study Participants in the Three Visits

No.	Section	Tick
1	A	
2	B	
3	C	
4	D	

Faculty of Medicine and Health Sciences

Assessment Phase

RESEARCH TOPIC:

EFFECTIVENESS OF PROTEIN SUPPLEMENTATION ON FRAILTY IMPROVEMENT, MUSCLE STRENGTH AND PHYSICAL PERFORMANCE AMONG PRE-FRAIL ELDERLY PEOPLE IN SELANGOR- MALAYSIA: A RANDOMIZED CLINICAL STUDY

RESEARCHER:

ALAA HUSSEIN NASSER ALRAWHANI

PROJECT SUPERVISOR:

ASSOCIATE PROFESSOR DR. SITI NUR 'ASYURA BINTI ADZNAM

DR. ZALINA ABU ZAID (UPM)

DR. NOR BAIZURA MD YUSOP (UPM)

DR HAKIMAH MOHAMMAD SALLEHUDDIN (UPM)

MR. MUHAMAD ARIFF ABD RAHMAN (HPUPM)

MS. JAZLINA SYAHRUL (HPUPM)

DATE: | | | | | | | | |

RESPONDENT CODE: | | | | | |

RESPONDENT CODE

This questionnaire contains **THREE** sections, namely section A (respondent's background), section B (respondents health status), section C (frailty status).

PART A: Respondents Background
INSTRUCTIONS: Please tick (/) the appropriate answer

		Note
1.	**GENDER** 1. ☐ Male 2. ☐ Female	
2.	**Age:**	
3.	**RACE:** 1. ☐ Malay 2. ☐ China 3. ☐ India 4. ☐ Other:	
4.	**MARRIAGE STATUS:** 1. ☐ Single 2. ☐ Married 3. ☐ Divorced 4. ☐ Widowed	
5.	**EDUCATION STATUS:** 1. ☐ Never went to school 2. ☐ Religious school/ lodge 3. ☐ Primary school 4. ☐ Lower secondary school 5. ☐ Upper secondary school 6. ☐ Certificate / college/ university	
6.	**HOMESTAY STATUS:** 1. ☐ Alone 2. ☐ With husband/ wife 3. ☐ With husband/ wife and siblings 4. ☐ With siblings 5. ☐ Others (please specify: _____)	

RESPONDENT CODE

7.	**WORK STATUS:** 1. ☐ Not working 2. ☐ Housewives 3. ☐ Retiree (please state previous occupation _____) 4. ☐ Retired but still working (please specify current occupation _____) 5. ☐ Work (please specify _____)	
8.	**FINANCIAL RESOURCES FOR LIVING (Can mark more than 1)** 1. ☐ Salary 2. ☐ Pension 3. ☐ Husband/Wife 4. ☐ Siblings 5. ☐ Grandsons 6. ☐ Other: _____	
9.	**TOTAL HOUSEHOLD INCOME** RM: _____	
10.	**Life Style Habits:** **Do you have one of these habits: (can be more than one)** ☐ **Smoking** ☐ **Drinking Alcohol** ☐ **Other please specify……..**	

RESPONDENT CODE

PART B: HEALTH STATUS SCREENING
INSTRUCTIONS: Please tick (/) the appropriate answer

		Note
PAST MEDICAL HISTORY:		
1.	**Please circle each condition that you have been told you have (or had):** ☐Cancer ☐Diabetes ☐Kidney Disease ☐Liver Disease ☐Stroke ☐High Blood Pressur ☐Heart Disease ☐Angina/Chest Pain ☐COPD ☐Ulcers ☐ Fibromyalgia ☐Osteoporosis ☐Osteoarthritis ☐Rheumatoid Arthritis ☐Sexually Transmitted Disease ☐Allergies/Asthma ☐ Lung disease ☐Other: ……	
2.	**Do you have or have you had a recent illness or infection (explain if yes)?** ……..	
3.	**During the past month, have you often been bothered by feeling down, depressed, or hopeless?** ☐ **YES** ☐ **NO**	
CURRENT HEALTH STATUS		
4.	**I am currently experiencing:** ☐Fever/chills/sweats ☐Poor balance (falls) Anxiety ☐ Headaches ☐Unexplained weight loss ☐Numbness or Tingling ☐Changes in appetite ☐Difficulty swallowing ☐Depression ☐Shortness of breath ☐Dizziness ☐Changes in bowel or bladder function ☐Nausea /Vomiting ☐Increased pain at night	
5.	**SURGERIES:** Type of surgery and specific date or your age at surgery: _____	

RESPONDENT CODE

6.	**HOSPITALIZATIONS:** List hospitalizations, including dates of and reasons for hospitalization: _____ _____	
7.	**MEDICATIONS:** List any prescription medications (with dosage and frequency of use) you are now taking: _____ _____	
8.	**ALLERGIES:** Are you allergic to any substance of food or medicine? ☐ YES ☐ NO if YES specify please:	
9.	**Fractures:** Do you have any sustained fracture (hip, vertebrata) in past six months: ☐ YES ☐ NO if YES specify please: ..... **Have you applied any surgery (hip, abdominal area) in past six months:** ☐ YES ☐ NO if YES specify please:	

RESPONDENT CODE

ASSESSMENT FORM

FIRST VISIT SECOND VISIT THIRD VISIT

DATE:

RESPONDENT CODE:

Part A: Anthropometric Measurements

	First Read	Second Read
Body weight		
Height		
Mid arm circumference		
Triceps skinfold thickness		
Total body fat		
Skeletal Muscle mass		
Calf circumference		
Leg Lean Mass		

RESPONDENT CODE

PART B: FRAILTY STATUS SCREENING:

		Point
1.	**Weight loss / weight loss:** (a) During the past 6 months, was your BMI less than 18.5 kg/m2? 0.□ No. 1.□ Yes (b) During the past 6 months, have you experienced unplanned weight loss (not due to diet or exercise) of more than 10 pounds (4.5kg)? 0.□ No. 1.□ Yes **Subjects who chose the answer "1" for one of questions (a) or (b), had frailty criteria.** **If so, mark / in the frailty criterion space next to it.** **(Ng et al., 2015).**	
2.	**Fatigue:** **During the past week, how often did feel the following?** (a) Feeling of difficulty when wanting to do something 0.□ Rarely/ none (1 day) 1.□ Sometimes (1-2 days) 2.□ Often (3-4 days) 3.□ At all times (5-7 days) (b) Having trouble getting on with life 0.□ Rarely/ none (1 day) 1.□ Sometimes (1-2 days) 2.□ Often (3-4 days) 3.□ At all times (5-7 days) **Subjects who chose the answer "2" or "3" for one of questions (a) or (b), had frailty criteria (Fairhall et al., 2008; Radloff, 1977). If so, mark / in the frailty criterion space next to it.**	
3.	**Muscle weakness:**	

RESPONDENT CODE

	Hand grip strength (kg) on the dominant hand using Jamar Hand Dynamometer: Determinants for grip strength (kg): **Men** **Women** 0. ☐ ≥ 26kg 0. ☐≥18kg 1. ☐ <26kg 1. ☐<18kg **Male or female subjects who had an answer of "1", had frailty criteria (Fairhall et al., 2008; Fried et al., 2001). If so, tick / in the frailty criterion space next to it.**	☐
4.	**Walking speed:** Time period (seconds) taken using a stopwatch to walk 4 meters (13.12 feet): _____ seconds **Subjects with a time period of ≤ 0.8 m/s to walk along 4 meters, had frailty criteria (Fairhall et al., 2008). If so, mark / in the frailty criterion space next to it.**	☐
5.	**Low Activity:** **In the past three months, I did not perform weight-bearing physical activity (any activity that forces to work against gravity, e.g.:** ☐ **Weight training, jogging, climbing stairs, tai chi, yoga, racquet sports, hiking etc.)** **OR** ☐ **Spent >4 hours per day sitting.** ☐ **Went for a short walk ≤1 hour per month** **(Cesari et al., 2006; Fairhall et al., 2008).**	☐
	Total Score of Frailty Criteria	**/5**
Frailty Criteria Score Scale: ≥ 3 *frailty Criteria* = *Frail* 1 – 2 *frailty Criteria* = *Pre-frail* 0 *frailty Criteria* = Normal		

RESPONDENT CODE

PART C: PHYSICAL PERFORMANCE TEST

SHORT PHYSICAL PERFORMANCE BATTERY PROTOCOL AND SCORE SHEET

All of the tests should be performed in the same order as they are presented in this protocol

1.	**BALANCE TESTS** If participant did not attempt test or failed, circle why: Tried but unable _____ 1 Participant could not hold position unassisted _____ 2 Not attempted, you felt unsafe _____3 Not attempted, participant felt unsafe_____ 4 Participant unable to understand instructions _____5 Other (specify) _____ 6 Participant refused _____7	
	A. Side-by-Side Stand Held for 10 sec ❒ 1 point Not held for 10 sec ❒ 0 points Not attempted ❒ 0 points Number of seconds held if less than 10 sec: …… sec If 0 points, end Balance Tests	
	B. Semi-Tandem Stand Held for 10 sec ❒ 1 point Not held for 10 sec ❒ 0 points Not attempted ❒ 0 points Number of seconds held if less than 10 sec: …… sec If 0 points, end Balance Tests	
	C. Tandem Stand Held for 10 sec ❒ 1 point Not held for 10 sec ❒ 0 points Not attempted ❒ 0 points Number of seconds held if less than 10 sec: …… sec If 0 points, end Balance Tests	

RESPONDENT CODE

2.	**GAIT SPEED TEST** Length of walk test course: Four meters ❒ Three meters ❒ What is the time for the faster of the two walks? Record the shorter of the two times _ _. _ _ sec [If only 1 walk done, record that time] _ _. _ _ sec If the participant was unable to do the walk: ❒ 0 points **For 4-Meter Walk:** If time is more than 8.70 sec: ❒ 1 point If time is 6.21 to 8.70 sec: ❒ 2 points If time is 4.82 to 6.20 sec: ❒ 3 points If time is less than 4.82 sec: ❒ 4 points **For 3-Meter Walk:** If time is more than 6.52 sec: ❒ 1 point If time is 4.66 to 6.52 sec: ❒ 2 points If time is 3.62 to 4.65 sec: ❒ 3 points If time is less than 3.62 sec: ❒ 4 points	
	A. First Gait Speed Test 1. Time for 3 or 4 meters _ _. _ _ sec 2. If participant did not attempt test or failed, circle why: Tried but unable 1 Participant could not walk unassisted 2 Not attempted, you felt unsafe 3 Not attempted, participant felt unsafe 4 Participant unable to understand instructions 5 Other (Specify) 6 Participant refused 7 Complete score sheet and go to chair stand test 3. Aids for first walk……None ❒ Cane ❒ Other ❒	

RESPONDENT CODE

	B. Second Gait Speed Test 1. Time for 3 or 4 meters _ _. _ _ sec 2. If participant did not attempt test or failed, circle why: Tried but unable 1 Participant could not walk unassisted 2 Not attempted, you felt unsafe 3 Not attempted, participant felt unsafe 4 Participant unable to understand instructions 5 Other (Specify) 6 Participant refused 7 Complete score sheet and go to chair stand test 3. Aids for first walk……None ❒ Cane ❒ Other ❒	
3.	**CHAIR STAND TEST** Scoring the Repeated Chair Test Participant unable to complete 5 chair stands or completes stands in >60 sec: ❒ 0 points If chair stand time is 16.70 sec or more: ❒ 1 points If chair stand time is 13.70 to 16.69 sec: ❒ 2 points If chair stand time is 11.20 to 13.69 sec: ❒ 3 points If chair stand time is 11.19 sec or less: ❒ 4 points	
	A. Single Chair Stand A. Safe to stand without help Yes ❒ No ❒ B. Results: Participant stood without using arms ❒ → Go to Repeated Chair Stand Test Participant used arms to stand ❒ → End test; score as 0 points Test not completed ❒ → End test; score as 0 points C. If participant did not attempt test or failed, circle why: Tried but unable 1 Participant could not stand unassisted 2 Not attempted, you felt unsafe 3	

RESPONDENT CODE

	Not attempted, participant felt unsafe 4 Participant unable to understand instructions 5 Other (Specify) 6 Participant refused 7	
	B. Repeated Chair Stands A. Safe to stand without help Yes ❒ No ❒ B. If five stands done successfully, record time in seconds. Time to complete five stands _ _. _ _ sec C. If participant did not attempt test or failed, circle why: Tried but unable 1 Participant could not stand unassisted 2 Not attempted, you felt unsafe 3 Not attempted, participant felt unsafe 4 Participant unable to understand instructions 5 Other (Specify) 6 Participant refused 7 Scoring the Repeated Chair Test Participant unable to complete 5 chair stands or completes stands in >60 sec: ❒ 0 points If chair stand time is 16.70 sec or more: ❒ 1 points If chair stand time is 13.70 to 16.69 sec: ❒ 2 points If chair stand time is 11.20 to 13.69 sec: ❒ 3 points If chair stand time is 11.19 sec or less: ❒ 4 points	
	Test Scores **Total Balance Test score _____ points** **Gait Speed Test score _____ points** **Chair Stand Test score _____ points** **Total Score _____ points (sum of points above)**	

Compliance Log for Twice Daily Consumption

Date: _____

Subject ID: _____

Group: A [ ] / B [ ]

Catatan:

1- Sila pastikan untuk mengisi log harian untuk mengikuti kepatuhan anda terhadap ujian.
2- Jika anda **terlepas** dos pagi, anda boleh mengambilnya pada waktu tengah hari atau anda boleh mengambil dua sudu pada waktu petang.
3- Jangan terdedah kepada **sinar matahari atau udara**. Ia lebih baik untuk menyimpan supplement di dalam almari dapur anda.
4- Sila pastikan anda mengukur dos dengan tepat (penuhkan sudu dan sapukan untuk mendapatkan 15g setiap dos).
5- Anda boleh mencampurkan supplement dengan air atau minuman apa-apa sahaja. Air panas tidak digalakkan.
6- **Jangan lupa untuk membawa** log ini bersama anda dalam lawatan anda yang seterusnya.

Notes:

1- It is important to complete the daily log to track your adherence to the trial.
2- If you **miss** taking the morning dose, you have the option to take it in the afternoon or take two spoons in the evening instead.
3- Avoid exposing the supplement to **sunlight or air**. It is recommended to store it in your kitchen cabinet.
4- Please ensure accurate measurement of the dose (fill the spoon and level it to obtain 15g per dose).
5- The supplement can be mixed with water or any beverage, but hot water should be avoided.
6- **Remember to bring** this log with you to your next visit.

Appendix C.3

Compliance Log Book for the Participants

Week __/__	Day	Morning Dose	Evening Dose	Total Daily Dose	Week __/__	Day	Morning Dose	Evening Dose	Total Daily Dose
1	1				2	8			
	2					9			
	3					10			
	4					11			
	5					12			
	6					13			
	7					14			
3	15				4	22			
	16					23			
	17					24			
	18					25			
	19					26			
	20					27			
	21					28			
5	29				6	36			
	30					37			
	31					38			
	32					39			
	33					40			
	34					41			
	35					42			
Congratulation! We completed week 6. Please Don't forget to bring this log with you tomorrow.									

Week __/__	Day	Morning Dose	Evening Dose	Total Daily Dose	Week __/__	Day	Morning Dose	Evening Dose	Total Daily Dose
7	43				8	50			
	44					51			
	45					52			
	46					53			
	47					54			
	48					55			
	49					56			
9	57				10	64			
	58					65			
	59					66			
	60					67			
	61					68			
	62					69			
	63					70			
11	71				12	78			
	72					79			
	73					80			
	74					81			
	75					82			
	76					83			
	77					84			
Congratulation! We completed the whole trial!! Please Don't forget to bring this log with you tomorrow.									

RESPONDENT CODE

3-Day Food Intake Record

Sila berikan kami gambaran tentang corak pemakanan biasa anda. Sila catatkan segala MAKANAN dan **MINUMAN** yang anda **MAKAN** selama **3 hari (2 hari biasa dan 1 hari hujung minggu)**. Termasuk semua makanan ringan dan minuman, serta waktu makan atau minum anda. Adalah penting bahawa anda tidak mengubah corak pemakanan biasa anda pada hari-hari ini. Sila cuba untuk menjadi setepat yang mungkin dengan mencatat semua makanan dan minuman yang anda makan dan minum pada hari yang dipilih. Sila lengkapkan laporan ini 3 hari sebelum sesi pertemuan di klinik dietetik. Kerjasama anda amat dihargai.

Please provide us with an idea of your usual pattern of eating. Please keep a record of everything you **EAT** and **DRINK** for **3 days – 2 weekdays and 1 weekend day**. Include all meals, snacks, and beverages, and the time of day you are eating or drinking. It is important that you do not change your usual eating pattern on these days. Please try to be as accurate as possible by recording all of the foods and beverages you eat and drink on the selected days. Please fill this report 3 days before the assessment visit in the dietetics clinic. Your cooperation is highly appreciated.

Panduan/Directions:

1. Catat waktu makan/minum pada hari tersebut. /Record the time when you eat/drink in the day.

2. Catat SEMUA makanan, minuman dan perasa tambahan yang anda ambil. /Record ALL foods, beverages and condiments you consume.

3. Kirakan jumlah/porsi (contoh. oz., cawan, sdm., tsp., 1 porsi) yang anda ambil dari setiap makanan dengan merujuk gambar di bawah. /Estimate the amount/servings (e.g. oz., cup, Tbsp., tsp., 1 serving) you consume of each food by refer the picture below.

1 cawan/cup = 250mL = 8 fluid oz

1 sudu makan /tablespoon (Tbsp) = 15g

1 ons/ounce (oz) = 30g

1 sudu teh/teaspoon (tsp) = 5g

4. Catat di mana makanan tersebut diambil. /Record where the food was consumed.

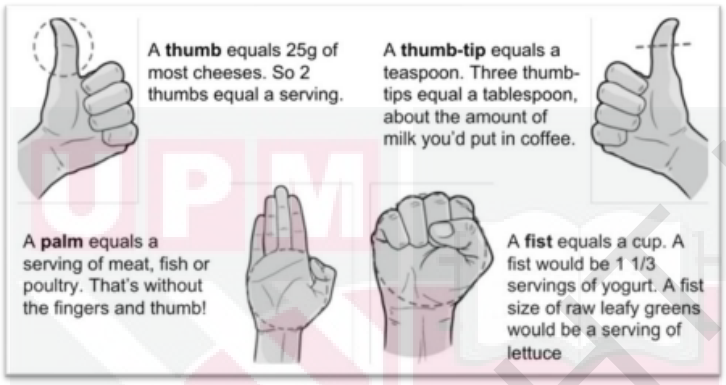
5. Untuk HIDANGAN KOMBINASI seperti Lasagna, Nasi Lemak, Sambal atau sup sertakan deskripsi bahan utama. /For COMBINATION DISHES such as Lasagna, Nasi Lemak, Sambal, or soups include a description of the main ingredients.

6. Sertakan MAKANAN RINGAN yang telah makan. Gunakan ruangan "catatan" untuk mencatat sebarang INFORMASI TAMBAHAN PRODUK. /Include SNACK FOODS eaten. Use the "notes" column to record any additional PRODUCT INFORMATION

Appendix C.4

3-day Diet Record of the study

RESPONDENT CODE



Example:

Date: 3/15/2015		**Day of the week: Sunday**		**Work Day: Yes/No**
Time	**Meal/Snack**	**Food**	**Estimated Amount/ Servings**	**Location/Activity**
7:30 am	Breakfast	Tuna, Egg, Cheese Sandwich on WhiteBread Orange Juice	2 slices white bread 2Tbsp tuna 1 slice cheddar cheese 1cup (8oz)	Home, sitting on couch watching morning news
10:00 Am	Snack	Blueberry Muffin	½ large muffin	Office meeting
1:00 pm	Lunch	Salad (lettuce, tomato, cucumber, cheddar cheese, croutons,ranch dressing) Diet Coke	2 cups lettuce ¼ cup tomato ¼ cup cucumber ¼ cup cheddar cheese ¼ cup croutons 2 Tbsp. ranch dressing 16oz diet coke	Ruby Tuesdays with coworkers
2:00 pm	Snack	Vanilla birthday cake with buttercream icing	3 in square	Office break room, with coworkers
5:00 pm	Snack	Apple	1 medium	Car, driving home from work
7:00 pm	Dinner	Baked chicken breast without skin Green beans Mashed potatoes with butter Water	4oz chicken breast ½ cup green beans ½ cup mashed potatoes 1 Tbsp. butter 2 cups (16oz)	Kitchen table, with family
9:00 pm	Snack	Popcorn	1 handful	Watching TV, with family

RESPONDENT CODE

Day 1 Food/Beverage Intake:

Tarikh/*Date:*		Hari/*Day:*		
Masa/ ***Time***	**Waktu makan/** ***Type of meals***	**Makanan/** ***Food***	**Jumlah/ Sajian** ***Amount/ Servings***	**Lokasi/Aktiviti** ***Location/ Activity***

RESPONDENT CODE

Day 2 Food/Beverage Intake:

Date:		Day of the week:		Work Day: Yes/No
Time	**Meal/Snack**	**Food**	**Amount/ Servings**	**Location/Activity**

RESPONDENT CODE

Day 3 Food/Beverage Intake:

Date:		Day of the week:		Work Day: Yes/No
Time	**Meal/Snack**	**Food**	**Amount/ Servings**	**Location/Activity**

Appendix C.5

The Statement of the Remuneration Receipt by the Participants

AKUAN PENERIMAAN WANG

NAMA PROJEK : ***Keberkesanan suplemen protein ke atas penambahbaikan keuzuran, kekuatan otot dan prestasi fizikal dalam kalangan orang tua pra-lemah di Selangor-Malaysia: sebuah kajian klinikal terawak***

Saya seperti nama dan nombor kad pengenalan di bawah mengaku telah menerima wang sejumlah _____ sebagai _____ bagi sesi penilaian kesihatan untuk tujuan program 'Frailty Improvement Through Protein Suppelementation (FIPS)' dalam kalangan warga emas.

Berikut adalah perincian butiran pembayaran gaji yang diterima:

RM _____/sesi x _____ sesi = RM _____

NAMA PENERIMA :

NO K/P :

TARIKH :

Tandatangan penerima:

_____

Nama:

Tarikh:

Tandatangan Ketua Projek:

_____

Nama:

Tarikh: