Muscle strength and muscle mass in older adults: A focus on protein intake, distribution, and sources

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Abstract

Background: Ageing and obesity, which impair muscle protein synthesis (MPS), are associated with muscle mass and muscle strength loss in older adults. It is recognised that adequate protein intake, distribution and sources contribute to increased MPS and muscle mass in older adults. However, little is known about protein intake, distribution, and sources in New Zealand (NZ) older adults. Furthermore, it is unclear whether dietary protein influences muscle strength.

Objectives: This thesis explored muscle strength, muscle mass and dietary protein intake, distribution and sources in community-dwelling older adults living in NZ. To meet this objective, the role of obesity in the relationship between muscle mass and muscle strength was examined. This was followed by an investigation of protein intake, distribution and sources, and their association with muscle strength.

Methods: Data were obtained from the Researching Eating Activity and Cognitive Health (REACH) study, a cross-sectional study aimed at investigating dietary patterns and associations with cognitive function and metabolic syndrome in older adults aged 65 to 74 years. Isometric grip strength was measured using a hand grip strength dynamometer (JAMAR HAND). Body fat percentage and appendicular skeletal muscle mass (ASM) (sum of lean mass in the arms and legs) were assessed using dual-energy X-ray absorptiometry (Hologic, QDR Discovery A). The ASM index was calculated by ASM (kilograms, kg) divided by height (meters, m) squared. Dietary intake was collected using a 4-day food record, and the data was entered into FoodWorks 10. Data on absolute daily protein intake (grams, g) were generated. According to the peaks of protein consumption throughout the day, days were divided into three meals: breakfast, mid-day, and the evening meals. Protein sources were classified as meat and fish; plant; or dairy and egg protein sources based on the primary type of protein found in food. The relative protein intakes (g/kg) per day, meal, and source were calculated by dividing the absolute protein intake (g) by each participant's body weight (kg).

Statistical analyses: A linear regression analysis was performed to determine the association between muscle mass and muscle strength. This analysis was conducted on males and females based on obesity classifications using body fat percentage (obesity ≥ 30% males, ≥ 40% females). The relative protein intake was compared against a cut-off value of 1.2 g of protein per kg body weight (g/kg BW) per day. The distribution of protein across the three meals was expressed as the coefficient of variance (CV), the average of total protein intake per main meal and the number of meals exceeding 0.4 g/kg BW of protein across the day. Sources of protein intake were assessed at breakfast, mid-day and the evening meals. Results are presented as a percentage of the total protein intake for each meal. Finally, linear regression analyses were conducted separately in males and females to investigate the relationships between BMI-muscle strength and protein intake, distribution and sources, accounting for relevant confounders.

Results: Muscle mass was a significant predictor of muscle strength in non-obese participants. However, in participants with obesity, muscle mass was no longer a significant predictor of muscle strength.

More than half of the participants had a protein intake of < 1.2 g/kg BW per day (62% females, 57% males). Protein intake was unevenly distributed throughout the day (CV = 0.48 for males and females) and was inadequate for reaching 0.4 g/kg BW at breakfast (for both males and females) and at the mid-day meal for males. The main sources of protein at breakfast were milk (28%), breakfast cereals (22%), and bread (12%); at the mid-day meal, bread (18%), cheese (10%) and milk (9%); and at the evening meal, meat provided over half the protein (56%).

In females, relative protein intake was positively associated with muscle strength adjusted BMI (BMI-muscle strength) ($r^2 = 0.15$, $\rho < 0.01$). Protein derived from either dairy and egg ($\rho = 0.03$); and plant sources ($\rho < 0.01$) was related to BMI-muscle strength but not protein from meat and fish ($\rho = 0.55$). Greater frequency of protein consumption of at least 0.4 g/kg BW per meal was associated with BMI-muscle strength ($\rho = 0.01$), but the coefficient of variance for protein intake distribution was not related to BMI-muscle strength ($\rho = 0.47$).

There was no relationship between BMI-muscle strength and total daily protein intake, protein from meat and fish; dairy and egg; and plant-based sources, or distribution defined as frequency of protein consumption of at least 0.4 g/kg BW per meal or CV in male older adults.

Conclusions: Obesity should be considered when measuring associations between muscle mass and muscle strength in older adults. A higher BMI-adjusted muscle strength was associated with consuming more protein each day and a higher frequency of consumption of a meal containing at least 0.4 g/kg BW; and from dairy and egg; and plant food sources in female older adults. There was no correlation between protein intake, distribution and sources and muscle strength in males. Protein intake was less than 1.2 g/kg BW per day and 0.4 g/kg BW per meal for a large proportion of older adults. At breakfast and the mid-day meals the main sources of protein were from cereals and dairy products, and from meat sources at the evening meal. Further research is needed to investigate how best to optimise protein intake to increase and maintain muscle mass and muscle strength in older adults from the general population.

Acknowledgements

This research was conducted with the support of several people. First and foremost, I would like to thank my supervisors at Massey University's School of Sport, Exercise and Nutrition: Associate Professor Kathryn Beck, who seeded the vision for this thesis and provided substantial content and professional guidance throughout its development; Associate Professor Cathryn Conlon, who reviewed and advised on the thesis's content; and Professor Pamela von Hurst, who provided guidance and feedback throughout this process. My supervisors' expert advice, scientific ideas, and mentorship have been invaluable throughout this process.

Several people contributed to the timely and effective completion of the Researching Eating Activity and Cognitive Health (REACH) study. The entire REACH research team deserves special recognition, especially Owen Mugridge and Cassie Slade for managing participant recruitment and data collection; and Karen Mumme, Cherise Pendergrast, Kimberley Brown, Harriet Guy, Angela Yu, and Nicola Gillies for assisting with data collection and data entry.

I would like to recognise all the volunteers who have participated in the REACH study for their dedication and commitment to the research.

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List of abbreviations

ACC	Accident Compensation Corporation
ANZSSFR	Australian and New Zealand Society for Sarcopenia and Frailty Research
AA	Amino acid
ASM	Appendicular skeletal muscle mass
ALM	Appendicular lean mass
BW	Body weight
BMI	Body mass index
BIA	Bioelectrical impedance analysis
CSA	Cross-sectional area
CT	Computed tomography
DXA	Dual-energy X-ray absorptiometry
EAR	Estimated average requirement
EFSA	European Food Safety Authority
EWGSOP	European Working Group on Sarcopenia in Older People
FFM	Fat-free mass
FFQ	Food frequency questionnaire
HGS	Handgrip strength
IAAO	Indicator amino acid oxidation
IDAA	Indispensable dietary amino acid
IQS	Isokinetic quadriceps strength
LM	Lean mass
LBM	Lean body mass
MAMA	Midarm muscle area
MeSH	Medical subject headings
MPS	Muscle protein synthesis
MPB	Muscle protein breakdown
MRI	Magnetic resonance imaging
NHANES	National Health and Nutrition Examination Survey

NZ	New Zealand
RDI	Recommended daily intake
RDA	Recommended daily allowance
REACH	Researching Eating Activity and Cognitive Health
RNI	Recommended Nutrient Intake
SMM	Skeletal muscle mass
UK	United Kingdom
US	United States
USDA	United States Department of Agriculture
WHO	World Health Organisation

List of manuscripts and conference presentations

This is a thesis with publications. Three publications were written as well as oral and poster conference presentations.

Published manuscript	
Paper I Incorporated as chapter 3	Hiol, A. N., von Hurst, P. R., Conlon, C. A., Mugridge, O., & Beck, K. L. (2021). Body composition associations with muscle strength in older adults living in Auckland, New Zealand. *PloS ONE 16*(5): e0250439. https://doi.org/10.1371/journal.pone.0250439.
Manuscript under review	
Paper II Incorporated as chapter 4	Hiol, A. N., von Hurst, P. R., Conlon, C. A., Mumme, K. D., & Beck, K. L. (2023). Protein intake, distribution, and sources in community-dwelling older adults living in Auckland, New Zealand. *Nutrition and Healthy Aging*.
Manuscript under review	
Paper III Incorporated as chapter 5	Hiol, A. N., von Hurst, P. R., Conlon, C. A., & Beck, K. L. (2023). The association between muscle strength and protein intake, sources, and distribution in older adults living in Auckland, New Zealand. Submitted to *Journal of Nutritional Science.*
Published oral presentations	
Abstract I	Hiol, A. N., von Hurst, P. R., Conlon, C. A., & Beck, K. L. (2019). Body composition and associations with muscle strength in older adults living in Auckland, New Zealand. *Proceedings*, 37(1), 51. https://doi.org/10.3390/proceedings2019037051.
Abstract II	Hiol, A. N., von Hurst, P. R., Conlon, C. A., de Seymour, J. V., & Beck, K. L. (2020). Prevalence of low muscle mass, muscle strength and upper-body muscle quality in community-living older adults living in Auckland, New Zealand. Symposia. *The Journal of Frailty & Aging* 9 (Suppl 1), 1–45. https://doi.org/10.14283/jfa.2020.8.
Published poster presentation	
Abstract III	Hiol, A. N., von Hurst, P. R., Conlon, C. A., & Beck, K. L. (2022). Protein sources across the day in community-dwelling older adults living in Auckland, New Zealand. *Medical Sciences Forum*, 9, 40. https://doi.org/10.3390/msf2022009040.

Researchers' contributions

This research was completed as part of the Researching Eating Activity and Cognitive Health (REACH) study – a cross-sectional study in 371 community-dwelling older adults aged 65 to 74 years living independently. The primary outcome of the REACH study was to investigate dietary patterns and associations with cognitive function and metabolic health in community-dwelling older adults. Participants completed a health and demographics questionnaire, a food frequency questionnaire, a food record over 4 days and an International Physical Activity Questionnaire. Anthropometric measurements including weight, height, waist and hip circumference were performed. Blood pressure was measured, and a fasting blood sample taken for lipid profile, glucose, Haemoglobin A1c, metabolomics and genotyping. Muscle strength was measured using handgrip strength. Dual-energy X-ray absorptiometry (DXA) was used to determine body composition. The Montreal Cognitive Assessment (MoCA) and the Computerised Mental Performance Assessment System were used to assess cognitive function. The American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement criteria were used to determine the incidence of metabolic syndrome (Grundy et al., 2005).

The REACH study was funded by a Health Research Council of New Zealand Emerging Researcher Grant 17/566 - Beck: Optimising cognitive function: the role of dietary and lifestyle patterns. Associate Professor Kathryn Beck, Associate Professor Cathryn Conlon, and Professor Pamela von Hurst conceptualised, designed and conducted the REACH study, alongside the wider REACH study team. Several people assisted with the REACH study, including Owen Mugridge and Cassie Slade who managed the recruitment of participants and data collection; Cherise Pendergrast, Kimberley Brown, Harriet Guy, Angela Yu, and Nicola Gillies who assisted with data collection and data entry; and Karen Mumme who was responsible for data management. The PhD candidate undertook an internship at Massey University from January to June 2018. During that time and during her PhD candidature, she worked full time on REACH study preparation and data collection in relation to the research objectives. The PhD candidate's key responsibilities and roles are outlined below.

Task	Task details	Specific activities undertaken by the PhD candidate
Massey University doctoral scholarship application	Defined the research topic	Reviewed the literature on muscle strength, muscle mass and dietary protein to design the research proposal
	Stated the problem	
	Literature review	
	Methodology and research design	
Study preparation	Procedures for measuring muscle strength, height, and weight	Developed a standard operating procedure for measurement of handgrip strength
		Generated a standard operating procedure for measurement of height and weight
	Food diary instructions	Involved in the development of a food diary instructions for participants to complete food diaries
Data collection	Muscle strength measurements	Undertook handgrip strength and anthropometric measurements on participants
	Anthropometric measurements	
	Health and demographic questionnaire	Assisted participants with completing health and demographic questionnaire
	Blood processing	Processed blood as part of REACH study wider objectives

Data handling and analysis	Food diary data processing	Entered food record data using FoodWorks 10 – this included creating and adding new recipes of dishes and food to the database
		Sorted database by timing of intake and sources of protein consumed: each participant provided a detailed description of all food and beverages consumed (food, beverage, name, brand, variety, and preparation methods), portion sizes, and the timing of intake over 4 days-period. There were over 50,000 lines of food and beverages lines in the database
	Handgrip strength, anthropometric, health and demographic data processing	Developed a data cleaning procedure to ensure the removal of duplicate, incorrect, or incomplete data from the dataset. Formatted, organised, and/or combined data sets into single Excel spreadsheets for statistical analysis. Developed a data transformation procedure that included coding, definitions, and assumptions made regarding the data
	Statistical analysis	Undertook all statistical analysis to complete the thesis with guidance from supervisors
Writing of thesis	Introduction, literature review, manuscripts 1, 2, and 3; discussion and conclusions	Interpreted results and wrote all of the thesis components. See appendix A, B and C for contribution forms

Chapter One – Introduction

This chapter outlines the scope and justification for this thesis by introducing the significance of muscle strength, muscle mass and dietary protein in the context of an aging population with a high risk of falls and injuries. Following this, the aims and objectives are provided along with the thesis outline.

1.1. Introduction

Both globally and in New Zealand (NZ) the proportion of older adults is increasing (Wan He et al., 2016). In 2020, 16% of NZ's population was aged 65 or older (Statistics New Zealand, 2020). This percentage is expected to increase to more than 21% by the year 2048 (Crothers, 2021). With an increasing older population, there is a high proportion of people experiencing falls and fall-related injuries (Health Quality & Safety Commission, 2016; Peel, 2011; Rubenstein & Josephson, 2002). Low muscle strength is an emerging global health concern because it is the leading causes of falls in older adults (American Geriatrics Society et al., 2001; Moreland et al., 2020; Rubenstein, 2006). However, little is known about predictors of muscle strength among NZ's older adults.

Cross-sectional studies have demonstrated that muscle mass is correlated with muscle strength (Barbat-Artigas, Plouffe, et al., 2013; Beliaeff et al., 2008; Chen et al., 2013; Hayashida et al., 2014; Reed et al., 1991). Furthermore, longitudinal studies investigating changes in muscle strength and muscle mass in older adults have shown that loss of muscle mass is a major factor in the decline in muscle strength with age (Frontera et al., 2000; Newman et al., 2003). However, there is also evidence that changes in muscle mass explain only a small proportion of the changes in muscle strength in older adults (Goodpaster et al., 2006; Hughes et al., 2001). Obesity may interact with the relationship between muscle mass and muscle strength, leading to different degrees of association.

The homeostasis of muscle mass is regulated by a dynamic turnover between muscle protein synthesis (MPS) (anabolic) and muscle protein breakdown (MPB) (catabolic). Studies have suggested that anabolic impairment to ingested protein is an important factor influencing age-related muscle mass loss (Fujita, 2007; Moore, 2014; Volpi et al., 2001). In addition, it is recognised that obesity in older adults can impair the responsiveness of MPS to protein ingestion (Beals et al., 2018; Guillet et al., 2009; Murton et al., 2015). This impaired MPS is likely to affect the intramuscular lipid content (or muscle quality), which is also associated with muscle strength (Goodpaster et al., 2001). However, there is limited evidence on the role of obesity on the relationship between muscle strength and muscle mass in older adults. With increasing rates of obesity in New Zealand and globally (Wan He et al., 2016), a greater understanding of obesity's role when investigating associations between muscle mass and muscle strength is warranted.

Dietary protein impacts on muscle mass and muscle strength in older adults. Per day and per meal stimulation of MPS have a saturable dose relationship with the quantity and quality (i.e., source) of protein consumed (Churchward-Venne et al., 2016; Gorissen et al., 2015). To maximise MPS stimulation, it has been suggested that older adults should consume ≥ 1.2 g of protein per kg body weight (g/kg BW) per day (Rafii et al., 2015), which has been shown to slow muscle mass loss in older adults aged 70 to 79 years (Houston et al., 2008; Scott et al., 2010). However, findings on the association between protein intake and muscle strength in cross-sectional and longitudinal studies have been contradictory. Handgrip strength (HGS) is commonly used to predict overall strength in older adults (Ekstrand et al., 2015; Takahashi et al., 2017). There is evidence of an association between relative protein intake (protein intake based on body weight) and HGS adjusted by body weight (BW) or body mass index (BMI) (Celis-Morales et al., 2018; Fanelli Kuczmarski et al., 2018; Isanejad et al., 2016). Other studies have found no association between relative protein intake and absolute HGS (Granic et al., 2018; Mishra et al., 2018; Ten Haaf et al., 2018). The inconsistent findings could be attributed to the use of different HGS indexes such as absolute HGS versus relative HGS with adjustment for BW or BMI.

A maximum stimulation of MPS rate in older adults has been observed at a protein intake of 30 g or 0.4 g/kg BW per meal (Moore et al., 2015; Paddon-Jones & Rasmussen, 2009). Studies have demonstrated that an evenly distributed intake of protein across the day of 30 g or 0.4 g/kg BW per meal resulted in higher MPS than did an uneven protein distribution (Mamerow et al., 2014; Paddon-Jones & Rasmussen, 2009). Furthermore, cross-sectional evidence from large numbers of participants has demonstrated that a more even distribution of protein is associated with greater muscle mass in older adults (Farsijani et al., 2016; Farsijani et al., 2017; Loenneke et al., 2016). Because protein distribution is a new concept, there is no consistent definition (Aoyama et al., 2021; Hudson et al., 2020). Protein distribution is commonly estimated as the coefficient of variance (CV) of the protein intake, or the number of meals exceeding 30 g or 0.4 g/kg BW. However, the use of different methods to estimate protein distribution makes it difficult to make conclusions regarding the relationship between protein distribution and muscle strength in older adults (Bollwein et al., 2013; Gingrich et al., 2017; Kim et al., 2018; Mishra et al., 2018; Ten Haaf et al., 2018). Investigating associations between muscle strength and protein distribution using different estimates of protein distribution commonly used in the literature may assist in clarifying the relationship.

High-quality animal proteins such as meat, dairy and eggs have a greater ability to enhance MPS than plant-based proteins (Gorissen et al., 2016; van Vliet et al., 2015; Yang et al., 2012). However, not all animal-based protein sources are comparable in terms of protein quality properties, which determine the amplitude and duration of the muscle protein synthetic response (Schaafsma, 2000; Smith & Gray, 2016; van Vliet et al., 2015). Studies have demonstrated that higher intake of animal-based protein (but not plant-based protein) was associated with higher muscle strength in older adults (Isanejad et al., 2015; McLean et al., 2016). However, these studies did not address whether either animal-based protein sources, (i.e., meat, fish, dairy, or eggs), which differ in quality, is associated with muscle strength.

In international cohorts, a high proportion of older adults' intake of protein has been shown to be < 1.2 g/kg BW (Houston et al., 2017; Isanejad et al., 2016; Mendonça et al., 2018). However, there is no data on the prevalence of protein intakes of < 1.2 g/kg BW in older adults in NZ.

Furthermore, few studies have investigated whether older adults are meeting protein intake recommendations of 30 g or 0.4 g/kg BW per meal. Two international cohort studies indicated that a high proportion of older adults were not consuming two or more meals containing at least 30 g or 0.4 g/kg BW of protein at each meal (Gaytán González et al., 2020; Loenneke et al., 2016). Studies are now beginning to consider the protein sources consumed at specific times of the day in order to understand how to increase protein intake at each meal and improve protein distribution throughout the day in older adults (Smeuninx et al., 2020; Tieland et al., 2015). A study in the Netherlands reported that in community-dwelling older adults (mean age 77 years), protein was obtained mainly from plant-based protein sources at breakfast and the lunch meal, and animal-based protein sources at dinner (Tieland et al., 2015). However, such work has not yet been undertaken in NZ.

In conclusion, further work is required regarding the role of obesity when investigating associations between muscle mass and muscle strength in older adults. It is also important to understand how older adults in New Zealand are consuming protein. This includes total protein intake, distribution of protein across the day and sources of protein (both across the day and at mealtimes). A greater understanding of how protein is consumed and associations with muscle strength adjusted appropriately for body composition is warranted. This knowledge will enable targeted nutrition education and interventions to be developed to help optimise protein intake in older adults.

1.2. Research objectives

The aim of this research is to explore muscle strength, muscle mass, and dietary protein intake in community-dwelling older adults living in Auckland, NZ. Specific objectives are to:

- Explore the role of obesity in the relationship between muscle mass and muscle strength in community-dwelling older adults living in NZ;

- Investigate protein intake, distribution, and sources in community-dwelling older adults living in NZ;

- Investigate muscle strength and its relationship with protein intake, sources, and different estimates of protein distribution throughout the day in community-dwelling older adults living in NZ.

1.3. Thesis outline

This thesis includes six chapters – an introduction, literature review, three manuscripts, and a discussion chapter. As each manuscript is presented in the form of a journal article adapted from its published version, there may be some repetition throughout the thesis.

Chapter one introduces the importance of the research topic in a global aging population. Previous literature and research gaps regarding muscle strength, muscle mass and dietary protein are identified, leading to the research aims and objectives.

Chapter two provides a thorough review of all relevant evidence regarding muscle strength, muscle mass, and dietary protein intake, distribution, and sources in older adults.

Chapter three, the first manuscript, examines the relationship between muscle mass and muscle strength, accounting for obesity in older adults living in Auckland, NZ.

Chapter four, the second manuscript, is a comprehensive investigation regarding protein intake, distribution, and sources in older adults living in Auckland, NZ.

Chapter five, the third manuscript, explores associations between muscle strength and protein intake, sources, and distribution in older adults living in Auckland, NZ. Note that chapters three, four and five are participants from the same study population.

Chapter six, discusses the findings of the three manuscripts, including their significance and relevance, the methodological strengths and limitations of the studies, and suggested future research on muscle strength, muscle mass, and dietary protein in older adults. This chapter ends with final conclusions regarding the research findings.

1.4. References

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Chapter Two – Literature review

This chapter begins with describing the aging global population and the incidence of falls, which is linked to the rationale for the research focus on muscle strength in older adults. Due to the contradictory evidence on the relationship between muscle mass and muscle strength and associations with obesity, studies that examine the potential interaction of obesity within that relationship will be discussed in depth. Proteins are the most abundant component of muscle mass, however the muscle's ability to stimulate protein synthesis following protein ingestion decreases as we age and with obesity. Therefore, current evidence on the role of protein intake, distribution, and sources in overcoming age-related muscle protein synthesis impairment will be reviewed, as well as any controversies in the literature discussed. Gaps in the literature that investigate protein intake, distribution, and sources in both the New Zealand and global contexts are identified. Finally, an overview of the literature on protein intake, distribution, and sources, and their association with muscle mass and muscle strength in older adults is provided.

2.1. Search strategies

Studies were identified through the following databases: Science Direct, PubMed, Google Scholar, and Scopus. Key concepts from the research questions were identified (Table 2. 1). Then search terms for each concept which identify controlled vocabulary and free text terms were created (McGowan et al., 2016). All terms within each concept were joined with **OR**. Finally, each concept was joined with another using **AND**. Papers were limited to those in English and published in full. To increase the search breadth, no additional filters for publication year or age group were applied.

Table 2. 1. Literature review search strategies

	Concept 1 Falls	AND	Concept 2 Muscle strength	AND	Concept 3 Older adults	AND	Concept 4 Muscle mass	AND	Concept 5 Obesity	AND	Concept 6 Protein intake
Controlled vocabulary terms/ Subject terms (MeSH terms)	Falls, injury		Muscle strength, muscle weakness		Aged, elderly		Skeletal muscle, intramuscular lipid		Adiposity		Protein intake, protein distribution, protein sources

	OR		OR		OR		OR				OR
Free text terms/natural language terms (Synonyms, UK/US terminology, medical/laymen's terms, acronyms/abbreviation, more narrow search terms)	Falls, injury, injuries, accident		Muscle strength, muscle weakness, handgrip strength, knee extensor strength, quadriceps muscle, arm strength, leg strength, upper strength, lower strength, leg muscle power		Aged, elderly, older, young-old, old-old, adults, senior, aging, ageing		Muscle mass, lean mass (LM), lean body mass (LBM), fat-free mass (FFM), appendicular lean mass (ALM), appendicular skeletal muscle (ASM), skeletal muscle mass (SMM), appendicular skeletal muscle index (ASMI), muscle cross-sectional area (CSA)		BMI, body fat percentage, fat mass, fat		Protein intake, protein distribution, protein sources, protein recommendation, protein need, protein timing, protein quality, food sources of protein, animal protein, animal-based protein, plant protein, plant-based protein, vegetable protein, dairy-eggs protein
Abbreviations: MeSH = Medical Subject Headings, BMI = Body Mass Index, UK = United Kingdom, US = United States.											

2.2. Ageing and falls

Population ageing is occurring worldwide, leading to a growing proportion of older adults (United Nations, 2019; Wan He et al., 2016). Previously, in 2015, the world population percentage for adults aged 65 and over was 8.5% (n = 617 million) (Wan He et al., 2016). According to the most recent United Nations World Population Prospects estimations, 9% (n = 703 million) of the world's population consisted of people aged 65 years and older in 2019, and this proportion is expected to increase to 16% by 2050 (United Nations et al., 2019).

The same trend is expected in New Zealand (NZ), a country of 4.9 million people. Currently, in 2020, 16% of New Zealand's population is aged 65 or older, with women accounting for more than half (53.4%) (Statistics New Zealand, 2020). This percentage is expected to increase to more than 21% by the year 2048, and to more than 24% by 2073 (Crothers, 2021).

One of the primary concerns with an ageing population is the incidence of falls and fall-related injuries (Health Quality & Safety Commission, 2016; Peel, 2011; Rubenstein & Josephson, 2002). An epidemiological review across different countries revealed that 20 to 33% of older adults (aged 65 years and over) in community settings experienced a fall once a year, and in the oldest group (85 years and older), up to 60% experienced a fall within a 12-month period (Peel, 2011). Recently, Moreland et al. (2020) examined trends in falls and fall-related injuries among older adults aged ≥ 65 years in the United States. They found that in 2018, 27.5% of older adults reported at least one fall in the past year, and 10.2% reported a fall-related injury. In NZ, there is a lack of studies reporting the prevalence of falls for older adults. However, it is clear that fall-related injuries are a public health concern for older adults. For example, data indicated that in New Zealand in 2016, the rate of one or more Accident Compensation Corporation (ACC) claims was for a fall-related injury. More specifically, the Health Quality and Safety Commission New Zealand reported "In 2016, 216,000 people aged 50 years and over had one or more ACC claims for a fall-related injury accepted" (Health Quality & Safety Commission, 2016). It was not stated how many claims were rejected.

Falls cause physical injuries as well as serious social and psychological consequences such as fear of recurring falls, social isolation, and depression, the cost of which are difficult to estimate (Hadjistavropoulos et al., 2011; Zijlstra et al., 2007). Healthcare costs for physical fall-related injuries such as contusions, lacerations, dislocations, hip fractures, and traumatic brain injuries, on the other hand can be estimated (Garrett, 2008; Stevens & Sogolow, 2005; Thompson et al., 2006). A study that estimated the community-based costs associated with minor falls in older adults in New Zealand found that the mean cost per fall was NZD$422 (Garrett, 2008). A higher cost of $600 per fall in New Zealand has been estimated in 2011 (de Raad, 2012). Of all the physical injuries, hip fractures are the most common injury resulting from falls, and also represent the costliest injury for New Zealand's ageing population (Robertson & Campbell, 2012). In New Zealand, a hip fracture with three weeks in hospital costs $47,000, and a hip fracture with complications and discharge to an aged residential care facility costs $135,000 (de Raad, 2012). Because all these estimations are over 10 years old, costs will have certainly risen since they were calculated.

There is a wealth of evidence on risk factors for falls (Barker et al., 2009; Hendriks et al., 2008; Rubenstein, 2006). A review of 16 studies indicated that the most common locomotor system and physiological (intrinsic) risk factors that contribute to falls were related to muscle weakness (Rubenstein, 2006). In addition, sarcopenia which is a consequence of ageing and is associated with insulin resistance, type 2 diabetes, and metabolic syndrome further increases the risk of cardiovascular disease and stroke in older adults (Chung et al., 2013). These conditions limit older adult's capacity to engage in physical activity, leading to further declines in muscle strength and an increase in the rate of falls in older adults.

The predicted number of New Zealanders who are likely to live to older age will place a significant burden on future fall-related injuries and healthcare costs. Hence, this emphasises the need to prioritise further research that addresses muscle weakness in older adults.

2.3. Muscle strength in older adults

Muscle weakness, also known as *dynapenia*, is a loss of muscle strength that occurs with age and is not caused by a neurological or muscular disease (Clark & Manini, 2012). *Strength* can be defined as the maximum force exerted by a muscle (Buchner et al., 1992). Muscle strength tends to peak between the second and third decades of life and then significantly decline (Figure 2. 1). The rate of decline is higher with advanced age, menopause, decreased intensity and duration of physical activity, the presence of chronic diseases, and certain lifestyle factors such as smoking (Kalyani et al., 2014; Kehayias et al., 1997; Marcell, 2003; Shah et al., 2022). Additionally, the timing of muscle strength loss varies by anatomical location, with lower extremity muscle loss occurring later than upper extremity muscle loss (Buckley et al., 2018; Burr, 1997).

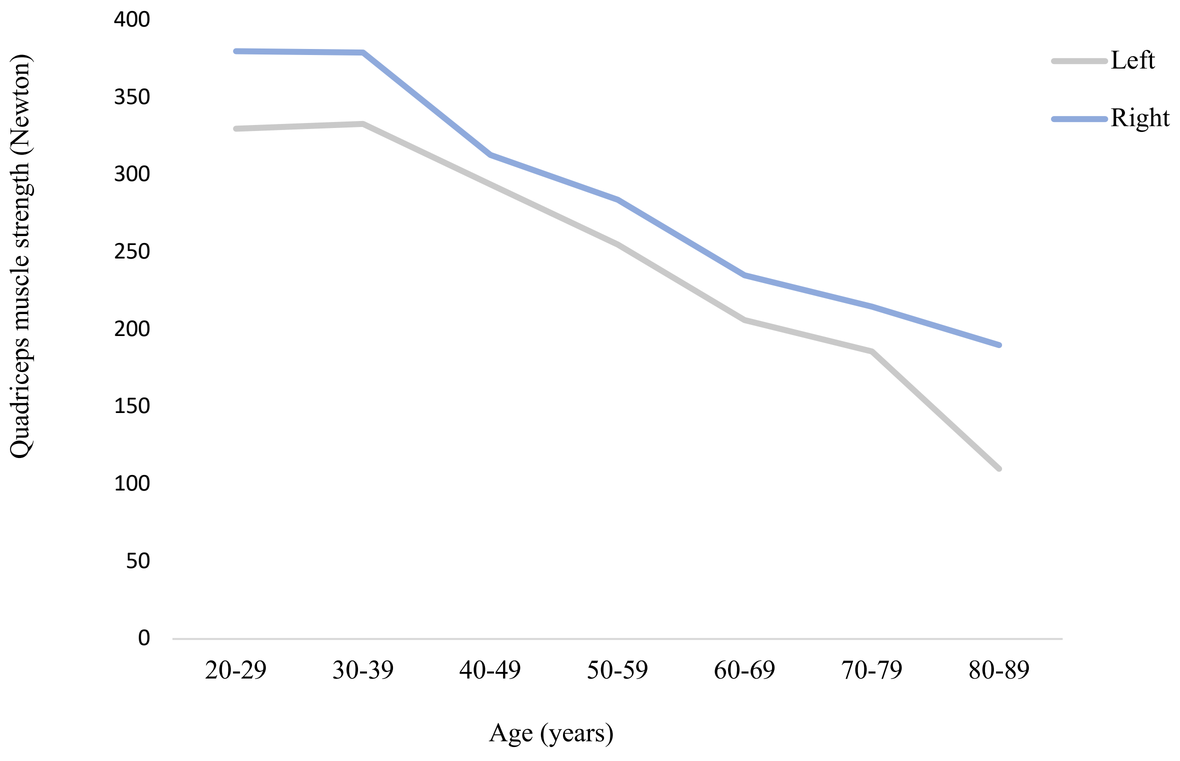

Figure 2. 1. Declining quadriceps muscle strength in adults. Data from Al-Abdulwahab (1999).

2.3.1 Assessment of muscle strength

There are a few methods to assess muscle strength. These include handgrip (isometric strength), knee flexion/extension (isotonic strength), and peak torque (isokinetic strength) (Roberts et al., 2011). Handgrip strength is a simple measurement of muscle strength that is commonly used to predict overall muscle strength (Ekstrand et al., 2015; Takahashi et al., 2017) and will be focussed on within this literature review. It is used for screening older adults with low muscle strength in the diagnosis of frailty (Dudzińska-Griszek et al., 2017), sarcopenia (Cruz-Jentoft et al., 2010) and malnutrition (Zhang et al., 2017). Low handgrip strength has also been associated with higher hospital readmission, increased risk of fractures, reduced physical functioning, and increased risk of falls (Bohannon, 2008; Ibrahim et al., 2018).

There are several protocols for measuring handgrip strength (Balogun et al., 1991; Firrell & Crain, 1996; Incel et al., 2002; O'Driscoll et al., 1992; Roberts et al., 2011), but the protocol by Roberts et al. (2011) is the most comprehensive and evidence-based. They recommend using a calibrated Jamar dynamometer with the handle in the second position. The individual being tested sits with his or her forearm and wrist in a neutral position supported by an armrest, his or her elbow flexed 90 degrees, and his or her shoulder at 0 degrees abduction / flexion. Three measurements must be taken for each hand, beginning with the right and alternating right/left.

The Foundation for the National Institutes of Health Sarcopenia Project (FNIH), Asian Working Group for Sarcopenia (AWGS), European Working Group on Sarcopenia in Older People (EWGSOP) and Sarcopenia Definition and Outcomes Consortium (SDOC) have produced consensus statements on cut-off values for low muscle strength using handgrip strength measurements (Table 2. 2). Because muscle strength adjusted BMI has been proposed as the ideal marker for muscle strength (Keevil et al., 2015; Kim et al., 2019; Kim et al., 2017; Tang et al., 2018; Villareal et al., 2004), the FNIH also propose using BMI-handgrip strength cut-off values of < 1.001 in men and < 0.56 in women (Cawthon et al., 2014).

Based on the consensus statement of the Australian and New Zealand Society for Sarcopenia and Frailty Research (ANZSSFR), clinicians and researchers in Australia and New Zealand are recommended to use the cut-off values for low handgrip strength proposed by the European Working Group on Sarcopenia in Older People 2 (EWGSOP2), with the cut-off now at < 16 kg for women and < 27 kg for men (Cruz-Jentoft et al., 2019; Daly et al., 2022). However, EWGSOP2 has not provided threshold values for BMI-handgrip strength.

Table 2. 2. Consensus statements on cut-off values for determining low muscle strength in older adults.

Working group	**Cut-off points by sex**	
	Females	Males
European Working Group on Sarcopenia in Older People (EWSSOP) (Cruz-Jentoft et al., 2010)	< 20 kg	< 30 kg
Foundation for the National Institutes of Health Sarcopenia Project (FNIH) (Studenski et al., 2014)	< 16 kg	< 26 kg
Asian Working Group for Sarcopenia (AWGS) (Chen et al., 2014)	< 18 kg	< 26 kg
European Working Group on Sarcopenia in Older People (EWSSOP2) (Cruz-Jentoft et al., 2019)	< 16 kg	< 27 kg
Sarcopenia Definition and Outcomes Consortium (SDOC) (Bhasin et al., 2020)	< 20 kg	< 35.5 kg

2.3.2. Prevalence of low muscle strength

Using the EWGSOP2 cut-off values (< 16 kg for women and < 27 kg for men), a nationally representative sample of Brazilians aged 65 years and older revealed a 28.2% prevalence of low muscle strength (Borges et al., 2020). Other studies, which used the older cut-off values defined by the EWGSOP (< 20 kg for women and < 30 kg for men), found the prevalence of low muscle strength to be 33.9% among Mexicans aged 50 years and older (Rodríguez-García et al., 2018), 22.5% among Europeans aged 70 years and older (Bertoni et al., 2018), 44% in Americans aged 65 years and older (Duchowny et al., 2018), and 71% in community-dwelling New Zealanders aged 75 years and older (Chatindiara et al., 2019). Different cut-off values used in the literature make it difficult to compare studies. However, low muscle strength in older adults appears to be a global issue.

2.4. Muscle strength and muscle mass in older adults

2.4.1. Muscle mass, protein, and ageing

Muscle is the most abundant tissue in the human body (40 to 50% of total body weight) (Karagounis & Hawley, 2010) and includes skeletal, smooth, and cardiac muscle. Muscle mass is the limb lean mass that covers the limb bones and is surrounded by limb fat. In this review, the terms skeletal muscle mass (SMM), appendicular skeletal mass (ASM), appendicular lean mass (ALM), fat-free mass (FFM), lean mass (LM) and muscle area, all refer to muscle mass. Lean mass is increased, decreased, and maintained through the regulation of the net protein balance between muscle protein synthesis (anabolic) (MPS) and muscle protein breakdown (catabolic) (MPB) (Bennet et al., 1979; Bohé et al., 2003; Bohé et al., 2001; Fujita, 2007). A significant increase in MPS and/or a decrease in MPB, such that the net protein balance remains positive, results in lean mass accretion (Bennet et al., 1979; Bohé et al., 2003; Bohé et al., 2001; Burd et al., 2011; Fujita et al., 2007).

Protein ingestion stimulates the process of MPS. Protein is a macronutrient composed of various amino acids, that can be found in both plant (e.g., soy, rice, peas, oats, wheat, legumes, beans and nuts) and animal-based foods (i.e., meat, dairy and eggs). Protease enzymes break down foods into short strings of amino acids (peptides) and single amino acids. This process starts in the stomach and progresses to the small intestine, where amino acids are absorbed into the bloodstream and used for protein synthesis (Cuthbertson et al., 2005; Fry et al., 2011; Groen et al., 2015; Pennings et al., 2011; Volpi et al., 2003).

With ageing, MPS stimulation after protein ingestion decreases (Colley et al., 2011; Cuthbertson et al., 2005; Katsanos et al., 2005, 2006; Paddon-Jones et al., 2004; Volpi et al., 2001). Cuthbertson et al. (2005) was the first to demonstrate that basal MPB rates were similar between younger and older people, but MPS in older adults was less responsive to the ingestion of protein (anabolic resistance). This anabolic resistance to protein was subsequently confirmed by others (Katsanos et al., 2005, 2006; Paddon-Jones et al., 2004).

The aetiology of anabolic resistance with ageing is not entirely understood but is proposed to be mediated by impairments in several physiological processes (Boirie et al., 1997; Burd et al., 2013; Dickinson et al., 2013; Rasmussen et al., 2006; Timmerman et al., 2010; Volpi et al., 1999). A reduced rate of amino acid absorption into the bloodstream and/or a greater retention of amino acids in the stomach and the small intestine may limit the availability of amino acids for MPS (Boirie et al., 1997; Volpi et al., 1999). In addition, a decline in amino acid transporters, that mediate transfer of amino acids into and out of cells, may limit the delivery of amino acids to the muscle and the uptake of amino acids by the muscle (Dickinson et al., 2013; Rasmussen et al., 2006; Timmerman et al., 2010).

The aged-anabolic impairment to protein results in muscle mass decline in older adults. A decrease in skeletal muscle mass of one to two percent per year has been observed in men and women (Kehayias et al., 1997; Marcell, 2003). In older adults aged 70 to 79 years, Goodpaster et al. (2006) observed that the loss of leg lean mass was about 1% per year, regardless of sex or ethnicity.

Similarly, Frontera et al. (2000) reported a reduction in muscle cross-sectional area of 1.4% per year in older adults after 50 years. This was also confirmed by von Haehling et al. (2010), who identified a loss of muscle mass of 1 to 2% per year after the age of 50.

2.4.2. Assessment and prevalence of low muscle mass

Body composition assessment is an important tool for the identification of common nutrition-related conditions and provides valuable information about responses to intervention. However, quantifiable and clinically meaningful changes in body composition take time to occur; therefore, there is a need for multiple assessments over time and this is determined based on the individual, the intervention, and the goals to be achieved.

There are a wide range of methods to quantify muscle mass, which require assumptions with variable degrees of accuracy. Total body counting (using radioactive potassium), and neutron activation are two direct measurements of muscle mass available to the researcher/clinician. Fat-free mass can be estimated using the amount of naturally radioactive potassium 40 (40K) in the body, assuming a constant concentration of potassium in FFM (Ellis, 2000). Currently, the only available detectors which can measure radioactive potassium are in the United States, which precludes its use in research outside of US. Neutron activation quantifies the body nitrogen to predict the amount of protein in the body to further analyse components of FFM. This method has been reported to be highly accurate, however it involves high levels of neutron radiation exposure and therefore has not been used in large-scale population research (Haas et al., 2007).

Indirect measures include anthropometric (limb circumference measurement), dual-energy X-ray absorptiometry (DXA), bioelectrical impedance analysis (BIA), computed tomography (CT), magnetic resonance imaging (MRI), and ultrasound are used to measure muscle mass (Cohn et al., 1983; Heymsfield et al., 1982; Heymsfield et al., 1990; Reeves et al., 2004). Anthropometric measures are vulnerable to error and therefore are not recommended for routine use in muscle mass assessment (Cruz-Jentoft et al., 2010).

Computed tomography and MRI are considered gold standard for skeletal muscle mass estimation in research, mainly because of their precision in separating fat from other soft tissues (Cruz-Jentoft et al., 2010). However, CT scans utilise radiation and the high cost of both CT and MRI scans limits their use (Chien et al., 2008). Ultrasound has been proposed as a simple alternative for measuring skeletal muscle mass in clinical practice, but it is not standardised and does not yet have validated cut-off values for determining low muscle mass (Perkisas et al., 2021; Stringer & Wilson, 2018). Bioelectrical impedance analysis is portable, inexpensive, easy to use, and can be used for both bedridden and ambulatory patients (Jung et al., 2020). However, the appendicular lean mass equations are both population and device-specific, which limits its accuracy (Gonzalez et al., 2018). The most common method of estimating skeletal muscle mass used in both clinical and research settings is DXA. It has the advantages of being able to distinguish fat, bone mineral, and lean tissues with good precision, and minimal radiation exposure (Cruz-Jentoft et al., 2010). However, DXA instruments are relatively expensive and are not portable, which may limit their use in large epidemiological studies (Cruz-Jentoft et al., 2010).

Heymsfield et al. (1990) first demonstrated that using DXA, the calculation of skeletal muscle mass (SMM), appendicular skeletal mass (ASM), or appendicular lean mass (ALM) as the sum of limb lean mass (LM) minus the sum of limb fat and bone mass was a highly accurate method of quantifying human skeletal muscle mass, while being safe and accessible. To determine the cut-off value for low muscle mass, Baumgartner et al. (1998) proposed controlling SMM, ASM, or ALM for height squared to calculate an index of relative skeletal muscle mass, because of the strong relationship between muscle mass and height. They determined that cut-off values for low muscle mass (ASM/height^2) were < 7.26 kg/m^2 for men and < 5.45 kg/m^2 for women.

Different definitions and cut-off values for low muscle mass have been developed for DXA and BIA measurements, making it difficult to compare studies. However, the ANZSSFR, recommend clinicians and researchers in Australia and New Zealand use the cut-off values for low muscle mass revised by the EWGSOP2 to be ASM/height^2 < 7 kg/m^2 for men and < 5.5 kg/m^2 for women (Cruz-Jentoft et al., 2019; Daly et al., 2022).

Table 2. 3 compares studies investigating the prevalence of low muscle mass in adults and older adults living in developed countries. A common finding is that low muscle mass is highly prevalent in older people and that it worsens with age.

Table 2. 3. Prevalence of low muscle mass in older adults: a comparison of different definitions and cut-off values.

Author et al. (years) Country	**Muscle mass assessment method**	**Muscle mass calculation and cut-off values**	**Sex**	**N**	**Age (years)**	**Prevalence**
Baumgartner et al. (1998) Mexico	DXA	ALM/ht^2 male ≤ 7.26 kg/m^2 female ≤ 5.45 k/m^2	Male and female	883	61 to 70 71 to 80 ≥ 80	13% 24% 50%
Morley et al. (2001) US	DXA	ALM /ht^2 male ≤ 7.26 kg/m^2 female ≤ 5.45 k/m^2	Male and female	199	< 70 ≥ 80	12% 30%
Janssen et al. (2002) Canada	BIA	Ratio of muscle mass/total body mass male ≤ 31.5% female ≤ 22.1%	Male Female	2224 2278	≥ 60 ≥ 60	7% 10%
Newman (2003) US	DXA	ALM /ht^2 male ≤ 7.23 kg/m^2 female ≤ 5.67 kg/m^2	Male Female	1435 1549	70 to 79	20% 20%
Waters et al. (2010) New Zealand	DXA	ASM index male < 7.2 kg/m^2 female < 5.4 kg/m^2	Male Female	183	56 to 93	4% 12%
Kim et al. (2012) Korea	DXA	ASM/weight Class I Class II	Male Female	4486 5999	≥ 20	9.7% 11.8%

Abbreviations: ALM = appendicular lean mass; ASM = appendicular skeletal mass; ht = height; DXA = dual-energy X-ray absorptiometry; BIA = bioelectrical impedance analysis; Class I was from 1 to 2 SD below the mean for young adults and Class II was below 2 SD.

2.4.3. Relationship between muscle mass and muscle strength

Cross-sectional studies have shown that muscle mass and muscle strength are positively correlated (Barbat-Artigas, Plouffe, et al., 2013; Beliaeff et al., 2008; Chen et al., 2013; Hayashida et al., 2014; Reed et al., 1991). Age and sex seem to interact with the relationship between muscle mass and muscle strength, leading to a weaker degree of association when classified by sex and age groups (Table 2. 4). Due to the limitations of these cross-sectional studies in detecting causality, only associations can be described.

In the Health, Aging and Body Composition study, Newman et al. (2003) reported that lower muscle strength in older men and women aged 70 to 79 years was predominantly due to lower muscle mass. Similarly, Frontera et al. (2000) found that quantitative loss of muscle mass was a major contributor to the decline in muscle strength seen in men of advanced age (initial mean age 65 years). These studies suggest that efforts to maintain muscle mass may have a significant impact on maintaining strength in older adults. However, several studies have shown that changes in muscle mass explain only a small portion (~5%) of the changes in strength in older adults (Goodpaster et al., 2006; Hughes et al., 2001). Also, there is evidence that older adults may have low muscle strength despite maintaining or increasing muscle mass and the reverse is also true (Alemán-Mateo et al., 2014; Park et al., 2006; Raue et al., 2009).

As previously stated, the aged-anabolic impairment to ingested protein is the most important factor influencing muscle loss in older adults (Colley et al., 2011; Fujita, 2007; Volpi et al., 2001). Obesity in older adults is also known to impair the responsiveness of MPS to protein ingestion (Beals et al., 2018; Guillet et al., 2009; Murton et al., 2015). This weakened MPS is likely to increase intramuscular lipid content (or poor muscle quality), body fat percentage (%BF) and decline in muscle oxidative capacity (Gallagher et al., 2000; Goodpaster et al., 2006; Hughes et al., 2002; Jacobs et al., 2014). Lipids which accumulate within the muscle cell potentially playing a role in the development of insulin resistance, which is associated accelerated peripheral inflammation and functional deficits in skeletal muscle (Consitt et al., 2009). These conditions are associated with both lower muscle strength and muscle mass in older adults (Goodpaster et al., 2001; Visser et al., 2005).

Also, obesity classified using BMI limits the degree of association to which higher muscle mass translates into greater muscle strength. Chen et al. (2013) found that obese older adults (BMI $\geq$ 30 kg/m^2) had lower strength than non-obese older adults with the same muscle mass. Thus, obesity may interact with the relationship between muscle mass and muscle strength, leading to different degrees of association.

Table 2. 4. Cross-sectional studies investigating the relationship between muscle mass and muscle strength in older adults.

Author et al. (years) Country	**Sex**	**N**	**Age (years)**	**Key findings**
Reed et al. (1991) US	Male and female	218	65^+	Midarm muscle area (MAMA) was correlated with upper arm strength (r = 0.68). By sex, correlation between MAMA and arm muscle strength was lower in both groups (r = 0.36 for males; r = 0.23 for females). Midthigh muscle area was correlated with thigh muscle strength (r = 0.37 for males; r = 0.19 for females)
Beliaeff et al. (2008) Canada	Male and female	904	65 to 84	In both legs and arms, muscle mass was correlated with muscle strength (r = 0.21 for legs; r = 0.49 for arms)
Chen et al. (2013) US	Male and female	2647	50^+	Correlation between muscle mass and isokinetic quadriceps strength after adjusting for age and sex was 0.365
Barbat-Artigas, Plouffe, et al. (2013) Canada	Female	1219	75^+	Muscle mass was positively, but weakly associated with muscle strength (r values ranged from 0.16 to 0.22)
Hayashida et al. (2014) Japan	Male and female	318	65^+	Leg muscle mass was correlated with knee extension strength (r = 0.33 in males; r = 0.22 in females). Appendicular muscle mass was correlated with knee extension strength (r = 0.34 in males; r = 0.23 in females)

2.5. Protein intake, distribution, sources, and muscle protein synthesis

2.5.1. Protein intake, recommendations, and muscle protein synthesis in older adults

Protein recommendations for older adults refer to the amount of protein that is necessary to maintain the balance between MPS and MPB (Joint FAO/WHO/UNU Expert Consultation, 2007; National Health and Medical Research Council Australian Government Department of Health and Ageing, 2006). Recommendations of protein are given as either the Estimated Average Requirement (EAR) or the Recommended Dietary Intake (RDI) (note that the US/Canadian term is Recommended Dietary Allowance (RDA), and in the UK is Recommended Nutrient Intake (RNI)) (Department of Health Panel on Dietary Reference Values, 1991; National Academy of Sciences, 2005). The EAR is the average daily nutrient intake level estimated to meet the needs of half of the healthy individuals in a given life stage and gender group. The RDI is an estimate of the minimum daily average dietary intake level that meets the nutrient requirements of 97 or 98% of healthy individuals in a given life stage and gender group (Joint FAO/WHO/UNU Expert Consultation, 2007).

The RDA for protein has been established by many health agencies, including the World Health Organisation (WHO) and the European Food Safety Authority (EFSA) as 0.8 grams per kilogram of body weight (g/kg) of good quality protein for healthy adult men and for healthy non-pregnant women of all ages (EFSA Panel on Dietetic Products Nutrition, 2012; Joint FAO/WHO/UNU Expert Consultation, 2007). However, a meta-analysis demonstrated that the median daily protein requirement for older adults was higher than for younger adults (0.9 g/kg BW for females and 1.1 g/kg BW for males) (Rand et al., 2003). Also, Campbell et al. (2001) reported that an intake of 0.8 g/kg BW in men and women aged 55 to 77 years over 14 weeks resulted in metabolic and physiological adaptation to the dietary intake, suggesting that 0.8 g/kg BW was insufficient to meet their protein requirements. Based on these findings, in 2006, the Australia and New Zealand Nutrient Reference Values (NRVs) provided a RDI for protein for older adults (aged 70^{+} years) that was higher than for younger adults (aged between 19 and 70 years) (Table 2. 5). In 2015, Japan established a recommendation of 0.9 g/kg BW, which is higher than 0.8 g/kg BW/day (Tsuboyama-Kasaoka et al., 2013).

Table 2. 5. Nutrient Reference Values for protein for adults and older adults in Australia and New Zealand (National Health and Medical Research Council Australian Government Department of Health and Ageing, 2006).

Age	**EAR (g/kg BW)**	**RDI (g/kg BW)**	**RDI (g/day)**
Men			
19 to 70 years	0.68	0.84	64
More than 70 years	0.86	1.07	81
Women			
19 to 70 years	0.60	0.75	46
More than 70 years	0.75	0.94	57

Abbreviations: EAR = Estimated Average Requirement, RDI = Recommended Dietary Intake, BW = body weight.

All the above protein recommendations are based on a short-term net protein balance protocol (EFSA Panel on Dietetic Products Nutrition, 2012; Joint FAO/WHO/UNU Expert Consultation, 2007; National Health and Medical Research Council Australian Government Department of Health and Ageing, 2006). The net protein balance measurement is based on the concept that protein is the most nitrogen-containing substance in the body. As a result, nitrogen gain or loss from the body corresponds to nitrogen gain or loss from protein. This method has several limitations, including the difficulty of quantifying all routes of nitrogen intake and loss and standardising research methods for addressing nitrogen balance studies. Because of these constraints, protein requirements are likely to be underestimated (Bauer et al., 2013; Gaffney-Stomberg et al., 2009). In addition, Morse et al. (2001) demonstrated that shorter-term nitrogen balance protocols are insufficient to establish the RDA for protein of older women aged 70 years and older.

The indicator amino acid oxidation (IAAO) method has emerged as a new method of investigating protein requirements (Elango et al., 2008, 2012). This method assumes that when the amount of one essential or indispensable dietary amino acid (IDAA) is insufficient for protein synthesis, all other excess IDAAs, including the indicator amino acid, will be used as an energy substrate, and thereby oxidised.

A study using this method to estimate protein requirements in older adults, suggests that the RDA for protein in men and women over 65 years of age should be increased to 1.2 g/kg BW/day (Rafii et al., 2015). More specifically, Courtney-Martin et al. (2016) estimated an RDA of 1.2 g/kg BW/day for men aged 66 to 79 years, 1.2 g/kg BW/day for women aged 80 to 87 years, and 1.3 g/kg BW/day for women aged 65 to 85 years.

In recent years, several research experts have recommended that older adults should consume protein at a rate of 1.0 to 1.2 g/kg BW/day. At a Workshop on Protein Requirements in the Elderly, members of the European Society for Clinical Nutrition and Metabolism presented and debated the latest research studies on preserving muscle performance in the older adults. They recommend adults over the age of 65 years consume at least 1.0 to 1.2 g protein per kg per day (Deutz et al., 2014). Similarly, the PROT-AGE study group (representing the European Union Geriatric Medicine Society, the International Association of Gerontology and Geriatrics, the International Academy on Nutrition and Aging, and the Australian and New Zealand Society for Geriatric Medicine) recommended an average daily intake of 1.0 to 1.2 g/kg BW/day in healthy older adults (Bauer et al., 2013). Furthermore, Moore and Soeters (2015) observed that 0.4 g/kg BW/meal over three meals, equating to 1.2 g/kg/day, resulted in a maximal simulation of MPS in older adults.

2.5.2. Protein distribution and muscle protein synthesis in older adults

The maximum stimulation rate for MPS in older adults has been observed at a protein intake of 30 g per meal or 0.4 g/kg BW per meal (Moore & Soeters, 2015; Paddon-Jones & Rasmussen, 2009). Paddon-Jones and Rasmussen (2009) observed that 30 g of protein per meal was sufficient to maximally stimulate muscle protein synthesis and giving a dose higher than this did not further increase MPS simulation. Similarly, Moore and Soeters (2015) found that maximal MPS was achieved at 0.4 g/kg BW per meal in older adults. Therefore, it was hypothesised that three meals per day, with a regular protein intake of 30 g or 0.4 g/kg BW per meal, could maximally stimulate MPS more frequently, and thereby ensure a more positive net protein balance.

Paddon-Jones and Rasmussen (2009) demonstrated that, in older adults, an even distribution of protein intake at 25 to 30 g per meal resulted in higher MPS over 24 hours than a skewed protein distribution (Figure 2. 2). To be more specific, Mamerow et al. (2014) demonstrated that, in healthy adults aged between 25 and 55 years, MPS was about 25% higher in those with an even distribution of daily protein intake (30 g or 0.4 g/kg BW per meal) than in those with a skewed distribution of the same amount of daily protein (90 g or 1.2 g/kg BW per day). In contrast, Murphy et al. (2015) found that MPS responses did not differ between older adults subjected to uneven (breakfast, lunch, and pre-bed snack at < 0.22 g/kg BW each, dinner at ~ 0.94 g/kg BW) and even (≥ 30 g or ≥ 0.33 g/kg BW at each meal) protein intakes. The intake of 0.4 g/kg BW required for maximal MPS in older adults per meal may be a potential explanation for the discordant findings.

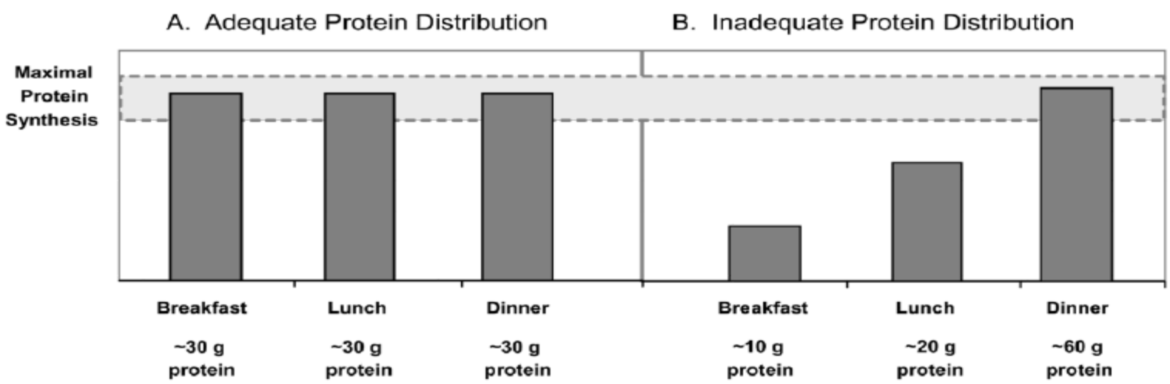

Figure 2. 2. Adequate protein distribution (A) and inadequate protein distribution (B). Adapted from Paddon-Jones and Rasmussen (2009).

2.5.3. Protein quality, sources, and muscle protein synthesis in older adults

The protein quality of a food is determined by its profile of essential amino acids and ease of digestibility and absorption in the body. These two properties define the amplitude and duration of the MPS response. Animal proteins contain all nine essential amino acids, whereas plant proteins are frequently missing or have very low amounts of one or more essential amino acids (Gilbert et al., 2011; Heimo Scherz et al., 2000).

In addition, animal-based proteins have often a greater protein digestibility corrected amino acid score (PDCAAS) than plant-based proteins (Berrazaga et al., 2019). Therefore, animal-based protein sources such as meat, fish, eggs, and milk are considered higher quality than plant-based protein sources such as cereals and legumes.

Several studies have been conducted to assess the impact of plant-based and animal-based proteins on MPS. According to these studies, high-quality animal proteins have a greater ability to improve MPS than plant-based proteins (Gorissen et al., 2016; van Vliet et al., 2015; Yang et al., 2012). van Vliet et al. (2015), in their review, stated that consumption of soy protein has been shown to result in lower MPS rates than consumption of whey, milk, or beef protein. Other studies have confirmed that plant-based proteins have lower anabolic properties than milk proteins (Gorissen et al., 2016; Yang et al., 2012).

Not all animal-based protein sources are comparable in terms of protein quality properties (i.e., essential amino acids content and digestibility) (Table 2. 6). Milk proteins, for example, have a higher leucine content (10.9% of total essential amino acid) than beef proteins (8.8%) (van Vliet et al., 2015). Egg proteins have a higher PDCAAS value than beef and fish (Schaafsma, 2000; Smith & Gray, 2016; van Vliet et al., 2015). Leucine is an important amino acid regulator of protein synthesis (Garlick, 2005; Lynch, 2001), and a higher leucine content in a food suggests that a lower amount of protein from that source is required to maximise MPS rates, whereas highly digestible protein sources will provide a higher proportion of absorbed amino acids.

Table 2. 6. Digestibility and amino acid concentrations of common animal protein sources. Adapted from van Vliet et al. (2015).

Animal sources	PDCAAS	Essential amino acids, % total protein	Leucine, % total protein
Milk	1.00	49	10.9
Whey	1.00	52	13.6
Egg	1.00	44	8.5
Beef	0.92	44	8.8

PDCAAS = Protein Digestibility Corrected Amino Acid Score.

In conclusion, to maximally stimulate MPS, it is recommended that older adults should consume ≥ 1.2 g/kg BW of high-quality protein and distributed across the day at ~ 30 g or 0.4 g/kg BW per meal (Gorissen et al., 2016; Mamerow et al., 2014; Moore & Soeters, 2015; Paddon-Jones & Rasmussen, 2009; Rafii et al., 2015; van Vliet et al., 2015; Yang et al., 2012).

2.6. Dietary assessment of protein: intake, distribution, and sources

Dietary intake can be quantified using prospective or retrospective methods. Table 2. 7 summarises the different methods for assessing dietary protein as well as their advantages and disadvantages. Prospective methods include food diaries (estimated or weighed), direct observation, and duplicate portion collection and analysis. Retrospective methods include 24-hour recall, the food frequency questionnaire (FFQ), and dietary history (Dao et al., 2019; Gibson, 2005). Direct observation and duplicate portion collection and analysis are time consuming, expensive and are not feasible for use in research studies involving large numbers of participants. The food diary is the gold standard dietary assessment method, the most accurate in estimating actual and habitual dietary intakes and a valuable tool for recording older people's dietary protein intakes because it is less reliant on memory (Gibson, 2005). Portion sizes can be weighed or estimated at the time of consumption (i.e., in real time, not from memory) and for a specific period. The reliability of the food diary is increased when several days are recorded, however this does increase the burden on the participant and the researcher or clinician. For population surveys of older adults, single or repeated 24-hour diet recall are the most feasible option and is commonly used in large surveys. However, this method is not suitable for participants with memory issues or for measuring meals in the distant past or irregularly consumed foods. The burden on study participants of a FFQ is lower than for other dietary assessment methods, but it can challenge participants cognitively, especially with quantitative FFQs where participants do not consume food items in the amounts specified on the FFQ (Cade et al., 2002). The diet history can be used to estimate usual intakes of food and drink, but there are multiple methodologies available, and it tends to be used more in clinical rather than research settings.

Table 2. 7. Dietary assessment methods and their advantages and limitations.

Method	Description	Uses and benefits	Limitations
Prospective methods			
Observation of dietary intake	Observe and calculate the actual food and beverage amounts and portion sizes consumed	- Observation by trained personnel at the household or institution level - Does not necessitate literacy skills	- Not suitable for people who eat out frequently - Expensive - Highly intensive for researchers
Food diary	All food and beverage quantities and portion sizes consumed are estimated (often using household measures) or weighed at the time of consumption	- Can assess both habitual and actual dietary intakes - Does not rely on memory - Provides information on food amounts, preparation, and timing - Weighing provides accurate portion sizes	- Estimated food diary relies on ability to estimate food quantities - Highly intensive for researchers - Burdensome for participants
Duplicate portion	The participant collects an equal portion of everything he/she eats and drinks over one or more days and the nutrient content is chemically analysed	- Not affected by memory or errors in estimating portion sizes - Errors associated with the use of food composition databases are removed	- Burdensome for participants - Highly intensive for researchers - The chemical analysis of the nutrient content of the duplicate portion in the laboratory is time consuming resulting in high costs

Retrospective methods			
24-hour recall	Participants are asked to recall all foods and beverages consumed over the past 24 hours or the preceding day	- Relatively quick and easy for the participant - Suitable for large-scale surveys	- Relies on memory of the participant; older adults may be unreliable - Requires a skilled interviewer
Food frequency questionnaire (FFQ)	An interview or self-directed questionnaire is used to comprehensively assess food intake over a given period	- High response rate and low participant burden - Relatively inexpensive - Can be self-administered by mail or email	- Accuracy of nutrient intake is lower than for other methods - No meal timing data - Relies on participant memory
Dietary history	Interview conducted in 24-hour recall style to obtain usual intake and overall habitual eating patterns	- Provides a good description of usual food and nutrient intakes - Accounts for seasonal variation	- High cost of analysis - Results are dependent on the skills of the interviewer - Methodology is difficult to standardise

Following dietary data collection, energy and nutrient intake can be determined typically through using food composition tables or databases. Protein intake can be represented as absolute grams (g), grams per kilogram body weight (g/kg BW), grams per kilogram of ideal body weight (g/kg IBW), or as a percentage of total energy (%TE). The one-size-fits-all approach for absolute protein intake does not account for differences in body weight, and in particular the differences often observed in weight between sexes in older adults (Moore, 2019). Therefore, assessment of relative protein intake is more feasible and more sensitive to the differences in habitual protein intake when comparing sexes, due to differences in body mass.

Due to protein distribution being a relatively new concept there is no consensus regarding its calculation. Cross-sectional studies and cohort studies assessing the distribution of protein intake use several different methods. Protein distribution is generally calculated as the coefficient of variance (CV) of protein intake, the percent or average of total daily protein intake per main meal, or the number of meals exceeding a certain amount of protein. The most common measure to define the distribution of protein intake is the unitless coefficient of variation (CV = standard deviation of protein intake of the three meals (g) / total protein intake for the three meals (g)). A CV of 0 indicating completely even protein intake and a positive CV indicating a more uneven protein distribution. Although the CV provides information on consistency of distribution, it does not provide information on the amount of protein consumed. As a result, individuals with an even distribution but insufficient total protein intake may produce misleading results. The second most common method of categorising dietary protein distribution is counting the number of meals per day that contain a specific amount of protein: usually between 25 g and 30 g of protein per meal. However, this method does not consider variations in body weight. The frequency of consuming ≥ 0.4 g/kg BW of protein across the day's meal is the least commonly used method in the literature but appears to be the most practical calculation of protein distribution in cross-sectional and longitudinal studies.

To date, a variety of approaches have been used in the literature to define eating occasions (meals and snacks). Table 2. 8 outlines their advantages and disadvantages. There are three main approaches to defining meals (Leech et al., 2015a, 2015b). These include classification by time of day, food-based classification and participant self-identification (using a list of meal labels such as breakfast, brunch, and lunch) (Leech et al., 2015b). When participants provide the time of food intake, meals can be calculated using the peaks of food consumption across the day (Leech et al., 2015a). This method takes into consideration potential influences such as local or cultural factors.

Table 2. 8. Summary of different approaches used to define eating occasions (Leech et al., 2015a, 2015b).

Approach	Description	Advantages	Disadvantages
Time-of-day	Participants report the time of day they consumed a food	- Simple to apply and comprehend - Emphasis on when foods are consumed	- May be biased, because the time intervals used to define meals are frequently based on the researcher's own understanding of eating patterns
Food-based classification	Food is first classified into food categories based on its nutritional profile. The type of eating occasion is determined by food category combinations	- Provides information on eating event pattern as well as nutritional quality	- The categorisation system is complicated - Differences in meal classification criteria may limit cross-study comparisons
Participant-identified	Participants report their own eating occasion, usually from a list of meal labels provided	- Refrains from using a priori definitions of eating occasions	- No standardisation of definition - Meal labels may be influenced by the researcher's prior knowledge of eating patterns, introducing researcher bias

2.7. Protein intake, distribution, and sources in community-dwelling older adults

Prevalence of low protein intake

Despite some evidence indicating that the RDA is an underestimation of protein requirements in older adults (Campbell et al., 2001; Rand et al., 2003), low protein has often been defined as an intake below RDA of 0.8 g/kg BW. More recent evidence has indicated that protein recommendations for older adults should be set higher between 1.0 to 1.2 g/kg/BW (Bauer et al., 2013). However, only a few studies have used these higher cut-off values when assessing the prevalence of low protein intakes in older adults (Houston et al., 2017; Isanejad et al., 2016; Mendonça et al., 2018). Overall, the prevalence of low protein intake using the 1.0 g/kg BW cut-off values was 34% in older adults aged between 70 to 79 years and 54% in adults aged 85^{+} years. A higher prevalence of low protein intake of less than 1.2 g/kg BW was observed in older elderly aged 85^{+} years (75%) than their younger counterparts aged between 65 to 72 years (22%) (Table 2. 9).

Table 2. 9. Prevalence of low protein intake.

Author et al. (years) Country	**Dietary assessment method**	**Sex**	**N**	**Age (years)**	**Prevalence of protein less than 1.0 g/kg BW**	**Prevalence of protein less than 1.2 g/kg BW**
Isanejad et al. (2016) Finland	3-day food record	Female	554	65 to 72	-	22%
Houston et al. (2017) US	FFQ	Male and female	1998	70 to 79	34%	-
Mendonça et al. (2018) UK	24-hour multiple pass dietary recall	Male and female	845	85^{+}	54%	75%

Abbreviations: BW = body weight, FFQ = Food frequency questionnaire, UK = United Kingdom, US = United States.

Although the prevalence of low protein intake using these cut-off values among New Zealand community-dwelling older adults is not known, current evidence suggests that protein intake for a high proportion of older adults will be less than 1.0 or 1.2 g/kg BW (Bennett et al., 2017; Ram et al., 2020; University of Otago and Ministry of Health, 2011). In the most recent nationally representative investigation of dietary intake in adult New Zealanders, undertaken in 2008/2009, between 1.7% of women and 1.6% of men aged 50 to 70 years consumed less than the EAR of protein (0.60 g/kg BW for females and 0.68 g/kg BW for males). For those over 70 years of age, 13.4% of women and 15.5% of men consumed less than the EAR of protein (0.86 g/kg BW for women and 0.75 g/kg BW for men) (University of Otago and Ministry of Health, 2011). Further to this, among participants in the LiLACS NZ study, Bennett et al. (2017) found that, in Māori adults aged 81 to 91 years, 35% of males and 24% of females did not meet the recommended protein intake of 65 g for men and 46 g for women. In the same study, Ram et al. (2020) reported that, in Māori adults aged 81 to 91 years, 36% of females and 43% of males consumed had less than the recommended protein intake (0.75 g/kg BW for females and 0.86 g/kg BW for males), and in non-Māori adults (aged 85^{+} years), 28% of females and 29% of males consumed less than this recommended protein intake. In the same cohort, Wham et al. (2016) observed that the median weight-adjusted protein intake for Māori and non-Māori males was 1.05 and 0.98 g/kg BW per day, respectively, and for Māori and non-Māori women was 0.87 and 0.91 g/kg BW, respectively. These findings suggested that protein intake may be insufficient in a nationally representative NZ population, when compared to the research recommendations that range between 1.0 and 1.2 g/kg BW.

Total protein intake and sources of protein

Insight into the distribution and sources of protein throughout the day would provide valuable information for developing effective dietary interventions to increase protein intake in older adults (Hung et al., 2019; Mendonça et al., 2018; Ram et al., 2020). Hung et al. (2019) found that in older adults in the European Union lower protein intake was more common in people who did not consume dairy products. In addition, Mendonça et al. (2018) demonstrated that participants with a low protein intake living in UK obtained relatively less protein from meat, but more from cereals, when compared with those who had a high protein intake.

In the LiLACS NZ study, Ram et al. (2020) indicated that bread was the main source of protein for Māori women and non-Māori men, and for women who had a low protein intake. These findings suggested that low protein intake across the day may be associated with a lower intake of dairy and meat products, which are relatively higher in protein when compared to other foods.

Protein distribution across the day

As has been previously reported in the literature, an adequate protein distribution to maximise stimulation of the MPS rate in older adults has been observed at a protein intake of 30 g or 0.4 g/kg BW per meal evenly distributed across the day (Moore & Soeters, 2015; Paddon-Jones & Rasmussen, 2009). International studies indicate that older adults have a low adherence for 30 g or 0.4 g/kg BW per meal thresholds (Gaytán González et al., 2020; Loenneke et al., 2016). Gaytán González et al. (2020) reported that 41% in Mexican older adults consumed zero meals, 39% one meal and 20% two or three meals containing at least 0.4 g/kg BW of protein, and that 61% older adults consumed zero meals, 34% one meal, and 5% two or three meals containing at least 30 g of protein. Similarly, Loenneke et al. (2016) found that 32% of free-living older adults in the US consumed zero meals, 52% one meal and 16% two or more meals containing at least 30 g of protein. To our knowledge, no other study has been published to date on meeting protein levels of 30 g or 0.4 g/kg BW per meal in older adults living in New Zealand. However, Ram et al. (2020) illustrated that the median protein intake (g/meal) for men but not women at the dinner meal was > 30 g protein for both Māori and non-Māori men i.e., 35.2 (20.1, 54.1) and 33.5 (21.9, 45.6) respectively.

In most Western countries, protein intake is unevenly distributed throughout the day (Bennett et al., 2017; Gaytán González et al., 2020; Gingrich et al., 2017; Paddon-Jones et al., 2015; Ram et al., 2020; Rousset et al., 2003). In older adults in New Zealand (Bennett et al., 2017; Ram et al., 2020), Netherlands (Tieland et al., 2015), the United Kingdom (Cardon-Thomas et al., 2017), and the United States (Paddon-Jones et al., 2015) the highest amount of protein is consumed at dinner.

However, in older adults in Mexico (Gaytán González et al., 2020), Germany (Gingrich et al., 2017), and France (Rousset et al., 2003) the highest amount of protein is consumed at lunch. Variation can be explained by the cultural and eating habits of each country.

Distribution and sources of protein

Evaluating protein sources at each meal could be an important step towards developing novel approaches for increasing protein intake per meal in older adults (Smeuninx et al., 2020; Tieland et al., 2015). Tieland et al. (2015) found that bread was the primary source of total protein intake in the community-dwelling in Dutch older adults at breakfast (44%) and lunch (23%); meat products contributed 36% of total protein intake at dinner. Furthermore, Smeuninx et al. (2020) observed that milk (68%) was the primary source of protein consumed at breakfast, bread (33%) for lunch, and meat (33%) for dinner. These findings suggested that bread is a common source of protein at breakfast and lunch, which could explain the low protein intake at these mealtimes in older adults. However, data on both the intake of specific sources of protein and the timing of intake of these protein sources are lacking in older adults living in New Zealand.

Sources of protein

Most dietary protein consumed worldwide is derived from plants (60%) (Faostat, 2016). Cereals are the most important plant-based food source, accounting for approximately 65% of plant proteins and 40% of total protein intake. Importantly, the contribution of plant and animal-based protein sources to total daily protein intake vary by continent or country of interest. Plant-based foods provide 77 and 66% of total protein intake in Africa and Asia, respectively, whereas animal-derived food sources contribute more to total dietary protein intake in the United States (56%), Europe (57%), and Oceania (65%) (Gorissen & Witard, 2018). Meat and dairy sources contribute the most to daily animal protein intake in the United States, Europe, and Oceania, whereas cereals contribute the most in Africa and Asia (Gorissen & Witard, 2018). Three food groups accounted for roughly three quarters of total protein intake in the Dutch National Food Consumption Survey-Older Adults (DNFCS).

These were 'meat and meat products' (28%), 'dairy products' (25%), and 'cereals and cereal products' (20%). The first two food groups contributed the most animal protein (43% and 40%, respectively), while 'cereals and cereal products' contributed the most plant protein (55%) (Ocke et al., 2013). In contrast, the 2008/2009 New Zealand Nutrition Survey found that the principal source of protein in the diet of older New Zealanders was all types of bread, including bagels, crumpets, and sweet buns (11.5% and 11.2% for males and females 50 to 70 years, respectively, and 14.3% and 14.2% for males and females older than 70 years, respectively) (University of Otago and Ministry of Health, 2011). However, the ability to compare protein sources between these two national surveys is difficult, given the differing categorisations of food groups. For example, bread appears to be high in protein due to how the bread food group were classified (i.e., bread-based dishes such as chicken sandwiches etc.) and meat sources appeared low contributors to protein intake because they were split into separate categories (beef, pork, poultry) in the NZ survey, whereas meat proteins were higher in the Dutch survey because all meat sources were combined into one category.

2.8. Protein intake, distribution, and sources; muscle mass; and muscle strength

2.8.1. Association between protein intake, muscle mass and muscle strength

Association between protein intake and muscle mass

Findings from cross-sectional and longitudinal studies on the association between protein intake and measure of muscle mass in older adults are consistent. In a cross-sectional study among older adults aged ≥ 50 years, Morris and Jacques (2013) found a positive association between protein intake and ALM in obese participants who met or exceeded the RDA for protein of 0.8 g/kg BW, but not in non-obese participants. This benefit accrued significantly in participants who engaged in muscle-strengthening exercise. Using the RDA of 46 g for women and 64 g for men, a similar positive association was reported between protein intake and leg LM among participants who met or exceeded the recommendation (Sahni et al., 2015).

These results suggest that achieving or exceeding the RDA for protein should have a significant effect on preserving muscle mass in older adults, particularly if accompanied with muscle-strengthening exercise. In a longitudinal study, Houston et al. (2008) found that older adults (aged 70 to 79 years) who consumed less than 0.8 g/kg BW of protein had 40% greater loss of muscle mass at a three-year follow-up compared with those who had a protein intake greater than 1.2 g/kg BW. In a Tasmanian cohort, older adults who did not meet the Australian and New Zealand recommendation of protein intake (64 and 81 g for men aged 51 to 70 and > 70 years, respectively, and 46 and 57 g for women in the same age groups) had 0.79 kg less muscle mass at a 2.6-year follow-up than those who met the protein intake recommendation (Scott et al., 2010). These results indicate that meeting the Australian and New Zealand RDI for protein is associated with a reduced rate of decline in muscle mass in older adults. However, as stated earlier older adults might benefit from daily higher protein intake of 1.2 g/kg BW than the current Australian and New Zealand RDIs for protein.

To further study the effect of increased protein intake on muscle mass in older persons, interventions in the form of whole meal or supplements have been undertaken. Over a 12-week period, Ten Haaf et al. (2019) found that older adults supplemented with 1.3 g/kg BW/day of protein increased muscle mass more than a placebo group who consumed 0.9 g/kg BW/day. In another study, Park et al. (2018) observed that older adults aged 70 to 85 years who consumed 1.5 g/kg BW/day of protein had significantly more muscle mass than those who consumed between 0.8 and 1.2 g/kg BW/day protein. Similarly, a randomised controlled trial found that older males following a diet providing twice the current Australian and New Zealand recommendation of protein (i.e., 1.6 g/kg BW/day) did not lose muscle mass, but instead increased it by 1.49 kg over 10 weeks (Mitchell et al., 2017).

Collectively, these findings suggest that greater consumption of protein might benefit older persons in terms of muscle mass. Longer-term research, on the other hand, have yielded contradictory results. Tieland et al. (2012) for example, found no effect on muscle mass after 24 weeks of supplementing with 15 g of milk protein at breakfast and lunch. Furthermore, there was no effect of increasing protein consumption to 1.3 g/kg on lean body mass during a 6-month period when compared to intakes of 0.8 g/kg of protein (Bhasin et al., 2018).

Long-term muscle mass improvements in these trials may be affected by factors other than protein quantity, such as protein distribution or protein sources consumed, which directly influence MPS response.

Association between protein intake and muscle strength

The ability for absolute or relative protein intake to predict muscle strength appears to be affected by sex and whether muscle strength is expressed in absolute or relative terms. Absolute protein intake (g) was found to be positively associated with absolute muscle strength (kg) in a longitudinal study that pooled their findings for men and women (McLean et al., 2016). When stratified by sex, this association remains significant for women but not for males (Mishra et al., 2018; Robinson et al., 2008). Sex differences in the relationship between absolute protein intake and absolute muscle strength observed in these studies may be explained by sex differences in muscle strength (Oksuzyan et al., 2010). More research is needed to determine the exact mechanism underlying the effect of sex disparities on associations between protein intake and muscle strength. In other studies, associations between relative protein intake and standardised muscle strength by BW or BMI have been observed (Celis-Morales et al., 2018; Fanelli Kuczmarski et al., 2018; Isanejad et al., 2016), but not with absolute muscle strength (kg) (Granic et al., 2018; Mishra et al., 2018; Ten Haaf et al., 2018; Wham et al., 2021). One exception was the Women's Health Initiative Study, where a higher biomarker-calibrated protein intake (1.18 g/kg BW) was associated with greater absolute muscle strength at baseline, and with reduced rates of decline in absolute handgrip strength (HGS) at a mean follow-up of 11.5 years (Beasley et al., 2013). The conflict between the findings of this study and others may be partially due to other research assessing protein intake using FFQ or food record measurements, whereas this study used a more accurate method of assessing protein intake. Protein intake calibration was derived from regression equations based on baseline FFQ data from the entire cohort, and doubly labelled water and 24-hour neutron activation quantified body nitrogen data collected from a representative sample as reference measures.

Overall, these findings suggest that higher absolute or calibrated protein intake is associated with higher absolute muscle strength, while higher relative protein intake is associated with higher relative muscle strength in older adults (Table 2. 10). As previously stated, the relative protein intake (g/kg) and muscle strength adjusted BW or BMI considered inter-individual variability in weight or obesity status, which may support the idea that muscle strength should be adjusted with BW or BMI when investigating its association with relative protein intake.

Table 2. 10. Associations between protein intake and handgrip strength in older adults.

Author et al. (years) Country	Muscle strength and dietary protein assessment methods	Study design	Sex	N	Age (years)	Key findings
Association between absolute protein intake and absolute handgrip strength						
Robinson et al. (2008) UK	Handgrip Jamar dynamometer FFQ	Cross-sectional	Male and female	2980	59 to 73	Grip strength (kg) was positively associated with protein intake (g/day) in females but not in males
McLean et al. (2016) US	Handgrip Jamar dynamometer FFQ	Prospective study over 6 years	Male and female	1764	29 to 85	Protein intake (g/day) was associated with reduced loss of strength (kg) when controlled for sex
Mishra et al. (2018) US	Handgrip Jamar dynamometer 24-hour multiple recall	Cross-sectional	Male and female	4123	51^{+}	Grip strength (kg) was positively associated with protein intake (g/day) in females but not in males

Association between relative protein intake and absolute muscle strength						
Beasley et al. (2013) US	Handgrip Jamar dynamometer FFQ 24-hour urinary nitrogen	Prospective study, with a mean 11.5 follow-up	Female	5346	50 to 79	Grip strength (kg) was positively associated with biomarker-calibrated protein intake (g/kg)
Mishra et al. (2018) US	Handgrip Jamar dynamometer 24-hour multiple recall	Cross-sectional	Male and female	4123	51+	Grip strength (kg) was not associated with protein intake (g/kg) in males or females
Ten Haaf et al. (2018) Netherlands	Handgrip Jamar dynamometer 24-hour multiple recall	Cross-sectional	Male and female	140	65+	Grip strength (kg) was not associated with protein intake (g/kg) when controlling for sex
Granic et al. (2018) UK	Handgrip Jamar dynamometer 24-hour multiple recall	Prospective study over 5 years	Male and female	722	85+	No association was found between protein intake (g/kg) and subsequent decline in muscle strength (kg) when controlling for sex
Højfeldt et al. (2020) Denmark	Handgrip Jamar dynamometer Quadricep muscle strength 3-day food record	Cross-sectional	Male and female	184	65+	Grip strength (kg) and quadricep muscle were not associated with protein intake (g/kg) in males or females

Wham et al. (2021) New Zealand	Handgrip Jamar dynamometer 24-hour multiple recall	Prospective study over 5 years	Male and female	554	81 to 91	Protein intake (g/kg) did not protect against loss of muscle strength (kg) when controlling for sex
Association between relative protein intake and relative muscle strength						
Isanejad et al. (2016) Finland	Handgrip Jamar dynamometer 3-day food record	Prospective study over 3 years	Female	554	65 to 72	Higher protein intake (g/kg) was associated with less decline in muscle strength adjusted BM
Celis-Morales et al. (2018) UK	Handgrip Jamar dynamometer 24-hour multiple recall	Cross-sectional	Male and female	146,816	40 to 69	Protein intake (g/kg) was associated with muscle strength adjusted BW in males and females
Fanelli Kuczmarski et al. (2018) US	Handgrip Jamar dynamometer 24-hour multiple recall	Cross-sectional	Male and female	2468	33 to 71	Protein intake (g/kg) was associated with muscle strength adjusted BMI when controlling for sex

Abbreviations: BM = body mass, BW = body weight, BMI = body mass index, FFQ = Food frequency questionnaire.

2.8.2. Associations between protein distribution, muscle mass, and muscle strength in older adults

The association between protein distribution and muscle mass

A recent meta-analysis concluded that protein distribution tends to be associated with muscle mass in older adults (Jespersen & Agergaard, 2021). Cross-sectional studies investigating associations between protein distribution and muscle mass have shown mixed results. Cross-sectional studies in older adults with small sample sizes (ranging from 97 to 184 participants) have not found significant relationships between protein distribution and muscle mass (Gingrich et al., 2017; Højfeldt et al., 2020; Ten Haaf et al., 2018; Valenzuela et al., 2013). These cross-sectional studies have defined protein distribution as CV, or as number of meals providing ≥ 0.4 g/kg BW or > 25 g of protein per meal (Gingrich et al., 2017; Højfeldt et al., 2020; Ten Haaf et al., 2018; Valenzuela et al., 2013). However, cross-sectional studies with larger numbers of participants have demonstrated a relationship between the protein distribution and muscle mass (Farsijani et al., 2016; Loenneke et al., 2016). A cross-sectional study of 1081 older adults (aged 50 to 85 years) found those who consumed two or more meals with at least 30 g protein showed higher leg muscle mass than those who consumed zero or one meals with 30g protein (Loenneke et al., 2016). Another cross-sectional study of 351 men and 361 women (aged 67 to 84 years) found a positive association between an even distribution of protein intake (CV ≤ 0.33) and muscle mass in males but not in females (CV ≤ 0.37). However, in the same cohort even protein intake distribution did not significantly prevent the decline of muscle mass when measured over 2 years (Farsijani et al., 2016). As indicated previously, the CV might give information about evenness, but it does not provide information about the amount of protein consumed. Thus, the consumption of meals containing less than 30 g or 0.4 g/kg, required for maximal MPS in older adults may be a potential explanation for this finding. A recent cross-sectional study found that consuming at least 0.4 g/kg of protein during the breakfast, lunch, and dinner meals was related to muscle mass in older females aged 60 and older (Johnson et al., 2022).

A recent randomised controlled trial with 14 participants found that, after an 8-week intervention involving an even (29 g or 0.39 g/kg of protein at each meal) or skewed (breakfast, lunch, and dinner each contain 13, 18, and 56 g of protein, respectively) dietary protein distribution, there was no difference between lean mass before and after the trial, and the different protein distribution patterns did not affect lean mass (Kim et al., 2018). It has been suggested that the null effect was due to sex differences in MPS.

The association between protein distribution and muscle strength

In their meta-analysis, Jespersen and Agergaard (2021) demonstrated that protein distribution was not likely to be associated with muscle strength in older adults. However, the application of different methods used to estimate muscle strength and different definitions of protein distribution makes it difficult to compare studies.

Using the CV to define protein distribution, Farsijani et al. (2017) found that a more even protein distribution was significantly associated with muscle strength scores at a 3-year follow-up for both men and women. In contrast, cross-sectional studies (Bollwein et al., 2013; Gingrich et al., 2017; Højfeldt et al., 2020; Ten Haaf et al., 2018) and a randomised controlled trial study (Kim et al., 2018) found no association between evenness of protein distribution and handgrip strength in older adults.

Using the threshold value of 30 g or 0.4 g/kg BW per meal, Loenneke et al. (2016) reported that older adults consuming two or more meals with at least 30 g protein showed higher knee extensor strength than those consuming zero or one meals containing this much protein. Another study recently found an association between muscle strength and the frequency of consuming ≥ 0.4 g/kg of protein across the day's meal in female older adults (Johnson et al., 2022). In contrast, Mishra et al. (2018) found that more frequent consumption of meals containing at least 25 g or 30 g of protein was not associated with increased handgrip strength after adjusting for potential confounders. Similarly, a recent study found no correlation between the frequency of consumption of a meal containing at least 0.4 g/kg BW or 30 g of protein and handgrip strength in older adults (Gingrich et al., 2017).

The two approaches in estimation of protein distribution may contribute to the inconsistent results regarding associations between protein distribution and muscle strength in older adults. To elucidate the relationship between protein distribution and muscle strength, there is a need for a study investigating the associations between one measure of muscle strength and different estimates of protein distribution commonly used in the literature.

2.8.3. Associations between protein sources, muscle mass, and muscle strength in older adults

The association between sources of protein and muscle mass

Different findings between studies conducted in Chinese and Western populations regarding the association between protein sources and muscle mass in older adults have been observed. In Western populations, a cross-sectional study in women aged 65 to 71 years found that intake of animal protein was associated with greater muscle mass, whereas intake of plant-based protein was not (Isanejad et al., 2015). A similar result was found in another cross-sectional study by Sahni et al. (2015) in men and women aged between 29 and 86 years. In the Health ABC Study, a longitudinal study among participants aged 70 to 79 years old, it was found that animal protein sources were more efficient in maintaining muscle mass than plant protein sources (Houston et al., 2008). In the Chinese population, it was found that those who consumed large amounts of protein from plant sources had higher muscle mass after four years, whereas there was no association with animal sources of protein (Chan et al., 2014). Recently, Li et al. (2019) found that higher total, animal, and plant protein intakes, regardless of animal-to-plant protein ratio, were associated with greater muscle mass in older adults. These discrepancies between results could be due to the quantity of different sources of protein consumed. In population studies in China, participants consumed more protein from plant-based sources, whereas in Western countries the range of sources was much greater for animal-based protein (Table 2. 11). These findings suggest that source of protein is not as important as total protein intake in determining muscle mass.

Randomised clinical trials have also been conducted to investigate the effects of a specific source of protein on muscle mass in older adults. Alemán-Mateo et al. (2014) showed that daily 210 g of ricotta cheese supplementation increased muscle mass in older men and women aged 60 or over. Likewise, another study found that fortified dairy supplements (250 ml of milk and to 200 g of yoghurt) resulted in a lower decline in midarm muscle circumference and a greater increase in the percentage of change in leg muscle mass in women aged 55 to 65 years (Manios et al., 2009). These findings suggest that supplementing with dairy products may help older adults maintain muscle mass.

Table 2. 11. The association between sources of protein and muscle mass

Author et al. (years) Country	**Study design**	**Sex**	**N**	**Age (years)**	**Total protein intake**	**Protein from animal-based**	**Protein from plant-based**	**Key findings**
Western populations								
Isanejad et al. (2015) Finland	Prospective study over 3 years	Female	554	65 to 72	92.0 ± 10.5 g	54.3 ± 6.7 g	*24.1 ± 4.2 g	Animal protein was associated with increased muscle mass, whereas plant-based protein consumption was not.
Sahni et al. (2015) US	Prospective study over 4 years	Male and female	2675	29 to 86	80 ± 27 g men 76 ± 26 g women	55 ± 22 g men 53 ± 21 g women	24 ± 9 g men 23 ± 9 g women	Participants in the highest quartiles of animal protein intake had greater leg lean mass than those in the lowest quartiles of intake. Plant protein intake was not associated with leg lean mass
Houston et al. (2008) US	Prospective study over 3 years	Male and female	2066	70 to 79	91.0 ± 27.1 g	60.7 ± 20.9 g	30.3 ± 12.5 g	Animal protein sources were more effective than plant protein sources in preserving muscle mass

Chinese populations								
Chan et al. (2014) China	Prospective study over 4 years	Male and female	2726	65+	-	1.21 ± 0.48 g/kg	0.89 ± 0.36 g/kg	After four years, older adults who consumed a high protein diet from plants had more muscle mass, whereas there was no association with animal protein sources
Li et al. (2019) China	Cross-sectional	Male and female	3213	40 to 80	109.1 ± 22.7 g 2.04 ± 0.35 g/kg	62.1 ± 18.3 g 1.16 ± 0.30 g/kg	47.0 ± 15.6 g 0.88 ± 0.28 g/kg	Animal and plant protein consumption were associated with higher muscle mass in older adults

*Protein from vegetable. Plant protein sources include cereals, vegetables, and fruits.

The association between sources of protein and muscle strength

Out of the studies that have investigated the association between protein intake and muscle strength, only a few have examined the association between protein sources and muscle strength in older adults. Isanejad et al. (2015) reported that higher intake of animal-based proteins (but not plant-based proteins) was associated with greater muscle strength in women aged 65 to 71 years. This finding was subsequently confirmed by McLean et al. (2016). Recently, Pikosky et al. (2022) demonstrated that increasing quartiles of both animal and plant dietary proteins were associated with increased handgrip strength in older adults. However, the increase became significant at a lower quartile for animal protein than for plant protein.

These findings suggested that a higher total protein intake from plant-based protein foods compared to animal, is required to influence muscle strength. Thus, animal and plant-based protein may be associated with greater muscle strength in older adults if there is a high protein intake from both sources.

2.9. Conclusions from the literature

Both globally and in New Zealand, the proportion of older adults is increasing. In the ageing New Zealand population, falls are both the most common and the costliest cause of injury. Muscle weakness has been identified as the biggest intrinsic risk factor for falls in older adults. To date, few studies have addressed the prevalence of low muscle strength in older adults and those that have use different cut-off values for handgrip strength measurement.

Ageing is accompanied with increased anabolic resistance to protein ingestion, resulting in loss of muscle mass in older adults. Age-related muscle mass loss has been proposed as a major factor in the strength decline seen with ageing. Changes in muscle mass, on the other hand, appear to account for only a part of the changes in strength observed in older adults. Age-related muscle mass loss is also accompanied by an increase in adiposity, both of which impair anabolic responses to protein ingestion. As a result, obesity may interact with the relationship between muscle mass and muscle strength, resulting in varying degrees of association.

The anabolic response to protein ingestion is impacted on by the amount, source, and distribution of dietary protein consumed, which varies with age, sex, ethnicity and level of physical activity. It is recognised that older adults should consume 1.2 g/kg BW of high-quality protein throughout the day (30 g or 0.4 g/kg BW per meal) to maximise muscle MPS. Protein intake for a high proportion of older adults was found to be less than 1.2 g/kg BW, insufficient for reaching 30 or 0.4 g/kg BW, and from low-quality sources at breakfast and lunch in the international literature. To the best of our knowledge, no other studies have investigated protein intake of less than 1.2 g/kg BW, 30 g or 0.4 g/kg BW/meal, and the sources of protein at eating occasions in older adults living in New Zealand.

It is widely acknowledged that in conjunction with both aerobic and resistance training older adults who consume high-quality protein throughout the day, with two or more meals containing at least 30 g of protein from animal and/or plant sources, maintain or gain muscle mass. However, the relationships between protein intake, sources, and distribution throughout the day and muscle strength in older adults are unclear. Some findings suggest that dietary protein is related to muscle strength, while others do not or are inconclusive. While protein is essential for the regulation of muscles, it is only one component of a well-balanced diet rich in nutrient-dense foods. Further research is needed to investigate this.

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Chapter Three – Body composition associations with muscle strength in older adults living in Auckland, New Zealand

Aging and obesity impair MPS, which is associated with muscle mass and muscle strength loss in older adults. This chapter is a manuscript based on an open access article published in the journal PLoS ONE that discussed the relationship between muscle mass and muscle strength in older adults living in Auckland, New Zealand, while accounting for obesity (Received: November 6, 2020; Accepted: April 7, 2021; Published: May 28, 2021).

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The formatting, layout, and referencing style of the published manuscript have been modified to align with this thesis.

3.1. Abstract

Background: Ageing is associated with decreases in muscle strength and simultaneous changes in body composition, including decreases in muscle mass, muscle quality and increases in adiposity.

Objective: Examine the relationship between muscle strength, muscle mass, and body fat percentage in older adults living in Auckland, NZ.

Methods: Adults (n = 369; 236 females) aged 65 to 74 years living independently were recruited from the cross-sectional Researching Eating Activity and Cognitive Health (REACH) study. Body fat percentage and appendicular skeletal muscle mass (ASM) (sum of lean mass in the arms and legs) were assessed using dual-energy X-ray absorptiometry (Hologic, QDR Discovery A). The ASM index was calculated by ASM (kilograms) divided by height (meters) squared. Isometric grip strength was measured using a hand grip strength dynamometer (JAMAR HAND).

Results: Linear regression analyses revealed that muscle strength was positively associated with the ASM index ($r^2 = 0.431$, $\rho < 0.001$). When exploring associations between muscle strength and muscle mass according to obesity classifications (obesity ≥ 30% males; ≥ 40% females), muscle mass was a significant predictor of muscle strength in non-obese participants. However, in participants with obesity, muscle mass was no longer a significant predictor of muscle strength.

Conclusions: Body fat percentage should be considered when measuring associations between muscle mass and muscle strength in older adults.

3.2. Introduction

Globally and in New Zealand the proportion of older adults is increasing (United Nations, 2019). In 2006, 12.3% of New Zealand's population was older than 65 years; this percentage is expected to increase to 19.9% by the year 2026 and to more than 26.3% by 2051 (Statistics New Zealand, 2007).

In the ageing New Zealand population, falls are both the most common and costliest cause of injury (Robertson & Campbell, 2012). In 2016 the rate of one or more Accident Compensation Corporation (ACC) claims for a fall-related injury was 216 per 1,000 in people aged 50 and over. People aged 85 and over were twice as likely as 50 to 64 years old to have an ACC claim for a fall-related injury (Health Quality & Safety Commission, 2016).

Evidence pooled from a large number of studies has shown that one of the most common risk factors for falls is low muscle strength (Robbins et al., 1989; Rubenstein, 2006; Rubenstein & Josephson, 2002). Muscle strength refers to the amount of force a body can produce to perform normal daily household, work related, and recreational activities. Muscle strength increases with age and then significantly decreases after 40 and 50 years old for women and men, respectively (Schlussel et al., 2008).

Ageing is also associated with changes in body composition, including decreases in muscle mass, muscle quality and increases in adiposity (Gallagher et al., 2000; Goodpaster et al., 2006). These changes occur simultaneously with a decline in muscle strength.

Cross-sectional studies have demonstrated that muscle mass is correlated with muscle strength (Chen et al., 2013; Hayashida et al., 2014; Reed et al., 1991). However, longitudinal studies have shown that changes in muscle mass explain only a small portion (~5%) of the changes in strength in older adults (Goodpaster et al., 2006; Hughes et al., 2001). This indicates that other factors may preserve muscle strength during ageing, one of which may be age-related changes in adiposity.

The prevalence of obesity in older adults living in New Zealand has increased (Ministry of Health, 2015; Ministry of social development, 2016). Body Mass Index (BMI) is commonly used to assess obesity as it is easily measured and does not require costly equipment. One cross-sectional study found that muscle strength was positively associated with BMI in underweight, normal, overweight and obese older men and women (Keevil et al., 2015). In contrast, another study found low muscle strength had a negative association with BMI (overweight/obese) in older adults (Borges et al., 2020). BMI has poor diagnostic accuracy for identifying older adults with obesity (Batsis, Mackenzie, et al., 2016; Batsis & Zagaria, 2018), which might account for the contrary findings. Body fat percentage (% BF) is a more accurate reflection of obesity in older adults. Several studies have demonstrated that higher body fat percentage is associated with lower muscle strength (Goodpaster et al., 2006; Kim et al., 2017) and lower muscle mass in older adults (Visser et al., 2005; Yoo et al., 2014). This finding suggests that body fat percentage may contribute to the relationship between muscle strength and muscle mass.

Body composition is related to muscle strength, however there is very limited evidence regarding the contribution of obesity classification based on body fat percentage when investigating the relationship between muscle strength and muscle mass in older adults. The aim of this research was to examine the relationship between muscle strength, muscle mass, and body fat percentage in older adults living in Auckland, NZ.

3.3. Materials and methods

3.3.1. Study design

This study was a secondary aim of the Researching Eating, Activity and Cognitive Health (REACH) Study. The main objective of the REACH Study was to investigate dietary patterns and associations with cognitive function and metabolic syndrome in older adults. Ethical approval was granted by the Massey University Human Ethics Committee: Southern A, Application 17/69 and all participants provided written informed consent.

Further information regarding the REACH study protocol can be found elsewhere (Mumme et al., 2019). The study took place in the Human Nutrition Research Unit at Massey University's Auckland Campus, New Zealand (NZ).

3.3.2. Study participants and procedures

Participants included men and women aged 65 to 74 years, living independently in Auckland, NZ. Exclusion criteria were a diagnosis of dementia or any condition which may impair cognitive function (i.e., traumatic head injury, stroke), medication which may influence cognitive function, colour blindness, or any other event in the last two years which had a substantial impact on dietary intake or cognitive function. Participants who registered their interest in the REACH study were provided with an information sheet and completed an online screening questionnaire to determine their eligibility to take part. If the inclusion criteria were met, participants were invited to participate in the study.

3.3.3. Data collection

All participants visited the Human Nutrition Research Unit on one occasion for collection of data as part of the wider REACH study. Socio-demographic information including age and sex were collected through written questionnaires. Data quality was ensured by checking questionnaires for completeness.

Height and weight measurements were undertaken using standardised techniques adapted from the International Society for the Advancement of Kinanthropometry (ISAK) protocol. Height was measured to the nearest 0.1 cm using a stadiometer (SECA). Weight was measured with the participant in light clothing, using floor scales (Wedderburn) (Appendix D). BMI was calculated using body weight in kilograms divided by height (metres) squared.

Body composition values were ascertained from a total body dual-emission X-ray absorptiometry (DXA) scan (Hologic, QDR Discovery A). The machine was checked and calibrated daily in line with the standard operating procedure recommendations.

All scanning and analysis procedures were performed by a trained operator. After removal of shoes and jewellery, participants adopted a supine position with arms to the side (Thurlow et al., 2018). Participants were then scanned as per established recommendations (Nana et al., 2012; Sawka et al., 2007), with the standard mode scan taking approximately eight minutes to complete. The values for body composition outcomes were determined from the ratio of soft tissue attenuation of two X-ray energy beams for each pixel containing a minimal amount of soft tissue but no significant bone. Body fat percentage was calculated by dividing total fat mass by the sum of bone, lean and fat mass (Kim et al., 2017). Regional analyses were performed and appendicular skeletal muscle mass (ASM) was calculated as the sum of mineral-free lean mass of the arms and legs (Heymsfield et al., 1997; Shafiee et al., 2018).The ASM index was calculated by ASM (kilogram) divided by height (meters) squared (Baumgartner et al., 1998). Low muscle mass was defined as an ASM index < 7 kg/m^2 (men) and < 5.5 kg/m^2 (women) (Cruz-Jentoft et al., 2019).

Isometric grip strength was measured in both hands using an adjustable hand grip strength dynamometer (JAMAR HAND) (Roberts et al., 2011). The participant was seated on a standard straight back chair without arm rests, and with elbow, hips and knees at 90° angles (Appendix E). All participants were instructed to squeeze the handle as hard as they could upon a verbal signal from the researcher. Verbal encouragement was provided throughout the period of effort which did not exceed 10 seconds. Three measurements were taken for each hand, alternating right/left to permit muscular recovery between replicate trials. Results were recorded in kilograms (kg), the mean of three trials for each hand was recorded and the highest value of the two means was used for further analyses (Mathiowetz et al., 1985; Roberts et al., 2011). Low muscle strength was defined as hand grip strength < 27 kg in men and < 16 kg in women (Cruz-Jentoft et al., 2019).

3.3.4. Statistical analysis

Continuous data were assessed for normality using Shapiro Wilcoxon tests and visual assessment of histograms. Descriptive statistics were reported as means ± SD for parametric data, and frequencies and percentages for categorical data.

Differences between groups were analysed using independent t-tests for parametric data, and the chi-square test of independence for categorical data.

The measurements were categorised into two groups according to sex. The measurements were further categorised according to body fat percentage into two groups. Using body fat percentage, obesity categories were defined as ≥ 30% fat (males) and ≥ 40% fat (females) (Waters et al., 2010). Classification of obesity using BMI was according to the categories suggested by the World Health Organization: underweight (BMI < 18.5kg/m^2), normal weight (18.5–24.9), overweight (25–29.9), and obese (30$^+$) in both men and women (Cole et al., 2000; World Health Organization Consultation on Obesity, 1998).

A multiple linear regression analysis was performed to determine body composition parameters predicting muscle strength in males and females. Adjusted r, standard error values, and multicollinearity statistics were used to identify the most appropriate equations. This analysis was undertaken in males and females according to obesity classifications based on body fat percentage.

All statistical analyses were completed using IBM SPSS Statistics for Windows, version 27.0 (IBM SPSS, Armonk, NY, USA). Results were considered significant at $\rho < 0.05$.

3.4. Results

Three hundred and sixty-nine participants (n = 236 females) were included in the analyses. Descriptive statistics for the study population according to sex are presented in Table 1. The mean ± SD age of participants was 69.7 ± 2.6 years. Males were taller, heavier and had a lower body fat percentage than females, but these differences were not significant. The mean BMI for this study population fell in the overweight BMI category (26.34 ± 4.6 kg/m^2). Using BMI categories, 16.5% and 16.1% males and females were classified as obese. In males and females, 9.8% and 23.7% respectively were classified as obese using body fat percentage categories (Waters et al., 2010). The prevalence of low muscle mass was 2.3% and 6.4%, and the prevalence of low muscle strength was 1.5% and 4.7% in males and females, respectively.

Appendicular skeletal muscle mass index and muscle strength were higher in males compared with females; this difference was significant for muscle strength only (Table 3. 1).

Table 3. 1. Characteristics of study participants by sex [a,b].

Characteristics		**Total**	**Males**	**Females**	**ρ-value**
		n = 369	n = 133	n = 236	
Age (years)		69.67 ± 2.57	70.16 ± 2.42	69.39 ± 2.62	0.151
Height (m)		1.67 ± 0.09	1.76 ± 0.07	1.63 ± 0.06	0.184
Weight (kg)		73.98 ± 15.05	83.09 ± 13.85	68.84 ± 13.18	0.578
BMI (kg/m^2)		26.34 ± 4.59	26.85 ± 4.01	26.06 ± 4.87	0.066
BMI categories n (%)	Underweight	3 (0.8)	1 (0.8)	2 (0.8)	0.019*
	Normal weight	148 (40.1)	40 (30.1)	108 (45.8)	
	Overweight	158 (42.8)	70 (52.6)	88 (37.3)	
	Obese	60 (16.3)	22 (16.5)	38 (16.1)	
Body fat percentage (%)		31.84 ± 7.47	24.48 ± 4.41	36.0 ± 5.34	0.067
Body fat percentage categories n (%)	Non-obese	299 (81.0)	120 (90.2)	179 (75.8)	< 0.01**
	Obese	69 (18.7)	13 (9.8)	56 (23.7)	
ASMI (kg/m^2)		7.59 ± 1.38	8.89 ± 7.59	6.85 ± 0.96	0.644
ASMI categories n (%)	Normal	351 (95.1)	130 (97.7)	221 (93.6)	< 0.01**
	Low	18 (4.8)	3 (2.3)	15 (6.4)	
Grip strength (kg)		30.56 ± 9.78	40.97 ± 7.67	24.69 ± 4.55	< 0.01**
Grip strength categories n (%)	Normal	356 (96.5)	131 (98.5)	225 (95.3)	< 0.01**
	Low	13 (3.5)	2 (1.5)	11 (4.7)	

[a]Continuous values are expressed as mean ± standard deviation. [b]Categorical values are expressed as frequency (percentage). Sex difference at **$\rho < 0.01$, *$\rho < 0.05$. BMI = body mass index. ASMI = appendicular skeletal muscle mass index. Classification of obesity using BMI was according to categories suggested by the World Health Organization: underweight (BMI < 18.5kg/m^2), normal weight (18.5–24.9), overweight (25–29.9), and obese (30$^+$) in both men and women. Using body fat percentage, obesity categories were defined as ≥ 30% fat (males) and ≥40% fat (females). Low muscle mass was defined as an ASM index < 7 kg/m^2 (men) and < 5.5 kg/m^2 (women). Low muscle strength was defined as handgrip strength < 27 kg in men and < 16 kg in women.

In both males and females, muscle strength was positively associated with appendicular skeletal muscle mass index (r^2= 0.431, $\rho < 0.001$) (Figure 3. 1).

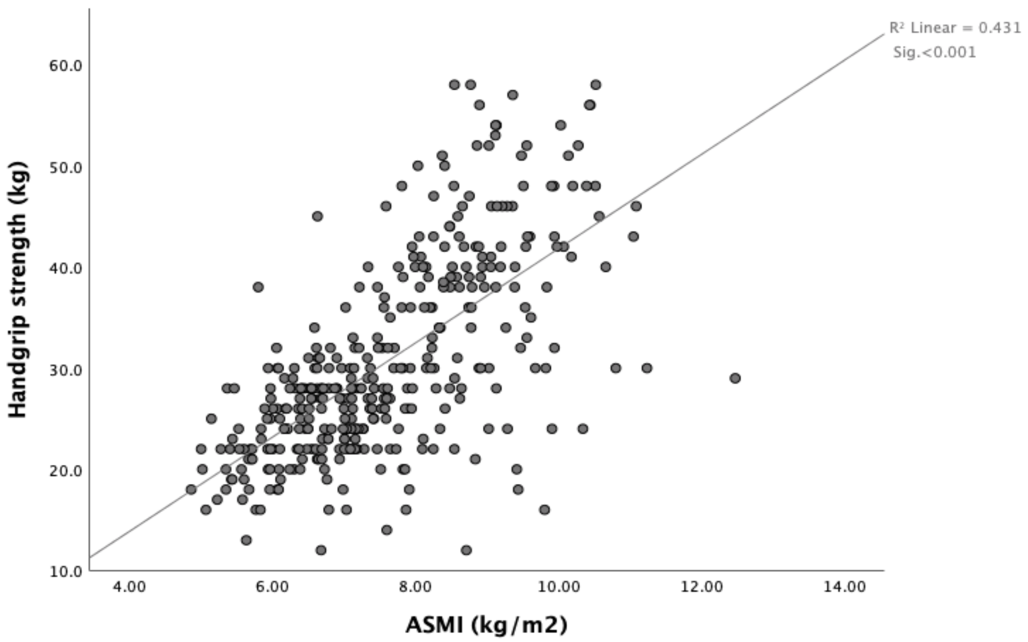

Figure 3. 1. Association between muscle strength and mass in females and males (n = 369).

When stratified by sex, the association was still significant, but lower (females $r^2 = 0.040$, $\rho = 0.002$; males $r^2 = 0.055$, $\rho = 0.006$) (Tables 3. 2 and 3. 3), with the addition of % BF increasing the ability of the model to predict muscle strength slightly (females $r^2 = 0.116$, $\rho < 0.001$; males $r^2 = 0.097$, $\rho < 0.001$).

Table 3. 2. Results of multiple linear regression modelling on the relationship between muscle strength, mass, and body fat percentage in older females.

Model	Coefficient (B)	Standard error B	95% CI	Standardised β	r^2	ρ-value
Model 1					0.04**	0.002
Constant	18.217	2.107	14.066, 22.368			
ASMI	0.945	0.304	0.345, 1.545	0.199		
Model 2					0.116**	< 0.001
Constant	24.613	2.480	19.727, 29.498			
ASMI	1.288	0.303	0.692, 1.885	0.271		
% BF	- 0.243	0.054	- 0.350, - 0.136	- 0.285		

ASMI = appendicular skeletal muscle mass index. % BF = body fat percentage. Regression equation model 1: muscle strength = 18.21 + 0.945 * ASMI. Regression equation model 2: muscle strength = 24.61 + 1.29 * ASMI - 0.24 * body fat percentage. **significant at ρ < 0.01.

Table 3. 3. Results of multiple linear regression modelling on the relationship between muscle strength, mass and body fat percentage in older males.

Model	Coefficient (B)	Standard error B	95% CI	Standardised β	r^2	ρ-value
Model 1					0.055**	0.006
Constant	24.921	5.834	13.380, 36.461			
ASMI	1.806	0.652	0.515, 3.097	0.235		
Model 2					0.097**	< 0.001
Constant	29.814	6.066	17.813, 41.815			
ASMI	2.276	0.669	0.953, 3.599	0.296		
% BF	- 0.370	0.152	- 0.670, - 0.071	- 0.213		

ASMI = appendicular skeletal muscle mass index. % BF = body fat percentage. Regression equation model 1: muscle strength = 24.92 + 1.81 * ASMI. Regression equation model 2: muscle strength = 29.81 + 2.28 * ASMI - 0.37 * body fat percentage. **significant at ρ < 0.01.

When exploring the association between muscle strength and muscle mass according to obesity classification using body fat percentage, muscle mass was significantly associated with muscle strength in non-obese males and females. However, in participants with obesity, muscle mass was no longer associated with muscle strength (Tables 3. 4 and 3. 5).

Table 3. 4. Results of multiple linear regression modelling on the effect of obesity in the relationship between muscle strength and mass in older females.

Exploratory variable			**Coefficient (B)**	**Standard error B**	**95% CI**	**Standardised β**	**r^2**	**ρ-value**
Body fat percentage categories	Non-obese	Constant	12.34	2.43	7.55, 17.13		0.138	< 0.001**
		ASMI	1.91	0.36	1.20, 2.62	0.371		
	Obese	Constant	21.0	4.50	11.97, 30.02		0.005	0.617
		ASMI	0.304	0.60	- 0.91, 1.51	0.068		
*Significant at ρ < 0.05; **significant at ρ < 0.01.								

Table 3. 5. Results of multiple linear regression modelling on the effect of obesity in the relationship between muscle strength and mass in older males.

Exploratory variable			**Coefficient (B)**	**Standard error B**	**95% CI**	**Standardised β**	**r^2**	**ρ-value**
Body fat percentage categories	Non-obese	Constant	21.71	6.03	9.78, 33.64		0.083	< 0.001**
		ASMI	2.22	0.68	0.88, 3.57	0.289		
	Obese	Constant	26.32	22.03	- 22.18, 74.81		0.024	0.615
		ASMI	1.20	2.32	- 3.91, 6.32	0.154		
*Significant at ρ < 0.05; **significant at ρ < 0.01.								

3.5. Discussion

In this cross-sectional study, we evaluated the relationship between muscle strength, muscle mass, and body fat percentage in older adults living in Auckland, NZ. The findings indicate that muscle strength was associated with muscle mass. The magnitude of this association was greater in males than females, with addition of body fat percentage slightly increasing the ability of the model to predict muscle strength. When exploring the association between muscle strength and muscle mass according to obesity classification using body fat percentage, muscle mass was associated with muscle strength in non-obese participants. However, this association was not observed in older adults who were classified as obese. This indicates that body fat percentage should be considered when measuring associations between muscle mass and muscle strength in older adults.

3.5.1. Prevalence of obesity, low muscle strength and low muscle mass

We found a higher prevalence of participants with obesity using body fat percentage classifications (18.7%) than using BMI classifications (16.3%). This result was as expected, as BMI has been shown to underestimate adiposity in older adults (Romero-Corral et al., 2008). A recent survey in New Zealand using BMI classifications reported that the prevalence of obesity in older adults between 65 to 74 years was 34.9% (Ministry of Health, 2019). The lower level of obesity reported in our population may reflect our recruitment inadvertently targeting healthy older adults.

We also identified 3.5% of participants had low muscle strength. The lack of studies reporting the prevalence of low muscle strength and the application of different cut-off values makes it difficult to compare studies. In this cohort, we applied the updated cut off values of low muscle strength defined by the European Working Group on Sarcopenia in Older People (EWGSOP2). A nationally representative sample of Brazilians aged 65 years and older using the same cut-off values as our study observed a higher prevalence of low muscle strength (28.2%) (Borges et al., 2020).

Other studies which applied the older cut off values defined by the European Working Group on Sarcopenia in Older People (EWGSOP), observed a higher prevalence of low muscle strength of 33.9% among Mexican people aged 50 years and older (Rodríguez-García et al., 2018), 22.5% among Europeans aged 70 years and older (Bertoni et al., 2018), 44% in a population of Americans aged 65 years and older (Duchowny et al., 2018) and 71% in a community-dwelling older New Zealanders aged 75 years and older (Chatindiara et al., 2019). The higher prevalence observed in these groups is possibly explained by the inclusion of people older than 74 years, and a potentially less healthy population than those participants included in our study.

The prevalence of low ASMI was 6.3% in females and 2.3% in males in our study using cut-offs of < 7 kg/m^2 and < 5.5 kg/m^2 for men and women respectively. Another study in New Zealand adults aged 56 to 93 years (Waters et al., 2010) found that 12% of females and 4% of males had low muscle mass using ASMI cut-offs of < 7.2 and < 5.4 kg/m^2 for males and females respectively. The higher percentage in the study appears to be explained by the inclusion of adults over the ages of 74 years.

3.5.2. Association between body composition and muscle strength

Our results provide evidence that muscle mass is positively associated with muscle strength in older men and women. This result aligns with the literature (Barbat-Artigas, Rolland, et al., 2013; Beliaeff et al., 2008) and suggests that efforts to maintain muscle mass should have a significant effect on preserving strength in older adults.

When stratified by sex, we observed strong evidence that muscle mass was significantly associated, but not a major contributor to muscle strength in older men and women. Muscle mass accounted for 5% of the variance in muscle strength in men and 4% in women. This finding was similar to another study which found that leg muscle mass accounted for 5% and 4% of the variance in quadriceps muscle strength in men and women, respectively (Beliaeff et al., 2008).

In a regression model it was shown that an increase of 1 unit in muscle mass will increase the value of muscle strength by 0.945 kg in females and 1.81 kg in males. After taking muscle mass and body fat percentage into account, % BF increased the ability of the model to predict muscle strength. A decrease of 1% body fat and increase of 1 unit muscle mass was shown to increase the value of muscle strength by 1.53 kg in females and 2.65 kg in males.

These results highlight not only the importance of increasing muscle mass, but also the importance of decreasing body fat percentage to preserve muscle strength in older adults. The cross-sectional nature of our data impedes any causal inference. Nevertheless, the results from our study provide justification for further prospective research that evaluates the effects of interventions, which are aimed at optimising body composition and muscle strength in older adults.

3.5.3. The role of obesity classification in the relationship between muscle strength and muscle mass

To our knowledge, this is the first study to investigate the role of obesity classification based on body fat percentage in the relationship between muscle strength and muscle mass. Results from multiple linear regression analyses provide evidence supporting the important role of obesity classification according to body fat percentage when investigating the relationship between muscle strength and muscle mass. Our study demonstrated that when obesity was classified using body fat percentage, muscle mass was significantly associated with muscle strength in non-obese older adults. However, an association between muscle strength and muscle mass was not observed in older adults categorised as obese.

The accumulation of intramuscular lipid content (or poor muscle quality), which is seen in people with obesity may explain the influence of obesity in the relationship between muscle strength and muscle mass. Goodpaster et al. (2001) reported that higher intramuscular lipid content is associated with lower muscle strength, independent of muscle mass (Goodpaster et al., 2001).

Also, accumulation of intramuscular lipid content is known to be associated with insulin insensitivity, inflammation, and functional deficits in skeletal muscle. It will be important in the future to continue to focus on understanding predictors of muscle strength in older adults with obesity in order to provide appropriate interventions to increase muscle strength.

There were significant strengths to our study. The relatively large sample size permits us to examine whether the relationship between muscle strength and muscle mass was similar in males and females. Also, it is possible that the inclusion of community-dwelling healthy older adults provides the opportunity to identify issues and promote preventative action in early old age. Furthermore, the use of DXA is an accurate measure of body composition. However, in contrast to magnetic resonance imaging (MRI) or computed tomography (CT), DXA cannot detect intramuscular fat from muscle mass nor distinguish the composition of muscle (Fragala et al., 2015; Lee et al., 2019).

This cross-sectional study limits the ability to detect causality; hence, only associations were discussed. Other limitations are the population group, which was not representative of the New Zealand population, as this cohort was composed of a convenience volunteer sample of men and women aged 65 to 74 years living in the community. The classification by body fat percentage for obesity may also be perceived as a limitation given the arbitrary nature of the cut-off points. Finally, we did not assess lower extremity muscle strength, which is a more direct predictor of falls. However, grip strength is associated with lower-body muscle strength (Bernardi et al., 2004) and a strong predictor of disability (Bohannon, 2008).

3.6. Conclusions

Muscle mass and body fat percentage were predictors of muscle strength in this cohort. Muscle mass was associated with muscle strength in non-obese older adults whereas, there was no association between muscle mass and muscle strength in older adults who were classified as obese. This indicates that obesity classification plays an important role in the relationship between muscle strength and muscle mass in older adults.

We suggest that this could be mainly attributed to muscle quality, which could be a contributor of muscle strength in older adults who are obese. Further research should focus on identifying predictors of muscle strength in older adults with obesity.

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Chapter Four – Protein intake, distribution, and sources in community-dwelling older adults living in Auckland, New Zealand

In older adults, adequate protein intake, distribution, and sources are known to increased MPS and muscle mass. However, there is a lack of evidence little about protein intake, distribution, and sources in New Zealand (NZ) older adults. This chapter is a manuscript investigating protein intake, distribution and sources in community-dwelling older adults in New Zealand (Received: May 19, 2022; Accepted: May 3, 2023).

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The formatting, layout, and referencing style of the published manuscript have been modified to align with this thesis.

4.1. Abstract

Background: To maximally stimulate muscle protein synthesis (MPS), older adults should consume ≥ 1.2 g/kg body weight (BW) of high-quality protein, evenly distributed across the day (~ 0.4 g/kg BW per meal).

Objective: To investigate protein intake, distribution, and sources in community-dwelling older adults.

Methods: Data (including a 4-day food record) were obtained from the Researching Eating, Activity, and Cognitive Health (REACH) study, a cross-sectional study in adults aged 65 to 74 years.

Results: Participants (n = 327, 65.4% female) had a median daily protein intake of 1.16 g/kg BW (males) and 1.09 g/kg BW (females). Over half of participants consumed less than 1.2 g protein/kg BW (62% females, 57% males). Protein intake was unevenly distributed throughout the day (CV = 0.48 for males and females) and was inadequate for reaching 0.4 g/kg BW per meal at breakfast and at the mid-day meal (males only). The main sources of protein at breakfast were milk (28%), breakfast cereals (22%), and bread (12%); at the mid-day meal, bread (18%), cheese (10%) and milk (9%); and at the evening meal, meat provided over half the protein (56%).

Conclusions: Protein intake for a high proportion of older adults was less than 1.2 g/kg BW per day, and unevenly distributed through the day. Protein was obtained mainly from cereals and dairy products at breakfast and the mid-day meal, and meat sources at the evening meal.

4.2. Introduction

Globally, the population is getting older. In New Zealand, people aged 65 and older currently constitute 15% of the population (Statistics New Zealand, 2019). This is expected to increase to 20% by 2026 and to more than 26% by 2051 (Statistics New Zealand, 2007). Muscle strength declines as people age (Al-Abdulwahab, 1999; Aniansson et al., 1986; Bassey & Harries, 1993; Granic et al., 2016; Schlussel et al., 2008), and low muscle strength has been found to be related to physical disability, poor physical performance, and increased mortality (Batsis, Germain, et al., 2016; Duchowny et al., 2018). We previously identified that higher muscle mass was associated with greater muscle strength in older adults in New Zealand (Hiol et al., 2021).

The homeostasis of muscle mass is regulated by a dynamic turnover between muscle protein synthesis (MPS) and muscle protein breakdown (Bennet et al., 1979; Bohé et al., 2003; Bohé et al., 2001; Burd et al., 2011; Fujita et al., 2007). Ageing is accompanied by a blunted MPS response to protein ingestion, resulting in a decrease in muscle mass in older adults (Colley et al., 2011; Fujita, 2007; Rooyackers et al., 1996; Volpi et al., 2001; Welle et al., 1993; Yarasheski et al., 1993). The impairment of age-related MPS can be reduced by the consumption of an adequate quantity of high-quality protein, which is distributed evenly throughout the day (Mamerow et al., 2014; Rafii et al., 2015; Traylor et al., 2018).

In Australia and New Zealand, the current recommended daily intake of protein is established as 0.9 g/kg body weight (BW)/day for females and 1.1 g/kg BW/day for males aged 70 years or older (National Health and Medical Research Council Australian Government Department of Health and Ageing, 2006). However, older adults may require ≥ 1.2 g/kg BW of protein to maximally stimulate MPS (Rafii et al., 2015; Traylor et al., 2018). Furthermore, Mamerow et al. (2014) demonstrated that, despite a protein intake of approximately 1.2 g/kg BW, MPS was 25% higher in those with a protein intake evenly distributed across three meals (~ 0.4 g/kg BW per meal) compared to those with an uneven distribution.

Protein quality refers to the protein's composition of essential amino acids and their ability to be digested, absorbed, and retained by the human body (Moore & Soeters, 2015; Wolfe et al., 2016). Proteins from animal sources contain all the essential amino acids whereas plant proteins are often missing or have low amounts of one or more essential amino acids (Gilbert et al., 2011; Heimo Scherz et al., 2000). Also, animal-based protein has a higher digestibility compared to plant-based protein (Berrazaga et al., 2019; Gilani et al., 2015). Therefore, animal-based protein sources such as meat, eggs and milk are considered to be of higher quality than plant-based protein sources such as cereals and legumes (Heimo Scherz et al., 2000; van Vliet et al., 2015).

In most Western countries, protein intake is unevenly distributed throughout the day, with a high proportion of older adults not reaching the threshold of three daily meals containing ≥ 0.4 g/kg BW of protein per meal (Cardon-Thomas et al., 2017; Gingrich et al., 2017). Hung et al. found a lower protein intake was more common in people who did not consume certain sources of protein, such as dairy products (Hung et al., 2019). Insight into the distribution and sources of protein throughout the day would yield valuable information for developing effective dietary protein interventions that enable maximal MPS and have the potential to increase and maintain muscle mass in older adults. In this study, we aim to investigate protein intake, distribution, and sources in community-dwelling older adults in New Zealand.

4.3. Materials and methods

Data for this study were obtained from the REACH study. The REACH study was a cross-sectional study with a main objective of investigating dietary patterns and their associations with cognitive function and metabolic syndrome in older adults (Mumme et al., 2019). The study was granted ethical approval by the Massey University Human Ethics Committee (Southern A, Application 17/69), and all participants gave written informed consent.

4.3.1. Study population

Participants were older adults aged between 65 and 74 years, proficient in English, and living independently (i.e., not in residential care) in Auckland, New Zealand. Participants were excluded if they had a diagnosis of dementia or any other condition which might impair cognitive function (e.g., head injury, stroke), were taking medication which may influence cognitive function, had colour blindness (due to cognitive testing requirements), or had experienced any other event in the last two years which may have had a substantial impact on dietary intake or on cognition. The details of the recruitment and assessment processes for the REACH study have been described elsewhere (Mumme et al., 2019).

4.3.2. Data collection

All participants visited the Human Nutrition Research Unit at Massey University in Auckland, New Zealand on one occasion for collection of data as part of the REACH study. Socio-demographic data (age, sex, ethnicity, level of education, and living situation) were collected by written questionnaire. Height and weight measurements were completed by trained researchers using standardised procedures from the protocol of the International Society for the Advancement of Kinanthropometry (ISAK) (Marfell-Jones et al., 2012). Body height (in cm, to the nearest 0.1 cm) was measured without shoes using a stadiometer (SECA). Body weight (in kg, to the nearest 0.1 kg) was assessed using floor scales (Wedderburn). Body Mass Index (BMI) was calculated using body weight in kilograms divided by height (in metres) squared.

Dietary intake was recorded using a food record completed in the week following the participant's appointment. This included four consecutive assigned days, with at least one weekend day. Participants were given food record forms, a food portion guide (Nelson et al., 1997), and watched a video explaining how to complete the food record. Participants were instructed to adhere to their usual dietary habits and provide a detailed description of all food and beverages consumed (food, beverage, name, brand, variety, and cooking method), portion sizes, and the time of intake (Appendix F).

Participants were asked to send their completed food record back to the research team by post within one month of the study visit. In cases where data were unclear or missing, a follow-up phone call was conducted, in which participants gave verbal clarification.

4.3.3. Data handling

The four-day food record were processed by trained nutritionists and dietitians using FoodWorks 10 (Xyris Pty Ltd, 2019). FoodWorks 10 is based on the *New Zealand Food Composition database* (Sivakumaran et al., 2017). A register of common food items was kept, ensuring consistency in data entry among the nutritionists. Where necessary, recipes (and the number of servings provided) were entered into FoodWorks 10. All food record entries were checked by a New Zealand registered dietitian for accurate data entry. Outliers identified using histograms were checked with the original food diaries to ensure accuracy.

Daily total energy and macronutrient intake data were generated. Average daily energy intake was considered implausible if energy intake was < 2100 kJ (500 kcal) or > 14 700 kJ (3500 kcal) for females and < 3360 kJ (800 kcal) or > 16 800 kJ (4000 kcal) for males (Willett, 2012). Percentages of energy from carbohydrates, total fats, and proteins were calculated (National Health and Medical Research Council Australian Government Department of Health and Ageing, 2006; Willett, 2012). Relative protein intake in g/kg BW was calculated by dividing absolute protein intake per day by each participant's body weight. To determine the percentage of participants who met dietary protein recommendations, relative protein intake were compared with the Australian and New Zealand estimated average requirements (EAR) for adults aged between 19 and 70 years (0.60 g/kg BW for females and 0.68 g/kg BW for males) and older adults aged 70 years or more (0.75 g/kg BW for females and 0.86 g/kg BW for males) (National Health and Medical Research Council Australian Government Department of Health and Ageing, 2006). Relative protein intake was also compared against a cut-off value of 1.2 g/kg BW (Rafii et al., 2015; Traylor et al., 2018).

To define meals consumed by participants, time of intake was split into one-hour slots. The time of day at which protein was consumed was assessed. Days were divided into three meals, according to the peaks of protein consumption across the day (Hung et al., 2019). Protein (in g/kg BW) was calculated at breakfast, the mid-day meal, and the evening meal. The distribution of protein across the three meals for each participant was expressed as the dimensionless coefficient of variation (CV) of protein intake at breakfast, the mid-day meal, and the evening meal (CV = standard deviation of protein intake of the three meals (g) / total protein intake for the three meals (g)). A CV of zero would indicate an even distribution of protein across the day (Bollwein et al., 2013).

All foods from the food record were initially grouped into 109 food groups, then further allocated to one of 27 food groups based on the main sources of protein intake identified in the 2008/09 *New Zealand Adults Nutrition Survey* (University of Otago and Ministry of Health, 2011). Each food group was classified as meat, plant, dairy, or egg protein according to the main type of protein it contained. For each meal, protein quantity (g) was assessed for each food group and presented as a percentage of total protein for that meal.

4.3.4. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, version 27.0 (IBM SPSS, Armonk, NY, USA). Descriptive statistics were generated for participants' characteristics and dietary intake data. Continuous data were assessed for normality using Shapiro Wilk tests and visual assessment of histograms. Data were reported as mean ± standard deviation if normally distributed, or median and interquartile range if non-normally distributed. Percentages were used for categorical data. Differences between groups were analysed using Mann–Whitney U tests for non-normally distributed scale data and a Chi-squared test of independence for categorical data. Results were considered significant at $\rho < 0.05$.

The association between high protein intake (≥ 0.4 g/kg BW per meal) and protein (g per meal) for each 27 food groups was analysed for each meal using separate univariable logistic regression analyses (enter method).

We applied a cut-off ρ -value of < 0.01 for inclusion in the multivariable model. A restricted prediction model was then generated by using a manual backward stepwise selection approach. At each step, variables with a ρ-value ≥ 0.05 were removed from the model until all remaining variables had ρ -values < 0.001.

4.4. Results

Three hundred and seventy-one participants took part in this study. Forty-one participants did not provide food record, and three had incomplete food record data, leaving 327 participants to be included in the analysis (Table 4. 1). The median age was 70 years (IQR 68-72). Most participants were of New Zealand European or European ethnicity (94.8%). Males were more likely to have a university education ($\rho < 0.01$) and to live with others ($\rho < 0.01$).

Table 4. 1. Characteristics of participants [a,b]

Characteristics	**Total**	**Males**	**Females**	**ρ-value**
n (%)	327	113 (34.6%)	214 (65.4%)	
Age, years	69.66 (67.90, 71.56)	70.26 (68.45, 71.94)	69.27 (67.50, 71.42)	0.01*
Ethnicity, n (%) NZ European / other	310 (94.8)	107 (94.7)	203 (94.9)	0.98
Māori / Pacific	8 (2.4)	3 (2.7)	5 (2.3)	
Asian	9 (2.8)	3 (2.7)	6 (2.8)	
Height, m	166.70 (160.20, 173.00)	176.90 (171.90, 180.90)	163.40 (158.50, 167.50)	< 0.01**
Body weight, kg	71.10 (63.20, 81.45)	81.45 (73.70, 89.30)	66.95 (59.52, 74.60)	< 0.01**
Body mass index, kg/m^2	25.62 (23.05, 28.18)	26.35 (24.32, 28.27)	25.26 (22.69, 28.08)	0.02*
Highest level of education, n (%) No qualification	6 (1.8)	1 (0.9)	5 (2.3)	< 0.01**
Secondary	68 (20.8)	14 (12.4)	54 (25.2)	
Post-secondary	136 (41.6)	44 (38.9)	92 (43.0)	
University	117 (35.8)	54 (47.8)	63 (29.4)	
Living situation, n (%) Alone	97 (29.7)	14 (12.4)	83 (38.8)	< 0.01**
With others	230 (70.3)	99 (87.6)	131 (61.2)	

[a]Continuous values are expressed as median (25th, 75th percentile). [b]Categorical values are expressed as frequency (percentage). Sex difference at **ρ < 0.01, *ρ < 0.05.

Daily energy, macronutrient, and protein intake in older adults are shown in Table 2. In terms of percentage of energy intake (EI), females consumed significantly less carbohydrate (ρ = 0.01) but more total fats (ρ = 0.04) than males.

Absolute protein intake per day was lower for females (74.71 g/day) than for males (95.86 g/day) (ρ < 0.01) and accounted for 16.9% and 16.7% of their energy intakes, respectively (ρ = 0.45).

Median relative protein intake was 1.2 (0.9, 1.4) g/kg BW in males and 1.1 (0.9, 1.3) g/kg BW in females (ρ = 0.37). Two (3.7%) males and no females aged 65 to 70 years consumed less than the EAR for protein, while 13 (22%) males and 8 (8.9%) females older than 70 years consumed less than the EAR (National Health and Medical Research Council Australian Government Department of Health and Ageing, 2006). Over half of the participants did not meet the intake of ≥ 1.2 g/kg BW to maximally stimulate MPS (61.7% females and 56.6% males) (Table 4. 2).

Table 4. 2. Median daily energy, macronutrient, and protein intake in older adults by sex [a,b].

Nutrient	**Total**	**Males**	**Females**	**ρ-value**
Energy intake, kJ/day	7911 (6717, 9271)	9239 (7920, 10730)	7434 (6344, 8460)	< 0.01**
Carbohydrate, g/day	186.9 (147.1, 225.9)	222.9 (185.2, 262.6)	173.1 (136.4, 201.4)	< 0.01**
Carbohydrate %EI	39.38 (34.34, 44.39)	41.61 (35.11, 45.62)	38.60 (34.03, 43.57)	0.01*
Total fat, g/day	76.67 (63.60, 94.12)	90.84 (69.45, 107.71)	72.91 (61.71, 86.36)	< 0.01**
Total fat %EI	36.71 (32.93, 40.89)	35.69 (31.38, 40.11)	37.41 (33.89, 41.13)	0.04*
Protein intake, g/day	79.21 (67.26, 97.19)	95.86 (77.52, 107.16)	74.71 (63.41, 87.86)	< 0.01**
Protein %EI	16.83 (15.12, 18.99)	16.68 (14.89, 18.76)	16.89 (15.28, 19.09)	0.45
Protein intake, g/kg BW/day	1.12 (0.94, 1.35)	1.16 (0.94, 1.40)	1.09 (0.94, 1.32)	0.37
Protein intake [c] < 0.60 g/kg BW for females or < 0.68 g/kg BW for males, n (%)	2 (1.1)	2 (3.7)	0 (0.0)	< 0.01**
Protein intake [d] < 0.75 g/kg BW for females or < 0.86 g/kg BW for males, n (%)	21 (14.1)	13 (22)	8 (8.9)	< 0.01**
Protein intake < 1.2 g/kg BW, n (%)	196 (59.9)	64 (56.6)	132 (61.7)	< 0.01**

[a]Continuous values are expressed as median (25th, 75th percentile). [b]Categorical values are expressed as frequency (percentage). [c]The Australian and New Zealand estimated average requirements (EAR) for adults aged between 65 and 70 years (n = 178). [d]The Australian and New Zealand estimated average requirements (EAR) for older adults aged 70 years or more (n = 149). Sex difference at **ρ < 0.01, *ρ < 0.05. Abbreviations: Body weight (BW), energy intake (EI).

The time of day at which protein was consumed is shown in Figure 4. 1. The amount of protein consumed differed across hours of the day, with peaks between 05:30 and 11:29 hours (mostly breakfast), 11:30 and 16:29 (mostly the mid-day meal) and 16:30 and 21:29 (mostly the evening meal).

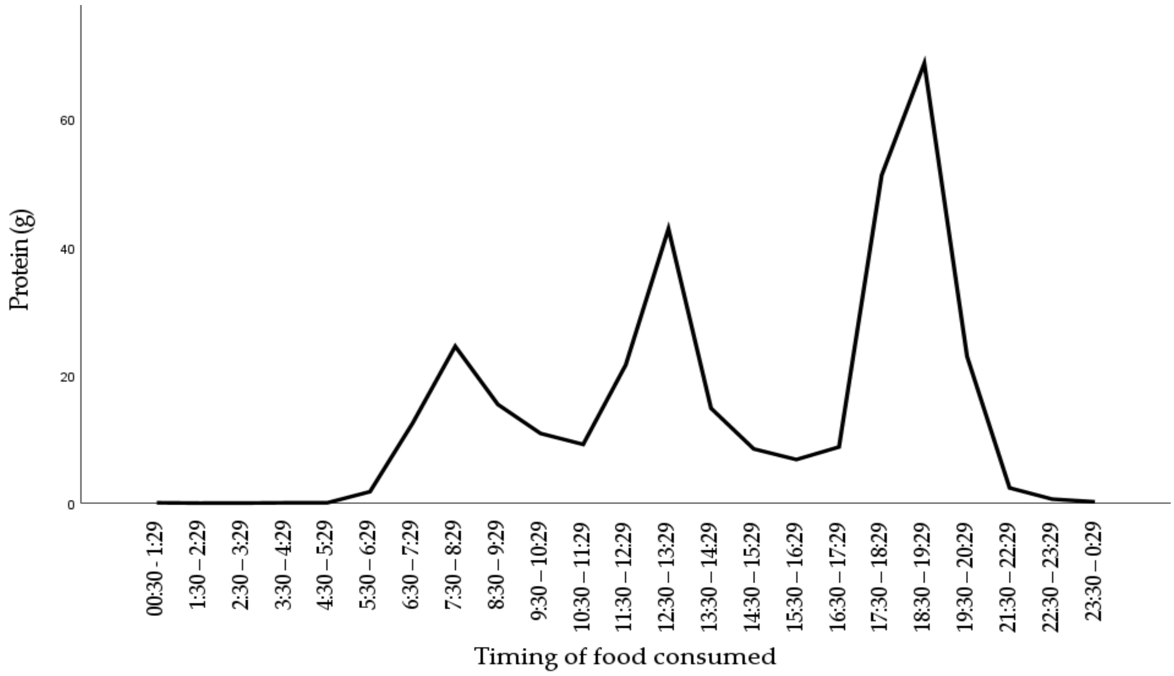

Figure 4. 1. Distribution of dietary protein intake across the day in community-dwelling older adults.

The dotted line represents the suggested protein intake for maximal muscle protein synthesis stimulation of 0.4 g/kg BW. Protein intake was unevenly distributed across the day, as indicated by a mean CV of 0.48 ± 0.22 in males and 0.48 ± 0.23 in females. For males, the mean ± SD protein intakes at breakfast, the mid-day meal, and the evening meal were 0.29 ± 0.14 g/kg BW, 0.31 ± 0.13 g/kg BW, and 0.57 ± 0.20 g/kg BW, respectively. For females, the mean ± SD protein intakes were 0.24 ± 0.12 g/kg BW, 0.35 ± 0.14 g/kg BW, and 0.55 ± 0.18 g/kg BW respectively (Figure 4. 2).

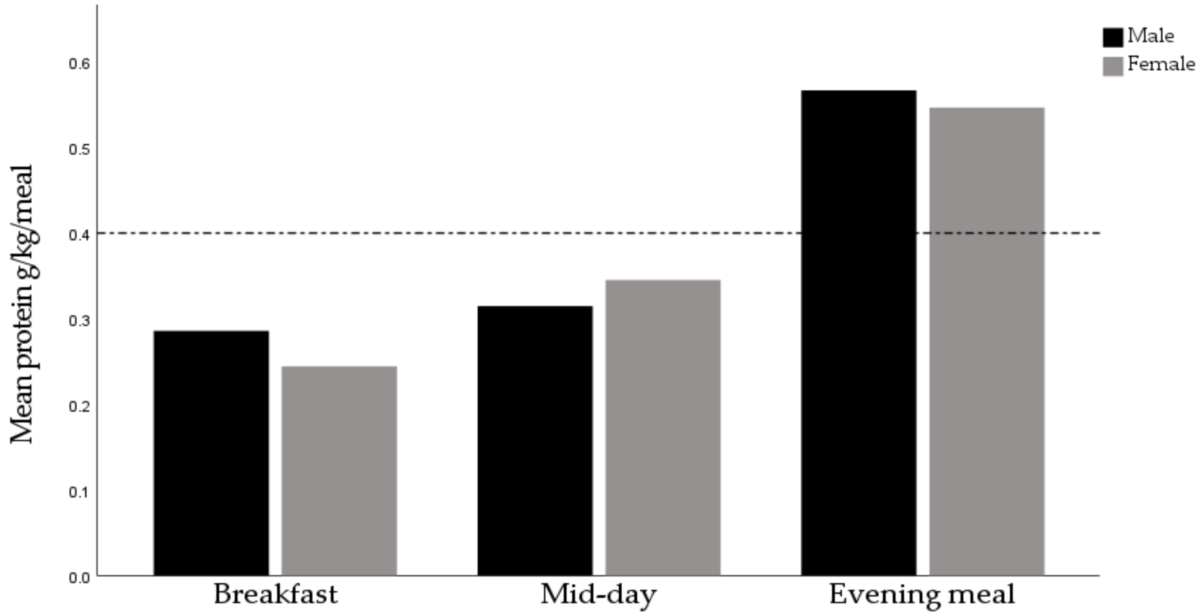

Figure 4. 2. Relative protein intakes for breakfast, the mid-day meal, and the evening meal, by sex.

Intake and relative contribution of protein from different food sources at each meal are shown in Table 4. 3. Plant proteins were the main protein at breakfast and the mid-day meal (53% and 49%, respectively), while meat was the main protein source at the evening meal (56%). At breakfast, participants primarily consumed protein from milk (5 g), breakfast cereal (4 g), and bread (2 g). Protein intake at the mid-day meal was mainly from bread (4 g), cheese (2 g), and milk (2 g). At the evening meal, the main sources of protein were red meat, poultry, and fish and shellfish (9 g, 6 g, and 6 g, respectively).

Table 4. 3. Mean intake and relative contribution of protein from different food sources at each meal.

Sources of protein	**Breakfast**		**Mid-day meal**		**Evening meal**	
	Protein intake, g/meal	Relative contribution of protein, %	Protein intake, g/meal	Relative contribution of protein, %	Protein intake, g/meal	Relative contribution of protein, %
Meat	**0.70 ± 1.93**	**3.07 ± 7.80**	**6.88 ± 7.01**	**25.73 ± 21.22**	**23.13 ± 11.98**	**55.99 ± 19.18**
Red meat	0.06 ± 0.54	0.28 ± 2.72	1.62 ± 4.11	5.27 ± 11.65	8.96 ± 9.07	21.22 ± 19.50
Poultry	0.04 ± 0.55	0.13 ± 1.38	1.46 ± 3.42	5.18 ± 11.49	6.26 ± 7.48	15.15 ± 16.89
Fish and shellfish	0.07 ± 0.49	0.39 ± 3.12	1.97 ± 3.32	7.73 ± 13.13	5.53 ± 6.70	13.58 ± 15.03
Processed meat	0.50 ± 1.62	2.13 ± 6.60	1.78 ± 3.11	7.30 ± 12.41	2.32 ± 3.92	5.88 ± 9.74
Animal fat	0.03 ± 0.96	0.15 ± 0.57	0.05 ± 0.15	0.22 ± 0.78	0.06 ± 0.16	0.16 ± 0.49
Dairy and eggs	**8.33 ± 5.70**	**44.05 ± 20.78**	**5.90 ± 4.46**	**25.40 ± 17.72**	**4.69 ± 4.03**	**12.54 ± 11.11**
Milk	5.31 ± 4.80	28.06 ± 20.08	2.05 ± 2.23	9.24 ± 10.68	1.28 ± 1.75	3.35 ± 4.78
Cheese	0.34 ± 1.00	1.79 ± 5.26	2.30 ± 3.10	9.49 ± 11.27	1.81 ± 2.59	4.59 ± 6.47
Yoghurt	1.22 ± 2.28	6.60 ± 10.73	0.21 ± 0.82	0.99 ± 3.73	0.36 ± 1.02	1.02 ± 3.10
Eggs and egg products	1.46 ± 2.79	7.60 ± 14.28	1.33 ± 2.28	5.69 ± 9.61	1.25 ± 2.07	3.57 ± 5.98

Plant	9.52 ± 5.46	52.88 ± 21.10	10.84 ± 5.27	48.87 ± 20.68	11.75 ± 5.38	31.48 ± 15.49
Bread	2.12 ± 2.71	12.15 ± 16.09	3.92 ± 3.05	17.46 ± 14.42	1.23 ± 2.04	3.39 ± 5.76
Breakfast cereal	4.25 ± 4.40	21.61 ± 19.46	0.17 ± 1.53	0.70 ± 5.83	0.06 ± 0.35	0.15 ± 0.85
Vegetables	0.18 ± 0.88	0.90 ± 3.65	1.14 ± 1.71	4.93 ± 7.35	3.45 ± 2.64	9.30 ± 7.38
Soups, bouillon	0.02 ± 0.27	0.08 ± 1.14	0.93 ± 3.00	3.84 ± 10.50	0.46 ± 1.47	1.38 ± 4.65
Fruits	0.83 ± 0.87	5.75 ± 9.01	0.74 ± 0.76	4.03 ± 8.24	0.47 ± 0.60	1.33 ± 1.86
Potato, kūmara, taro, and other root vegetables	0.03 ± 1.17	0.13 ± 0.69	0.26 ± 0.73	1.21 ± 4.87	1.34 ± 1.35	3.41 ± 3.28
Pasta, rice, other grains	0.06 ± 0.45	0.36 ± 2.43	0.40 ± 1.23	1.74 ± 6.00	1.41 ± 1.98	3.60 ± 5.02
Plant-based milks and cream	0.14 ± 0.60	0.97 ± 4.22	0.02 ± 0.14	0.08 ± 0.51	0.03 ± 0.20	0.10 ± 0.76
Soy products	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.38	0.17 ± 1.62	0.21 ± 1.10	0.69 ± 4.26
Nuts and seeds	0.85 ± 1.72	4.91 ± 9.91	0.89 ± 2.08	3.66 ± 7.48	0.81 ± 1.72	2.10 ± 4.36
Biscuits	0.10 ± 0.29	0.61 ± 1.75	0.18 ± 0.37	0.83 ± 1.79	0.18 ± 0.42	0.48 ± 1.13
Cakes and desserts	0.50 ± 1.14	2.93 ± 7.63	1.16 ± 1.80	5.64 ± 9.90	0.43 ± 0.86	1.22 ± 2.67
Snacks	0.16 ± 0.83	0.76 ± 3.01	0.53 ± 1.05	2.56 ± 6.05	0.50 ± 0.88	1.35 ± 2.57
Sugar and confectionery	0.17 ± 0.51	0.95 ± 2.64	0.18 ± 0.42	0.74 ± 1.74	0.37 ± 0.78	0.97 ± 2.13
Alcoholic beverages	0.00 ± 0.02	0.004 ± 0.06	0.05 ± 0.19	0.25 ± 1.05	0.26 ± 0.44	0.64 ± 0.98
Non-alcoholic beverages	0.08 ± 0.27	0.66 ± 3.50	0.09 ± 0.32	0.38 ± 1.19	0.06 ± 0.19	0.16 ± 0.48
Vegetable oils	0.01 ± 0.03	0.04 ± 0.22	0.02 ± 0.11	0.09 ± 0.74	0.04 ± 0.25	0.10 ± 0.65
Sauces and spices	0.01 ± 0.04	0.08 ± 2.29	0.13 ± 0.54	0.57 ± 2.52	0.44 ± 1.04	1.12 ± 2.53

Values are expressed as mean ± SD.

Table 4. 4 shows the results of the multivariable logistic regression models for prediction of protein intake ≥ 0.4 g/kg BW at breakfast (n = 39), the mid-day meal (n = 87), and the evening meal (n = 266). Of the 27 food groups, bread, breakfast cereal, milk, and yoghurt were predictors of having a protein intake ≥ 0.4 g/kg BW at breakfast. At the mid-day meal cheese, eggs and egg products, fish and shellfish, milk, nuts and seeds, poultry, red meat, and vegetables were significantly associated with high protein intake (≥ 0.4 g/kg BW per meal). The multivariable logistic regression model for prediction of protein intake ≥ 0.4 g/kg BW at the evening meal included poultry, red meat, fish and shellfish, and processed meat.

Table 4. 4. Final model for prediction of protein intake ≥ 0.4 g/kg BW at each meal.

	β [a]	S.E.	Wald	Sig. [b]	Exp (β) [c]
Model Breakfast					
Constant	-6.161	0.724	72.415	< 0.0001	0.002
Food group (g/day)[d]					
Bread	0.339	0.079	18.215	< 0.0001	1.403
Breakfast cereals	0.219	0.045	23.370	< 0.0001	1.245
Milk	0.214	0.045	22.510	< 0.0001	1.239
Yoghurt	0.289	0.076	14.478	< 0.0001	1.336
Model Mid-day meal					
Constant	-4.475	0.486	84.610	< 0.0001	0.011
Food group (g/day)[d]					
Cheese	0.210	0.055	14.586	< 0.0001	1.234
Eggs and egg products	0.268	0.069	14.920	< 0.0001	1.308
Fish and shellfish	0.195	0.048	16.450	< 0.0001	1.215
Milk	0.266	0.071	13.819	< 0.0001	1.304
Nuts and seeds	0.335	0.081	16.945	< 0.0001	1.399
Poultry	0.244	0.051	22.521	< 0.0001	1.276
Red meat	0.174	0.043	16.546	< 0.0001	1.190
Vegetables	0.294	0.086	11.831	0.001	1.342
Model Evening meal					
Constant	-1.127	0.355	10.096	0.001	0.324
Food group (g/day)[d]					
Red meat	0.117	0.025	22.657	< 0.0001	1.124
Processed meat	0.207	0.060	11.750	0.001	1.229
Poultry	0.129	0.030	18.188	< 0.0001	1.138
Fish and shellfish	0.166	0.037	19.999	< 0.0001	1.180

[a]β, unstandardised regression coefficient. [b]ρ- value < 0.01 considered significant. [c]Exp (β), exponentiation of the β coefficient, an odds ratio. S.E., standard error; Wald, Wald statistic. [d]Food items from the 4-day food record were collapsed into 27 food groups.

4.5. Discussion

This study aimed to investigate protein intake, distribution, and sources in community-dwelling older adults living in New Zealand. The current EAR for protein in Australia and New Zealand was met by most participants, whereas a high proportion of older adults did not meet the protein threshold of ≥ 1.2 g/kg BW per day. As expected, an uneven distribution of protein intake was observed, which was insufficient to reach 0.4 g/kg BW per meal at breakfast (for both males and females) and at the mid-day meal for males. We found milk, breakfast cereals, and bread were the main sources of protein at breakfast; bread, cheese, and milk at the mid-day meal, with meat providing over half the protein at the evening meal. In addition, we revealed that the probability of having a high protein intake at mealtimes increased with the consumption of bread, breakfast cereal, milk, and yoghurt at breakfast; cheese, eggs and egg products, fish and shellfish, milk, nuts and seeds, poultry, red meat, and vegetables at the mid-day meal; and poultry, red meat, fish and shellfish, and processed meat at the evening meal.

4.5.1. Prevalence of low protein intake

Protein intake can be presented as absolute grams (g), grams per kilogram body weight (g/kg BW), grams per kilogram of ideal body weight (g/kg IBW), or as a percentage of total energy (%EI). Recommended intakes for protein in older adults differ between the US (Trumbo et al., 2002), Japan (Tsuboyama-Kasaoka et al., 2013), Nordic countries (Nordic Council of Ministers, 2014), Australia and New Zealand (National Health and Medical Research Council Australian Government Department of Health and Ageing, 2006), which limits the interpretation of findings in the current study. A study conducted in age-advanced Māori (81 to 91 years) and non-Māori (85 years) showed that 36% and 43% Māori females and males consumed less than the EAR for protein (0.75 g/kg BW (females), 0.86 g/kg BW (males)) for adults aged 70 years or more This was lower in non-Māori (28% females, 29% males) (Ram et al., 2020). In the present study, no females (aged between 65 and 70 years) had a protein intake of < 0.60 g/kg BW, while 3.7% of males had a protein intake of < 0.68 g/kg BW. There was a higher prevalence of low protein intake (< 0.75 g/kg BW (for females), and < 0.86 g/kg BW (males)) in older adults aged 70 years or more (22% for males and 8.9% for females).

The higher prevalence of low protein in older adults aged 70 years or more may be due to the higher EAR set within the Australia and New Zealand guidelines.

We found 62% of females and 57% of males had a protein intake of < 1.2 g/kg BW. We also showed that protein intake was inadequate for reaching 0.4 g/kg BW per meal at breakfast for both males and females and at the mid-day meal for males. These findings suggest that a high proportion of older adults are at risk of insufficient protein for optimal MPS stimulation through the day.

4.5.2. Distribution of protein intake

Different approaches have been used to define meals (Leech et al., 2015b). In this study, we used a time-of-day approach, using the time of intake provided by participants. We found that the highest peaks for eating protein occurred between 05:30 and 11:29, 11:30 and 16:29, and 16:30 and 21:29 hours, which we defined as breakfast, the mid-day meal, and the evening meal, respectively. Using the same approach, Cardon-Thomas et al. (2017) found that similar peaks for eating protein occurred between 05:00 and 11:00 (breakfast), 11:00 and 16:00 (lunch), and 16:00 and 23:59 (dinner).

When we analysed the distribution of protein intake across breakfast, the mid-day meal, and the evening meal, we found an uneven distribution of protein intake (CV = 0.48 in both males and females), with the highest amount of protein being consumed at the evening meal and the lowest at breakfast. This finding is consistent with observations from British (Cardon-Thomas et al., 2017), Dutch (Tieland et al., 2015), and US (Paddon-Jones et al., 2015) studies of older adults. Studies from Mexico (Gaytán-González et al., 2020), Germany (Gingrich et al., 2017), and France (Rousset et al., 2003) in older adults also found an uneven distribution of protein intake, but with the highest amount of protein being consumed at the mid-day meal. Variation can be explained by the cultural and eating habits of each country.

4.5.3. Relative contribution of protein from different food sources

After eating a meal containing protein, approximately 50% of dietary protein-derived amino acids become available in circulation within 6 hours. These amino acids have two main functions in muscles: they stimulate MPS, and they provide the precursors for *de novo* MPS (Groen et al., 2015; Pennings et al., 2011). Animal-based proteins are considered higher quality proteins and are known to enhance MPS simulation in older adults compared with plant proteins (Gorissen et al., 2016; Heimo Scherz et al., 2000; van Vliet et al., 2015). Identifying dietary protein sources at each meal could be an important step towards developing strategies to increase MPS at each meal in older adults. In the present study, participants primarily consumed protein from milk, breakfast cereal, and bread at breakfast; bread, cheese, and milk at mid-day; and meat-derived proteins (red meat, poultry, fish and shellfish) at the evening meal. Breakfast cereals and most cereals-based products are low quality proteins due to their low digestibility-corrected amino acid score (Adhikari et al., 2022; Fanelli et al., 2021; Shewry, 2007). The addition of dairy products such as milk to the breakfast meal results in a meal that contains high-quality proteins, which might enhance MPS in older adults (Fanelli et al., 2021).

4.5.4. Sources of protein determining high protein intake

Hengeveld et al. (2019) demonstrated that food sources contributing to protein intake differ between people who consume low versus high intakes of protein. Those with a low protein intake obtained relatively less protein from meat, but more from cereals than those with a high protein intake. The same differences in meat and cereal consumption were found in the Newcastle 85^{+} Study (Mendonça et al., 2018). These findings suggested that higher protein intake across the day may be associated with the consumption of certain sources of protein.

To our knowledge, this is the first study investigating the association between sources of protein and high protein intake (≥ 0.4 g/kg BW) at each meal.

Our study found the consumption of bread, breakfast cereal, milk, and yoghurt were associated with high protein intake at breakfast; cheese, eggs and egg products, fish and shellfish, milk, nuts and seeds, poultry, red meat, and vegetables were associated with high protein intake at the mid-day meal; and poultry, red meat, fish and shellfish, and processed meat were associated with high protein intake at the evening meal. These findings emphasise the importance of using specific sources of protein in future intervention strategies for increasing protein intake at each meal and improving protein distribution across the day in older adults.

4.6. Strengths and limitations

There were significant strengths to our study. One of the main strengths was the use of food record, which are considered to be the gold standard of dietary assessment methods and are the most accurate in estimating actual habitual dietary intakes (Gibson, 2005). Our study also used the time-of-day approach to define the three meals in our population, as timing of meals is potentially influenced by local or cultural factors. The inclusion of community-dwelling healthy older adults in this study provided the opportunity to identify inadequate protein intake, which may help promote preventive action in early old age. However, the findings of this study also have various limitations. Firstly, our study population was not representative of the New Zealand population, therefore the results cannot be generalised to the rest of the New Zealand population. Our findings are representative of healthy, community-dwelling older adults, and so provide little insight into the dietary protein requirements of sub-populations of older adults, such as frail older adults, those in residential care, or those who are hospitalised. Finally, as with all dietary assessment instruments, food record is restricted to participant self-report. Although self-reported and subjective opinions provide valuable insights, they may be affected by social desirability bias and hypothetical bias, and, hence, may not accurately represent actual behaviour.

4.7. Conclusions

In this study, the protein intake for a high proportion of older adults was less than 1.2 g/kg BW, and was unevenly distributed through the day, being obtained from cereals and dairy products at breakfast and the mid-day meal, and meat sources at the evening meal. Identifying which sources of protein are associated with meeting a protein intake of 0.4 g/kg BW per meal has the potential to facilitate targeted dietary advice for older adults.

Future research should focus on the sources and timing of protein intake in order to develop effective protein dietary interventions that enable maximal MPS across the day, with the potential to increase muscle mass in older adults.

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Chapter Five – The association between muscle strength and protein intake, sources, and distribution in community-dwelling older adults living in Auckland, New Zealand

Muscle protein synthesis impairment is associated with muscle mass and muscle strength loss in older adults. It is widely acknowledged that adequate protein intake, distribution, and sources contribute to improved MPS and increased muscle mass in older adults. However, it is unknown whether dietary protein plays a role in muscle strength. Chapter five is a manuscript based on an article being reviewed by the journal Nutrients that examines the association between muscle strength and protein intake, sources, and distribution (submitted March 2023).

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The formatting, layout, and referencing style of the published manuscript have been modified to align with this thesis.

5.1. Abstract

Background: Protein intake, sources and distribution impact on muscle protein synthesis and muscle mass in older adults. However, it is less clear whether dietary protein influences muscle strength.

Objective: To explore associations between muscle strength and protein intake, sources of protein, and different estimates of protein distribution throughout the day in community-living older adults in New Zealand.

Methods: Data were obtained from the Researching Eating Activity and Cognitive Health (REACH) study, a cross-sectional study aimed at investigating dietary patterns, cognitive function and metabolic syndrome in older adults 65 to 74 years. Dietary intake was assessed using a 4-day food record, and muscle strength using a handgrip strength dynamometer (JAMAR HAND). Body mass index was used to adjust handgrip strength (BMI-muscle strength). Multiple linear regression analyses were performed separately in females and males to examine associations between BMI-muscle strength (dependent variable) and protein intake, sources and distribution.

Results: Participants (n = 325, 65.2% female) had a median daily protein intake of 1.2 (0.9, 1.4) g/kg body weight (BW) (males) and 1.1 (0.9, 1.3) g/kg BW (females). After adjusting for confounders, in female older adults, relative protein intake was positively associated with BMI-muscle strength ($r^2 = 0.25$, $\rho < 0.01$). Protein derived from either dairy and eggs ($\rho = 0.03$); and plant sources ($\rho < 0.01$) was related to BMI-muscle strength but not protein from meat and fish ($\rho = 0.55$). Greater frequency of protein consumption of at least 0.4 g/kg BW per meal was associated with BMI-muscle strength ($\rho = 0.01$), but the coefficient of variance (CV) for protein intake distribution was not related to BMI-muscle strength ($\rho = 0.47$). There was no relationship between BMI-muscle strength and total protein intake; protein from meat and fish, dairy and eggs, and plant-based sources; or evenness distribution defined as frequency of protein consumption of at least 0.4 g/kg BW per meal ($\rho = 0.35$) or CV ($\rho = 0.12$) in male older adults.

Conclusions: In female older adults, higher muscle strength was associated with consuming more protein each day; protein from dairy and eggs, and plant food sources; and frequently consuming higher amounts of protein at each meal. No associations between protein intake and muscle strength were observed in males. There may be sex differences when investigating associations between protein intake and muscle strength in older adults. Further research is needed to investigate these sex differences.

5.2. Introduction

Proteins are the most abundant component of skeletal muscle mass. Approximately 40% of the body weight of a healthy adult weighing 70 kg is skeletal muscle mass, which is composed of about 20% muscle protein (Frontera & Ochala, 2015). With ageing, the muscle's ability to stimulate protein synthesis is reduced, leading to a decline in muscle mass in older adults (Colley et al., 2011; Fujita, 2007; Volpi et al., 2001). The loss of muscle mass is suggested to be a key contributor to the decrease in muscle strength observed with age, and low muscle strength is recognised as the single largest intrinsic risk factor for falls (Frontera et al., 2000; Newman et al., 2003).

The cause of muscle protein synthesis (MPS) impairment with ageing is unknown, but it is assumed to be affected by impairments in several physiological processes (Boirie et al., 1997; Burd et al., 2013; Dickinson et al., 2013; Rasmussen et al., 2006; Timmerman et al., 2010; Volpi et al., 1999). It was initially thought that a reduced rate of amino acid absorption into the bloodstream and/or digestibility in the stomach and the small intestine may limit the availability of amino acids for MPS (Boirie et al., 1997; Volpi et al., 1999). Further research suggested that a decline in amino acid transporters, which mediate transfer of amino acids into and out of cells, may decrease amino acids that are delivered to the muscle and their subsequent uptake by the muscle (Dickinson et al., 2013; Rasmussen et al., 2006; Timmerman et al., 2010). Amino acid transfer and availability are both regulated by the quality and quantity of protein consumed per meal and per day.

The quality of protein in various foods is determined by their essential amino acid composition, total amount of each amino acid, and relative ease of digestion in the stomach and small intestine, as well as absorption. High-quality animal proteins (e.g., meat, fish, dairy and eggs) have a greater ability to enhance MPS and increase muscle mass than plant-based proteins (e.g., soy, pea, and wheat) (Gorissen et al., 2016; Heimo Scherz et al., 2000; van Vliet et al., 2015; Yang et al., 2012). Isanejad et al. (2015) reported higher total and animal (but not plant) protein intakes were associated with greater muscle strength in women aged 65 to 71 years. These findings were subsequently confirmed in women and men by McLean et al. (2016).

However, these studies did not consider whether the source of animal protein, i.e., meat, fish, dairy, or eggs, makes any difference. In terms of protein quality properties, not all animal-based protein sources are the same. Leucine, for instance, makes up 10.9% of all essential amino acids in milk proteins, compared to 8.8% in beef proteins; and compared to beef/fish, egg proteins have the highest value for protein digestibility (Schaafsma, 2000; Smith & Gray, 2016; van Vliet et al., 2015). In comparison, highly digestible protein source offers a higher proportion of absorbed amino acids, whereas a higher leucine content indicates that less protein from a given source is necessary to maximise MPS rates (Garlick, 2005; Lynch, 2001). As a result, the association between muscle strength and animal proteins may depend on the type of animal protein being consumed.

In older adults, a protein intake of 0.4 g/kg body weight (BW) per meal has been found to provide a pool of available amino acids that stimulates muscle protein maximally (Moore et al., 2015; Paddon-Jones & Rasmussen, 2009). Therefore, it is hypothesised that eating three meals per day, with a regular protein intake of 0.4 g/kg BW per meal, could increase MPS throughout the day, potentially increasing and preserving muscle strength in older adults. In a randomised controlled trial, Mamerow et al. (2014) demonstrated that, in healthy adults aged 25 to 55 years, MPS was approximately 25% higher in those with an even distribution of daily protein intake (0.4 g/kg BW per meal) compared with those who had a skewed distribution of the same amount of daily protein (1.2 g/kg BW per day). Distribution of protein is a relatively new concept. In previous studies distribution is estimated as the coefficient of variance (CV) of the protein intake, or the number of meals exceeding 0.4 g/kg BW. There are conflicting findings regarding the influence of protein distribution on muscle strength, which could be impeded by different distribution calculations.

The aim of this study was to examine muscle strength and its relationship with protein intake, sources, and different estimates of protein distribution throughout the day, in community-living older adults in Auckland, New Zealand.

5.3. Materials and methods

5.3.1. Study design

Data for this study were obtained from Researching Eating, Activity and Cognitive Health (REACH) study; the methodology has been described elsewhere (Mumme et al., 2019). In brief, REACH participants were adults aged between 65 to 74 years, proficient in English, and living independently (i.e., not in residential care) in Auckland, NZ. Ineligibility criteria included a diagnosis of dementia or any condition which may impair cognitive function (e.g., previous head injury, stroke), taking medication which may influence cognitive function, colour blindness (due to cognitive testing requirements), or experiencing any other event in the last two years which may have had a substantial impact on dietary intake or cognition.

The research protocol was approved by the Massey University Human Ethics Committee: Southern A, Application 17/69 and all participants provided written informed consent.

5.3.2. Data collection

Age, sex, polypharmacy status and smoking status data were collected using a health and demographic written questionnaire. Physical activity was assessed through the International Physical Activity Questionnaire - short form (Craig et al., 2003). A physical activity score was calculated using the Metabolic equivalent of a task (MET-min) where one minute of walking, moderate or vigorous activity equates to 3.3, 4.0 or 8.0 METs, respectively (Craig et al., 2003). Polypharmacy status was defined as five or more daily medications, and smoking status was described as both current and previous smoking (Masnoon et al., 2017).

Weight (in cm, to the nearest 0.1 cm) was measured using a stadiometer (SECA). Body weight (BW) (in kg, to the nearest 0.1 kg) was assessed using floor scales (Wedderburn). Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

Muscle strength was assessed using an adjustable handgrip strength dynamometer (JAMAR HAND). The handgrip dynamometer measures the maximum kilograms of force per trial, where three trials were undertaken for each of the right and left hands with a 15–20 second break between trials (Mathiowetz et al., 1985; Roberts et al., 2011). The mean of three trials for each hand was noted and the highest value of the two means was considered the final value. Because the Foundation for the National Institutes of Health (FNIH) showed that BMI-standardised handgrip strength is more strongly associated with falls and related injuries than absolute handgrip strength, we used handgrip strength adjusted for BMI (Tang et al., 2018). Low muscle strength was defined as BMI-handgrip strength < 1.00 m^2 in men and < 0.56 m^2 in women (Cawthon et al., 2014).

Dietary intake data was collected using a 4-day food record. Participants were asked to write down everything they ate and drank for four consecutive days, including at least 1 weekend day. They were advised to describe the amount of food consumed using household measurements. The dietary data collection method has been described in detail in chapter four (manuscript 2).

5.3.3. Data handling

Food record data was entered by trained nutritionists using FoodWorks 10, which is based on the New Zealand Food Composition database (Sivakumaran et al., 2017; Xyris Pty Ltd, 2019). Energy and macronutrient intake was generated as kilojoules and grams of intake per day respectively. Relative protein intake was calculated by dividing the amount of protein (g) consumed by body weight (kg).

Meals were categorised into ‘breakfast’, the ‘mid-day meal’ and the ‘evening meal’ based on the time of day at which protein was consumed. Protein in g and g/kg BW was calculated at breakfast, the mid-day meal, and the evening meal. The consumption of 0.4 g/kg BW of protein at each meal was reported.

Protein distribution was calculated for each participant as a:

- Coefficient of variance (CV = standard deviation of protein intake of the three meals (g) / total protein intake for the three meals (g)). A CV of zero indicates that protein is distributed evenly throughout the day. The smaller the CV, the more even the distribution and,

- The frequency of consuming ≥ 0.4 g/kg of protein across the day's meal. This was calculated by adding the number of meals at which individuals consumed ≥ 0.4 g/kg BW of protein. The value for frequency of protein consumption variable ranged from 1-3 (breakfast, the mid-day and the evening meal). A higher number represented a more even distribution.

All foods from the food record were allocated into one of 27 food groups based on the main sources of protein intake reported in the 2008/09 New Zealand Adult Nutrition Survey (University of Otago and Ministry of Health, 2011). The food groups were further classified under meat and fish; dairy and egg products; and plants according to the main type of protein they contained (Appendix G). Protein intake from meat and fish; dairy and egg products; and plants were calculated in g/kg BW.

5.3.4. Statistical analysis

All continuous variables were tested for normality with the Shapiro-Wilk test. Participant characteristics were displayed as median and interquartile range (IQR) for non-parametric continuous variables and as counts with percentages for categorical variables. Differences between groups were tested using the Mann-Whitney U test for non-normally distributed continuous data or the Chi-square test for categorical variables.

Multiple regression analysis were performed separately in females and males to examine associations between BMI-muscle strength (dependent variable) and:

- Relative protein intake per day. The confounding variables were energy intake (kJ), physical activity level (MET-minutes/week), age (years), polypharmacy status (yes or no) and smoking status (yes or no).

- Protein sources eaten each day: meat and fish (g/kg); dairy and egg products (g/kg); and plants (g/kg). These models accounted for the effects of energy intake (kJ), physical activity level (MET-minutes/week), age (years), polypharmacy status (yes or no) and smoking status (yes or no). Total protein intakes from meat and fish; dairy and egg products; and plants were included in the same regression model to adjust for one another.

- Different estimates of protein distribution throughout the day (CV and frequency consuming of meals containing ≥ 0.4 g/kg of protein per day). The models were controlled for total protein intake (g), energy intake (kJ), physical activity level (MET-minutes/week), age (years), polypharmacy status (yes or no) and smoking status (yes or no).

All statistical analyses were performed using IBM SPSS Statistics for Windows, version 27.0 (IBM SPSS, Armonk, NY, USA). All the probability values were two-tailed and were considered significant if a ρ-value was < 0.05.

5.4. Results

A total of 371 individuals participated in the REACH study. We excluded individuals who did not provide or had incomplete variables such as food record (44), handgrip strength (1) and physical activity (1). The final data set included 325 older adults (113 males and 212 females).

Table 5. 1 presents descriptive statistics according to sex for the study population. There were no significant differences between males and females in physical activity ($\rho = 0.88$), polypharmacy ($\rho = 0.31$), or smoking status ($\rho = 0.93$). Females were younger ($\rho < 0.01$) and had a lower height ($\rho < 0.01$), weight ($\rho < 0.01$), BMI ($\rho = 0.02$), and BMI-muscle strength ($\rho < 0.01$) compared with males. Males had a higher prevalence of low BMI-muscle strength (4.4%) than females (3.8%).

Table 5. 1. Participants' characteristics [a,b]

Characteristics	**Total**	**Females**	**Males**	**ρ-value**
n (%)	325	212 (65.2)	113 (34.8)	< 0.01**
Age, years	69.7 (67.9, 71.6)	69.3 (67.5, 71.4)	70.3 (68.5, 71.9)	0.01*
Physical activity, MET min/week	3088.0 (1679.5, 5079.0)	3126.0 (1618.0, 5121.0)	3066.0 (1778.7, 4942.0)	0.88
Height, m	1.7 (1.6, 1.7)	1.6 (1.6, 1.7)	1.8 (1.7, 1.8)	< 0.01**
Body weight, kg	71.2 (63.2, 81.5)	67.0 (59.6, 74.7)	81.5 (73.7, 89.3)	< 0.01**
BMI, kg/m^2	25.6 (23.1, 28.2)	25.3 (22.7, 28.1)	26.4 (24.3, 28.3)	0.02*
Polypharmacy status, Yes, n (%)	25 (7.7)	14 (6.6)	11 (9.7)	0.31
Smoker status, Yes, n (%)	71 (21.8)	46 (21.7)	25 (22.1)	0.93
BMI-muscle strength	1.1 (0.9, 1.4)	1.0 (0.8, 1.1)	1.6 (1.3, 1.8)	< 0.01**
Prevalence of low BMI-muscle strength	13 (4)	8 (3.8)	5 (4.4)	< 0.01**

[a]Continuous values are expressed as median (25^{th}, 75^{th} percentile). [b]Categorical values are expressed as frequency (percentage). Sex difference at $^{**}\rho < 0.01$, $^{*}\rho < 0.05$

Table 5. 2 shows the median protein intake, distribution, and sources for both females and males. Females consumed significantly less energy ($\rho < 0.01$), carbohydrate ($\rho < 0.01$), fat ($\rho < 0.01$), and absolute protein ($\rho < 0.01$) than males. Females had a lower relative protein intake per day (1.1 (0.9, 1.3) g/kg BW) to males (1.2 (0.9, 1.4) g/kg BW), but this was not significant ($\rho = 0.38$).

The evening meal provided the highest median protein intake (g/kg) at 0.5 (0.3, 0.7), followed by the mid-day meal at 0.3 (0.2, 0.4), and breakfast at 0.3 (0.2, 0.3). Protein distribution was uneven throughout the day as indicated by a median CV for protein distribution of 0.5 (0.3, 0.6) for both males and females. When comparing individual meals to the 0.4 g/kg threshold, 5% of females and 5% of males met the threshold for all three meals. Males and females had a median protein intake (g/kg) of 0.4 (0.3, 0.6) from plant sources, 0.4 (0.3, 0.5) from meat and fish sources, and 0.3 (0.2, 0.4) from dairy and egg sources.

Table 5. 2. Median protein intake, distribution, and sources [a,b]

		Total	Females	Males	ρ-value
Energy intake, kJ/day		7914.7 (6717.1, 9271.3)	7434.3 (6339.3, 8382.0)	9239.4 (7920.1, 10729.9)	< 0.01**
Carbohydrate, g/day		186.4 (147.1, 225.9)	172.9 (136.2, 201.2)	222.9 (185.2, 262.6)	< 0.01**
Total fat, g/day		76.7 (63.6, 94.1)	72.9 (61.5, 86.5)	90.8 (69.5, 107.7)	< 0.01**
Protein, g/day		79.4 (67.5, 97.2)	75.0 (63.5, 88.2)	95.9 (77.5, 107.2)	< 0.01**
Protein, g/kg BW/day		1.1 (0.9, 1.4)	1.1 (0.9, 1.3)	1.2 (0.9, 1.4)	0.38
Protein intake at each meal (g/kg BW)	Breakfast	0.3 (0.2, 0.3)	0.2 (0.2, 0.3)	0.3 (0.2, 0.4)	0.01*
	Mid-day	0.3 (0.2, 0.4)	0.3 (0.3, 0.4)	0.3 (0.2, 0.4)	0.14
	Evening	0.5 (0.3, 0.7)	0.5 (0.4, 0.7)	0.6 (0.4, 0.7)	0.30
Number of meals providing ≥ 0.4 g/kg of protein	0	44 (13.5%)	25 (11.8%)	19 (16.8%)	0.53
	1	188 (57.8%)	126 (59.4%)	62 (54.9%)	
	2	77 (23.7%)	51 (24.1%)	26 (23.0%)	
	3	16 (4.9%)	10 (4.7%)	6 (5.3%)	
CV		0.5 (0.3, 0.6)	0.5 (0.3, 0.6)	0.5 (0.3, 0.7)	0.96
Protein sources (g/kg BW)	Protein from meat and fish	0.4 (0.3, 0.5)	0.4 (0.3, 0.5)	0.4 (0.3, 0.6)	0.09
	Protein from dairy and eggs	0.3 (0.2, 0.4)	0.3 (0.2, 0.4)	0.2 (0.2, 0.3)	0.19
	Plant protein	0.4 (0.3, 0.6)	0.4 (0.3, 0.6)	0.5 (0.4, 0.6)	0.28

[a]Continuous values are expressed as median (25[th], 75[th] percentile). [b]Categorical values are expressed as frequency (percentage). Sex difference at **ρ < 0.01, *ρ < 0.05.

Table 5. 3 shows the association between relative protein intake and BMI-muscle strength in females and males. In females, BMI-muscle strength was positively associated with relative protein intake ($r^2 = 0.25$, $\rho < 0.01$). However, in males, relative protein intake was not a significant predictor of BMI-muscle strength.

Table 5. 3. Association between protein intake and muscle strength in females and males.

BMI-muscle strength (dependent variable)	**Coefficient (B)**	**Standard error (B)**	**95% CI**	**Standardised (β)**	**r^2**	**ρ-value**
Model females					0.25	< 0.01**
Constant	0.98	0.41	0.17, 1.80			0.02*
Protein intake (g/kg BW/day)	0.22	0.05	0.12, 0.33	0.30		< 0.01**
Energy intake, kJ/day	1.74E-5	0.00	0.00, 0.00	0.11		0.12
Physical activity, MET min/week	1.55E-5	0.00	0.00, 0.00	0.17		0.01*
Age, years	-0.01	0.01	-0.02, 0.00	-0.12		0.06
Polypharmacy	0.16	0.59	0.05, 0.28	0.18		0.01*
Smoking	-0.03	0.03	-0.10, 0.04	-0.06		0.34
Model males					0.19	< 0.01**
Constant	1.75	0.89	-0.01, 3.50			0.05
Protein intake (g/kg)	0.11	0.11	-0.10, 0.32	0.11		0.23
Energy intake, kJ/day	4.97E-5	0.00	0.00, 0.00	0.31		< 0.01**
Physical activity, MET min/week	1.23E-5	0.00	0.00, 0.00	0.11		0.23
Age, years	-0.02	0.01	-0.04, 0.01	-0.12		0.19
Polypharmacy	0.12	0.10	-0.07, 0.31	0.11		0.22
Smoking	0.03	0.07	-0.11, 0.16	0.03		0.71

CI = confidence interval. MET = metabolic equivalent of a task. MET minutes/week based on 3.3 MET for walking, 4.0 MET for moderate activity and 8.0 MET for vigorous activity. Female and male models are adjusted for energy intake (kJ), physical activity level (MET-minutes/week), age (years), polypharmacy status (yes or no) and smoking status (no or yes). Significant at **p < 0.01, *p < 0.05.

BMI-muscle strength in females was positively correlated with protein from dairy and egg products (ρ = 0.03), as well as plant proteins (ρ <0.01), but not with proteins from meat and fish (ρ = 0.55). Table 5. 4 shows there was no relationship between BMI-muscle strength and all protein sources in males.

Table 5. 4. Associations between sources of protein and muscle strength in females and males.

BMI-muscle strength (dependent variable)	**Coefficient (B)**	**Standard error (B)**	**95% CI**	**Standardised (β)**	**r^2**	**ρ-value**
Females					0.35	< 0.01
Constant	1.10	0.39	0.34, 1.86			0.01
Protein from meat and fish, g/kg BW/day	0.04	0.06	-0.09, 1.17	0.36		0.55
Protein from dairy and eggs, g/kg BW/day	0.21	0.10	0.02, 0.40	0.13		0.03
Protein from plant, g/kg BW/day	0.60	0.09	0.43, 0.78	0.49		< 0.01
Energy intake, kJ/day	-6.19E-6	0.00	0.00, 0.00	-0.04		0.58
Physical activity, MET min/week	1.15E-5	0.00	0.00, 0.00	0.13		0.03
Age, years	-0.01	0.01	-0.21, -0.00	-0.12		0.04
Polypharmacy	0.18	0.05	0.07, 0.28	0.19		< 0.01
Smoking	-0.04	0.03	-0.10, 0.02	-0.07		0.21
Males					0.20	< 0.01
Constant	1.71	0.90	-0.07, 3.48			0.06
Protein from meat and fish, g/kg BW/day	0.06	0.14	-0.21, 0.33	0.04		0.65
Protein from dairy and eggs, g/kg BW/day	0.00	0.24	-0.48, 0.48	0.00		0.99
Protein from plant, g/kg BW/day	0.32	0.20	-0.07, 0.71	0.17		0.10
Energy intake, kJ/day	4.47E-5	0.00	0.00, 0.00	0.28		0.01
Physical activity, MET min/week	1.26E-5	0.00	0.00, 0.00	0.11		0.22
Age, years	-0.02	0.01	-0.04, 0.01	-0.11		0.21
Polypharmacy	0.12	0.10	-0.08, 0.32	0.11		0.25
Smoking	0.02	0.07	-0.12, 0.16	0.03		0.75

CI = confidence interval. MET = metabolic equivalent of a task. MET minutes/week based on 3.3 MET for walking, 4.0 MET for moderate activity and 8.0 MET for vigorous activity. Female and male models are adjusted for energy intake (kJ), physical activity level (MET-minutes/week), age (years), polypharmacy status (yes or no) and smoking status (no or yes). Significant at **p < 0.01, *p < 0.05.

Table 5. 5 demonstrates associations between the CV of protein across main meals and the number of meals containing at least 0.4 g of protein/kg BW and BMI-muscle strength in females and males. In females the CV was not significantly correlated to BMI-muscle strength ($\rho = 0.47$), whereas meal frequency of 0.4 g/kg was significantly associated with BMI-muscle strength ($\rho < 0.01$). However, there was no relationship between BMI-muscle strength and CV of protein across main meals ($\rho = 0.12$) and number of meals containing at least 0.4 g of protein/kg BW ($\rho = 0.35$) in males.

Table 5. 5. Associations between protein distribution and muscle strength and in males and females.

BMI-muscle strength (dependent variable)	**Coefficient (B)**	**Standard error (B)**	**95% CI**	**Standardised (β)**	**r^2**	**ρ-value**
Model 1 females					0.20	< 0.01
Constant	1.07	0.42	0.25, 1.90			0.01
Number of meals providing at least 0.4 g /kg BW/day	0.08	0.02	0.04, 0.13	0.26		< 0.01
Protein intake, g/day	-0.00	0.01	-0.01, 0.00	-0.16		0.06
Energy intake, kJ/day	4.14E-5	0.00	0.00, 0.00	0.27		< 0.01
Physical activity, MET min/week	1.54E-5	0.00	0.00, 0.00	0.17		0.01
Age, years	-0.01	0.01	-0.02, 0.00	-0.11		0.07
Polypharmacy status	0.18	0.06	0.07, 0.30	0.20		0.00
Smoking status	-0.02	0.04	-0.90, 0.05	-0.04		0.54
Model 2 females					0.19	< 0.01
Constant	1.01	0.43	0.17, 1.86			0.02
CV of protein intake at main meals	-0.05	0.07	-0.19, 0.09	-0.05		0.47
Protein intake, g/day	-0.00	0.00	-0.00, 0.00	-0.04		0.64
Energy intake, kJ/day	4.39E-5	0.00	0.00, 0.00	0.29		< 0.01
Physical activity, MET min/week	1.75E-5	0.00	0.00, 0.00	0.19		< 0.01
Age, years	-0.01	0.01	-0.02, 0.00	-0.12		0.07

Polypharmacy	0.19	0.06	0.07, 0.31	0.21		< 0.01
Smoking	-0.01	0.04	-0.08, 0.06	-0.02		0.74
Model 1 males					0.19	< 0.01
Constant	1.83	0.94	-0.04, 3.67			0.05
Number of meals providing at least 0.4 g /kg BW/day	-0.05	0.05	-0.16, 0.06	-0.12		0.35
Protein intake, g/day	-0.01	0.00	-0.01, 0.00	-0.04		0.81
Energy intake, kJ/day	7.28E-5	0.00	0.00, 0.00	0.45		< 0.01
Physical activity, MET min/week	1.51E-5	0.00	0.00, 0.00	0.13		0.15
Age, years	-0.02	0.01	-0.04, 0.01	-0.13		0.16
Polypharmacy (yes or no)	0.14	0.10	-0.07, 0.34	0.12		0.19
Smoking status (no/ yes)	0.03	0.07	-0.11, 0.17	0.04		0.66
Model 2 males					0.20	< 0.01
Constant	2.01	0.90	0.22, 3.80			0.03
CV of protein intake at main meals	-0.21	0.13	-0.48, 0.05	-0.15		0.12
Protein intake, g/day	-0.00	0.00	-0.01, 0.00	-0.09		0.46
Energy intake, kJ/day	6.44E-5	0.00	0.00, 0.00	0.40		< 0.01
Physical activity, MET min/week	1.53E-5	0.00	0.00, 0.00	0.14		0.14
Age, years	-0.02	0.01	-0.04, 0.01	-0.12		0.19
Polypharmacy	0.10	0.10	-0.09, 0.30	0.10		0.29
Smoking	0.02	0.07	-0.12, 0.16	0.03		0.77

CV: coefficient of variance of protein intake (g) at breakfast, lunch, and dinner. CI = confidence interval. MET = metabolic equivalent of a task. MET minutes/week based on 3.3 MET for walking, 4.0 MET for moderate activity and 8.0 MET for vigorous activity. Female and male models are adjusted for energy intake (kJ), physical activity level (MET-minutes/week), age (years), polypharmacy status (yes or no) and smoking status (no or yes). Significant at **p < 0.01, *p < 0.05.

5.5. Discussion

In this cross-sectional study, we investigated the relationship between BMI-muscle strength and protein intake, sources, and distribution in female and male older adults living in Auckland, NZ. In females, the findings indicate that relative protein intake was associated with BMI-muscle strength. This relationship was independent of total energy intake, age, physical activity, smoking and polypharmacy status. We also demonstrated an association between BMI-muscle strength and protein intake from dairy, eggs and plant sources; but not with protein from meat and fish intake. We found the coefficient of variance of protein intake was not related to BMI-muscle strength. However, we demonstrated that greater frequency of protein consumption of 0.4 g/kg BW per meal was associated with BMI-muscle strength. In male older adults there were no associations between BMI-muscle strength and protein intake, sources, or distribution.

5.5.1. Associations between BMI-muscle strength and protein intake, sources, and distribution in females

We provide evidence that relative protein intake is positively associated with BMI-muscle strength in female older adults aged between 65 to 74 years. Our findings are consistent with previous cross-sectional studies indicating an association between relative protein intake and muscle strength adjusted by body weight (BW) or BMI in older adults (Celis-Morales et al., 2018; Fanelli Kuczmarski et al., 2018; Isanejad et al., 2016). In contrast, other studies found there was no association between relative protein intake and muscle strength (Granic et al., 2018; Mishra et al., 2018; Ten Haaf et al., 2018). One explanation for their lack of associations could be related to not adjusting muscle strength for BW or BMI.

Muscle strength adjusted BMI or BMI-muscle strength has been proposed as a new marker for muscle strength because it minimises the confounding effect of body weight (Keevil et al., 2015; Kim et al., 2019; Kim et al., 2017; Villareal et al., 2004).

Furthermore, low BMI-muscle strength represents not only low muscle strength but also obesity, both of which are closely related to impairment of MPS in response to ingested protein (Beals et al., 2018; Guillet et al., 2009; Keevil et al., 2015; Kim et al., 2019; Kim et al., 2017; Murton et al., 2015; Villareal et al., 2004). In this regard, we used BMI-muscle strength and demonstrated a relationship between relative dietary protein and muscle strength in female older adults.

In multiple regression analysis examining associations between protein sources and BMI-muscle strength, dairy and egg proteins (but not meat and fish) were found to be positively associated with BMI-muscle strength. Because the amount of protein consumed from both animal food group sources were comparable, these findings suggest that the protein quality properties of dairy and eggs, which have a higher leucine content and higher value for protein digestibility than meat and fish proteins, may explain the association found in this study. In contrast to previous research (Isanejad et al., 2015; McLean et al., 2016), an association between BMI-muscle strength and protein from plant sources was observed. The differences in findings could be attributed to differences in the amount of plant protein consumed. Participants in this cohort consumed a similar amount of protein from both animal and plant-based sources, whereas in other studies, the range of plant-based protein sources was much smaller.

When calculating the distribution of protein using the CV calculation, we found no association between the CV and BMI-muscle strength in female and male older adults. This result aligns with the literature (Bollwein et al., 2013; Gingrich et al., 2017; Kim et al., 2018; Ten Haaf et al., 2018), and suggests that the CV might give information about distribution of protein, but it does not provide information about the amount of protein consumed. Murphy et al. (2015) also demonstrated that MPS responses were not different in older adults subjected to uneven or even distribution. Given this, the consumption of meals containing less than 0.4 g/kg, required for maximal MPS in older adults may help to explain this finding. We demonstrated a positive association between the frequency of meals of $\geq$ 0.4 g/kg BW and BMI-muscle strength in female older adults. Two recent studies also investigated associations between muscle strength and consuming at least 0.4 g/kg BW in older adults (Gingrich et al., 2017; Johnson et al., 2022).

While both studies did not adjust muscle strength for BMI, Johnson et al. (2022) did account for BMI in their multiple regression analyses and found an association between absolute muscle strength and consuming at least 0.4 g/kg BW in female older adults. Gingrich et al. (2017), on the other hand found no relationship between the number of meals providing 0.4 g/kg BW and muscle strength. However, BMI and sex differences were not considered in their analysis. These findings confirm that the conflicting findings on the influence of protein distribution on muscle strength are impeded by different protein distribution calculations. In the wider literature it is necessary to reach an agreement on an appropriate protein distribution calculation.

5.5.2. Associations between muscle strength and protein intake, sources, and evenness distribution in males

There was no association between muscle strength and protein intake, sources, or distribution in older male adults. The unequally sized sex groups, with males having a sample size of 113 and females of 212, may lead to possibly insufficient power to detect associations between muscle strength and protein intake, sources, and distribution in males.

One of the main strengths of this study was the use of BMI-muscle strength, which has been shown to be more strongly associated with composite adverse outcomes such as falls compared with absolute muscle strength (Tang et al., 2018). An additional strength was the use of food record, which is considered as the gold standard of dietary assessment methods due to their accuracy in estimating actual dietary intakes (Gibson, 2005). Relative protein intake was used to account for differences in body weight, and particularly weight differences between sexes in older adults (Moore, 2019). In addition, several variables related to protein intake (quantity, sources, and distribution) were addressed concurrently in this study. Finally, adjustments were made for age, total energy intake, physical activity, polypharmacy and smoking status, all of which are important potential confounders in the relationship between muscle strength and dietary intake. Total protein intake was adjusted for when considering the distribution of protein consumed throughout the day. The study's limitations include its cross-sectional design, which limited its ability to detect causality; thus, only associations can be discussed.

5.6. Conclusions

In this study, we demonstrated that female older adults who consumed a high relative protein intake and frequently consumed higher amounts of protein at each meal had increased BMI-muscle strength. Protein from dairy and eggs, as well as plant sources, was associated with BMI-muscle strength, but not protein from meat and fish. In male older adults, however, there was no association between BMI-muscle strength and protein intake, sources, or distribution. These results indicate that there may be sex differences when investigating associations between dietary protein quantity, sources and distribution and muscle strength. Future research should investigate the factors that contribute to the sex differences in this relationship.

5.7. References

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Chapter Six – Discussion

This chapter describes and interprets the major findings of this thesis. The study's strengths and limitations are discussed, leading to recommendations for additional research, and final conclusions are drawn.

This thesis explored the muscle strength, muscle mass and dietary protein intake, distribution and sources in community-dwelling older adults living in NZ. The main findings indicate that: 1) Muscle mass was associated with muscle strength in non-obese participants. However, this association was not observed in older adults who were classified as obese; 2) In female older adults, higher muscle strength was associated with consuming more protein each day; protein from dairy and eggs, as well as plant food sources; and frequently consuming higher amounts of protein at each meal. No associations between muscle strength and protein intake, sources and distribution were observed in males; 3) Protein intake for a high proportion of older adults was less than 1.2 g/kg BW per day, 0.4 g/kg BW per meal through the day, and mainly from cereals and dairy products at breakfast and the mid-day meal, and meat sources at the evening meal.

6.1. Discussion of main results and further perspectives

Our results provide evidence that muscle mass was positively associated with muscle strength in older males and females. This result was predicted and is consistent with the literature (Barbat-Artigas, Plouffe, et al., 2013; Beliaeff et al., 2008; Chen et al., 2013; Hayashida et al., 2014; Reed et al., 1991). However, obesity appears to moderate this relationship. We demonstrated that muscle mass was associated with muscle strength in non-obese older adults (< 30% BF (males) and < 40% BF), whereas there was no association between muscle mass and muscle strength in older adults who were classified as obese (≥ 30% BF (males) and ≥ 40% BF (females). Chen et al. (2013) found that obese older adults (BMI ≥ 30 kg/m^2) had lower strength than non-obese older adults with the same muscle mass. These findings indicate that obesity status, as defined by % BF or BMI should be considered in further research investigating associations between muscle mass and muscle strength in older adults.

In light of these findings, BMI-muscle strength, which takes into account muscle strength but also obesity status, was considered more appropriate to use than absolute muscle strength when investigating the relationship between muscle strength and protein intake in older adults. We provide evidence that the relative protein intake (g/kg BW) is positively associated with BMI-muscle strength (handgrip strength adjusted for BMI, kg/m^2) in females. This result is consistent with previous research investigating the relationship between relative protein intake and the relative muscle strength adjusted BMI or BMI (Celis-Morales et al., 2018; Fanelli Kuczmarski et al., 2018; Isanejad et al., 2016). Other studies however have reported that relative protein intake was not associated with absolute muscle strength (kg) (Granic et al., 2018; Mishra et al., 2018; Ten Haaf et al., 2018; Wham et al., 2021). The observed differences in results could be attributed to the use of absolute muscle strength as opposed to relative muscle strength.

In linear regression analysis examining associations between protein sources and BMI-muscle strength, ‘dairy and eggs’ as well as ‘plant-based’ protein food groups were found to be positively associated with BMI-muscle strength in female older adults.

However, an association between BMI-muscle strength and protein from 'meat and fish' food groups was not observed. Because the amount of protein consumed from both animal food group sources were similar, these findings are likely attributed to the differences in their protein quality properties (leucine content and digestibility) and the presence of other nutrients such as vitamin D in dairy food group (Day et al., 2022). Other studies have reported that meat, fish, dairy and eggs (but not plant) sources of protein were associated with greater muscle strength (Isanejad et al., 2015; McLean et al., 2016). The differences in findings between these studies and ours could be attributed to differences in the amount of plant protein consumed. In this study, participants consumed a similar amount of protein from animal and plant sources, whereas in other studies, the protein intake from plant sources was much lower. Recently, Pikosky et al. (2022) demonstrated that increasing quartiles of both animal and plant dietary proteins were associated with increased muscle strength in older adults. However, this association was significant for animal-based protein sources at quartile 2, and only became significant at quartile 3 for plant-based proteins.

When calculating the distribution of protein using the coefficient of variance calculation, we found that the coefficient of variance of protein intake was not associated with BMI-muscle strength in female and male older adults. This result aligns with the literature (Bollwein et al., 2013; Gingrich et al., 2017; Kim et al., 2018; Ten Haaf et al., 2018), with a possible explanation being the consumption of meals containing less than 0.4 g/kg BW at breakfast and mid-day meals in these studies, which is the intake required for maximal MPS in older adults. Furthermore, we demonstrated that greater frequency of protein consumption of 0.4 g/kg BW per meal was associated with BMI-muscle strength in female older adults when we calculated the distribution of protein using the frequency of consuming 0.4 g/kg BW of protein across the day's meals. Two recent studies also investigated associations between muscle strength and consuming at least 0.4 g/kg BW older adults (Gingrich et al., 2017; Johnson et al., 2022). While both studies did adjust muscle strength for BMI, Johnson et al. (2022) accounted for BMI in their linear regression analyses and found an association between absolute muscle strength and consuming at least 0.4 g/kg BW in female older adults. Gingrich et al. (2017), on the other hand found no relationship between the number of meals providing 0.4 g protein/kg BW and muscle strength. However, BMI and sex differences were not considered in their analysis.

There was no correlation between BMI-muscle strength and protein intake, sources, or distribution in male older adults. Males have greater anabolic response to protein intake with greater muscle strength than females (Macaluso & De Vito, 2004). Consequently, males may require more protein in order to develop greater muscle strength than do females. In this population male protein intake was comparable to females, which may explain the association between muscle strength and relative protein intake in females but not males. Also, males had a sample size of 113, while females had a sample size of 212, which may result in insufficient power to detect associations between muscle strength and protein intake, sources, and distribution in males. Future research should investigate on factors that could contributed to the sex and body weight differences in this relationship.

We found that average protein intake was inadequate for reaching 0.4 g/kg BW per meal at breakfast for both males and females and at the mid-day meal for males. These findings are consistent with the literature (Gaytán González et al., 2020; Houston et al., 2017; Isanejad et al., 2016; Loenneke et al., 2016; Mendonça et al., 2018), which indicated that older adults and particularly males in our study have a low adherence for 0.4 g/kg BW per meal thresholds. Food preferences associated with ageing may be one barrier to consuming ≥ 0.4 g/kg BW per meal. We found that older adults tended to consume protein from breakfast cereals and bread products at breakfast and mid-day meals. Protein sources contributing to the evening meal were generally animal-derived in the form of red meat, poultry, fish and shellfish. When compared to other foods, breakfast cereals and bread are relatively low in protein per se, making it difficult to consume adequate amounts of these protein sources to provide a high amount of protein.

Our study demonstrated that the consumption of bread, breakfast cereal, milk, and yoghurt were associated with high protein intake at breakfast; cheese, eggs and egg products, fish and shellfish, milk, nuts and seeds, poultry, red meat, and vegetables were associated with high protein intake at the mid-day meal; and poultry, red meat, fish and shellfish, and processed meat were associated with high protein intake at the evening meal. These findings emphasise the importance of using specific sources of protein in future intervention strategies for increasing protein intake at each meal in older adults.

6.2. Strengths and limitations

There were significant strengths and limitations to our study. These include the study design, study population, measurements of body composition, measurements of muscle strength, and dietary assessment.

6.2.1. Study design

This research was completed as part of the REACH study, which had the main objective of investigating dietary patterns and their associations with cognitive function and metabolic syndrome in older adults. Because this was a cross-sectional study, we were able to collect data on muscle strength, muscle mass, protein intake, distribution, and sources from over 350 older adults. The nature of the data captured, however, limits our ability to determine the causality between muscle strength and muscle mass as well as the effect of protein intake, distribution, and sources on muscle strength in older adults.

6.3.2. Study population

Participants in this study were 65 to 74-year-olds, community-dwelling healthy older adults. Targeting this population group may allow for the promotion of preventive measures involving protein intake, distribution, and sources in early old age.

This research included a self-selected sample of older adults who lived independently and could travel to the testing facility. As a result, participants in this study may have been healthier than the general population because they were all living independently and are not necessarily representative of adults aged 65 to 74 years of age living in New Zealand.

Ethnic groups within the Auckland population, notably Māori groups, were not well represented in this sample. The outcomes of this thesis might not be applicable to all ethnic groups due to ethnic differences in protein intake, distribution, and sources. Thus, the study findings may have limitations in terms of generalisation to the overall older adult population.

6.3.3. Measurement of body composition

Using data from dual-energy X-ray absorptiometry (DXA), which is considered the gold standard measurement of body composition (Bernardi et al., 2004; Lee et al., 2019), we calculated muscle mass as described by Heymsfield et al. (1990). They demonstrated that the calculation of appendicular skeletal mass (ASM) as the sum of limb lean mass (LM) minus the sum of limb fat and bone mass was a highly accurate method of quantifying human skeletal muscle mass in older adults. In addition, we classified obesity status using DXA body fat percentage values in order to overcome the limitations of BMI classification in older adults. However, the classification may be perceived as a limitation due to the arbitrary nature of the cut-off points, as there is no consensus.

6.3.4. Measurement of muscle strength

Because lower extremity muscle strength loss occurs later than upper extremity muscle strength loss (Buckley et al., 2018; Burr, 1997), handgrip strength rather than low-body muscle strength, which is a more direct predictor of falls and related injuries, was used in this study. This gives us the advantage of being able to directly compare our findings with other studies' results as it is one of the most commonly used measures of muscle strength in older adults. Also, we adjusted the handgrip strength with body mass index, which is more strongly associated with falls and related injuries than absolute handgrip strength (Tang et al., 2018).

6.3.5. Dietary assessment

Dietary protein was measured using a 4-day food record, which provided the timing of intake used to define the three meals in our population, as meal timing can be influenced by local or cultural factors. Furthermore, when compared to other methods of assessing dietary intake, the food record reduces the risk of recall bias and improves portion size estimation because food items and portion sizes are recorded shortly after consumption. Future studies investigating the effects of dietary interventions on muscle mass and strength, should consider other lifestyle factors (such as exercise type and duration) and previous and current health status.

6.3. Further perspectives and conclusions

Based on this thesis, further research is recommended to develop methods, tools, practical guidance for researchers, and clarify some underlying concepts. For instance,

1. Because muscle mass was not associated with muscle strength in older adults with obesity, future research should focus on identifying predictors of muscle strength to provide appropriate interventions to increase muscle strength in older adults who are categorised as obese.

2. Future studies should determine whether muscle strength should be assessed as relative or absolute when investigating the relationship between muscle strength and dietary protein in older adults.

3. Because this study found that the relationship between muscle strength and protein distribution differed depending on the protein distribution calculation used, a consensus definition of protein distribution is required.

4. Because the last New Zealand Adult Nutrition Survey was reported more than 15 years ago, it may not reflect dietary protein trends that older adults are currently exposed to. An updated New Zealand Adult Nutrition Survey with good representation of older adults is required to further investigate protein intake, distribution and the source of protein at each meal.

5. A study of the relationship between muscle strength and protein intake, distribution and sources in a representative sample of older adults in New Zealand is needed to generalise the findings of this thesis to a larger population. Such findings will contribute to updating protein recommendations for older adults, with the potential to include specific sources and per meal protein guidance.

In conclusion, we have shown the role of obesity in the relationship between muscle strength and muscle mass. Efforts to maintain muscle mass and strength in older adults may need to be tailored differently for older adults according to their levels of obesity. Furthermore, our findings contribute to better understanding of protein intake, distribution and sources in healthy older New Zealand adults. Future research should focus on investigating whether increasing protein intake, per day and per meal, from high-quality sources would increase and maintain muscle mass and muscle strength in older adults from the general population.

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7. Appendices

Appendix A – Statement of contribution manuscript (chapter three)

DRC 16

MASSEY UNIVERSITY
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GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	HIOL ANNE NADINE
Name/title of Primary Supervisor:	Associate Professor Kathryn Beck
In which chapter is the manuscript /published work:	Chapter Three

Please select one of the following three options:

(●) The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

Hiol, A. N., von Hurst. P. R., Conlon. C. A., Mugridge, O., & Beck, K. L. (2021). Body composition associations with muscle strength in older adults living in Auckland, New Zealand. PLOS ONE 16(5): e0250439. https://doi.org/10.1371/journal.pone.0250439

( ) The manuscript is currently under review for publication – please indicate:

- The name of the journal:
- The percentage of the manuscript/published work that was contributed by the candidate:
- Describe the contribution that the candidate has made to the manuscript/published work:

( ) It is intended that the manuscript will be published, but it has not yet been submitted to a journal

Candidate's Signature:	
Date:	12-Sep-2022
Primary Supervisor's Signature:	KBeck
Date:	25-Oct-2022

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Appendix B – Statement of contribution manuscript (chapter four)

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(•) The manuscript is currently under review for publication – please indicate:

- The name of the journal:
  Nutrition and Healthy Aging
- The percentage of the manuscript/published work that was contributed by the candidate: 80.00
- Describe the contribution that the candidate has made to the manuscript/published work:
  Anne N. Hiol: Data curation, Methodology, Analysis, Writing – original draft, Writing – review & editing

( ) It is intended that the manuscript will be published, but it has not yet been submitted to a journal

Candidate's Signature:	
Date:	12-Sep-2022
Primary Supervisor's Signature:	K Beck
Date:	25-Oct-2022

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Appendix C – Statement of contribution manuscript (chapter five)

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Name of candidate:	HIOL ANNE NADINE
Name/title of Primary Supervisor:	Associate Professor Kathryn Beck
In which chapter is the manuscript /published work:	Chapter Five

Please select one of the following three options:

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- Please provide the full reference of the Research Output:

◉ The manuscript is currently under review for publication – please indicate:

- The name of the journal:

Nutrients

- The percentage of the manuscript/published work that was contributed by the candidate: 80.00

- Describe the contribution that the candidate has made to the manuscript/published work:

Anne N. Hiol: Data curation, Methodology, Analysis, Writing – original draft, Writing – review & editing

○ It is intended that the manuscript will be published, but it has not yet been submitted to a journal

Candidate's Signature:	
Date:	12-Sep-2022
Primary Supervisor's Signature:	K Beck
Date:	25-Oct-2022

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Appendix D – Protocol for all anthropometric measures (adapted from International Society for the Advancement of Kinanthropometry (ISAK) protocol)

- Written informed consent must be obtained from all individuals,
- Individuals should wear suitable clothing (no shoes, light clothing),
- All measurements should be taken in privacy,
- An explanation of the procedures should be given to each participant before measurements, are taken including what measurements will be taken and how,
- Take measurements from the side or behind where possible,
- Record measurements on a sheet. Where possible a recorder should be used to assist the measurer and enter data,
- For girth measurements two measurements should be taken at each site. A 3rd measure should be taken where the 2nd measure is not within 1cm of the first. The mean value is used in any further calculations if two measurements are taken. If three measurements are taken the median value is taken,
- Obtain a complete set of data before repeating the measurements for the second and third time,
- Take all measurements to 1 decimal place (eg. 67.6kg, 161.4cm),
- Order of measurements:
    - Measure height using stadiometer once (ensure calibrated prior to the measurement – dot needs to line up at 156.3cm)
    - Weight using Wedderburn scales once (these are the large scales plugged into the wall)
    - Measure waist and hip circumferences using a tape measure (Lufkin) (twice or 3 times if more than 1cm difference)

Height

Measure height using the appropriate standard operating procedure (check hair, feet, looking straight ahead, breathing normally – then read measurement).

Weight

Ensure participants are lightly clothed (remove heavy jackets, shoes, keys from pockets, etc). Ensure scales are placed on lino or a wooden board (not on carpet, if relevant). Use Wedderburn scales.

Appendix E – Standard operating procedure for handgrip strength

NOTE: This instrument is in the storage room of building 27.

1. Do blood pressure before grip strength.
2. Ensure participants are seated comfortably on a chair with their feet flat on the floor.

3. Explain that we are measuring their 'grip strength'.
4. Choose the hand to be tested.
5. Alter distance setting based on participant comfort.
6. Turn the dial to zero.
7. Participants should have their hand in the position shown in the picture above.
8. Set the JAMAR Hand Dynamometer to the second handle position from the inside. Lightly hold around the readout dial to prevent inadvertent dropping.

9. After the individual is positioned properly, say, "Squeeze as hard as you can…great…anymore…OK…STOP/RELAX! (e.g. provide encouragement for them to do their best).
10. Record the scores in Kg of two successive trials for each hand tested.
11. Alternate R,L,R,L ……give time to relax a little between each trial
12. Reset dial after each attempt.
13. If there is a large difference between readings on one hand (e.g. 5kg) try for a second time. Otherwise, two readings is sufficient.

Appendix F – Standard operating procedure for the 4-day food diary

- Show participants the 4-day food diary and food portion booklet and give them a brief run down on its purpose and how to fill it out.
- Set participants up to watch the DVD (which explains how to keep a food diary).
- On completion of watching the DVD, check whether the participant has any questions regarding the completion of the food diary.
- Emphasise the importance of being as honest and accurate as possible when filling out the food diary, as it will allow a more accurate assessment of their current diet.
- Assign participant dates that they need to complete the food diary (these should include at least one weekend day).
- Ask participants to return the food diary as soon as they have completed the last day of recording, via a stamped addressed envelope.
- Provide participants with the food portion booklet and scales to weigh food if needed / preferred (participants can weigh their food rather than estimate if they find this easier) and a courier envelope to return the scales in.
- When food diaries are returned go through the diary and contact participants to clarify any unclear, incomplete, or missing information.

Appendix G – Food groupings from derived from the 4-day food record

109 food groups	**27 food groups**	**Protein sources**
Potato e.g., boiled, mashed, baked, jacket, instant, roasted [1 medium or 1/2 cup]	Potato, kūmara, taro and other root vegetables	**Plants**
Hot potato chips, French fries, wedges [1/2 cup]		
Kumara, taro, green banana, cassava e.g., boiled, mashed, baked, roasted [1 medium or 1/2 cup]		
Other root vegetables e.g., yams, parsnip, swedes, beetroot, turnips [1 medium or 1/2 cup]		
Carrots [1 medium or 1/2 cup]	Vegetables	
Peas, green [1/2 cup]		
Green beans, broad beans, runner beans [1/2 cup]		
Broccoli, cauliflower, brussel sprouts, cabbage (all varieties) [1/2 cup]		
Salad vegetables e.g., lettuce, cucumber, celery, sprouts [1/2 cup]		
Green leafy vegetables e.g., spinach, silver beet, swiss chard, watercress, puha, Whitloof, chicory, kale, chard, collards, Chinese kale, Bok Choy, taro leaves (palusami) [1/2 cup]		
Tomatoes (all varieties) [1 medium or 1/2 cup]		
All other vegetables e.g., corn, pumpkin, mushrooms, capsicum, peppers, courgette, zucchini, gerkins, marrow, squash, asparagus, radish, eggplant, artichoke [1/2 cup]		
Onions, leeks, garlic [1 Tbsp]		
Beans (canned or dried) e.g., black beans, butter beans, haricot beans, kidney beans, cannellini beans, refried beans, baked beans, chilli beans [1/2 cup]		
Peas and lentils e.g., chickpeas, hummus, falafels, split peas, cow peas, dahl [1/2 cup]		
Apples, pears, nashi pears [1 medium]	Fruit	
Banana [1 medium]		

Citrus fruits e.g., orange, tangelo, tangerine, mandarin, grapefruit, lemon, lime [1 medium or 2 small]		
Stone fruit e.g., apricots, nectarines, peaches, plums, lychees [1 medium or 2 small]		
Avocado [1/4 avocado]		
Olives [4 olives]		
Strawberries, blackberries, cherries, blueberries, boysenberries, loganberries, cranberries, gooseberries, raspberries (fresh, frozen, canned) [1/2 cup]		
Dried fruit e.g., sultanas, raisins, currants, figs, apricots, prunes, dates [2 Tbsp]		
All other fruit e.g., feijoa, persimmon, tamarillo, kiwifruit, grapes, mango, melon, watermelon, pawpaw, papaya, pineapple, rhubarb [1 medium or 1/2 cup]		
Nuts e.g., peanuts, mixed nuts, macadamias, pecan, hazelnuts, brazil nuts, walnuts, cashews, pistachios, almonds [1 Tbsp]	Nuts and seeds	
Nut butters or spreads e.g., peanut butter, almond butter, pesto [1 tsp]		
Seeds e.g., pumpkin seeds, sunflower seeds, pinenuts, sesame seeds, tahini [1 Tbsp]		
White pasta, noodles e.g., spaghetti, canned spaghetti, vermicelli, egg noodles, rice noodles, instant noodles [1/2 cup cooked]	Pasta, rice, other grains	
Whole meal pasta, noodles [1/2 cup cooked]		
White rice [1/2 cup cooked]		
Brown rice [1/2 cup cooked]		
Couscous, polenta, congee, Bulgur wheat, quinoa e.g., tabbouleh [1/2 cup cooked]		
White bread and rolls including sliced and specialty breads such as focaccia, panini, pita, naan, chapatti, ciabatta, Turkish, English muffin, crumpets, pizza bases, wraps, tortilla's, burrito,	Bread	

roti, rewena bread [1 medium slice or 1/2 medium roll]		
Whole meal or wheat meal bread and rolls including sliced and specialty breads [1 medium slice or 1/2 medium roll]		
Whole grain or multi grain bread and rolls including sliced and specialty breads [1 medium slice or 1/2 medium roll]		
Bran based cereals, muesli, porridges – e.g., rolled oats, oat bran, oat meal, All Bran, Sultana bran [1/2 cup]	Breakfast cereals	
Weetbix, cornflakes or rice bubbles [2 weetbix or 1/2 cup]		
Sweetened cereals e.g., Nutrigrain, Fruit Loops, Honey Puffs, Frosties, Milo cereal, CocoPops [1/2 cup]		
Other breakfast cereals e.g., Special K, Light and tasty [1/2 cup]		
Biscuits, plain [2 biscuits]	Biscuits	
Biscuits, chocolate or cream filled [2 biscuits]		
Crackers e.g., crisp bread, water crackers, rice cakes, cream crackers, Cruskits, Mealmates, vitawheat [2 medium crackers]	Snacks	
Muesli or cereal bar (all varieties) [1 bar]		
Potato crisps [1/2 cup]		
Vegetable oils [1 tsp]	Fat from plants	
Coconut cream [1 Tbsp]		
Coconut oil [1 Tbsp]		
Sugar (all varieties) added by you to food / drinks [1 tsp]	Sugar and confectionery	
Jam, marmalade, honey, syrups, sweet spreads or preserves [1 tsp]		
Sweets, lollies [5-6 lollies]		
Chocolate (all other varieties) [4 squares]		
Vegemite [1 tsp]		
Margarine [1 tsp]		

Pancakes, waffles, sweet buns, scones, sweet muffins, fruit bread, croissants, doughnuts, brioche [1 serve]	Cakes and desserts	
Cakes, slices, pastries [1 medium serve]		
Non-milk based puddings e.g., pavlova, sweet pastries, fruit pies, trifle [1 medium serve]		
Fruit and vegetable juices (all varieties) [1 glass]	Non-alcoholic beverages	
Low calorie cordials [1 glass]		
Cordials including syrups, powders e.g., Raro [1 glass]		
Sports drinks e.g., Powerade [1 glass]		
Energy drinks e.g., Red Bull, V [1 glass]		
Diet soft/fizzy drinks e.g., Sprite Zero, Diet Coke, Coke Zero [1 glass]		
Soft/fizzy drinks e.g., Sprite, Coke [1 glass]		
Coffee (all varieties), tea and herbal tea, fruit tea		
Waters		
Beer, lager, cider (all varieties) [1 can or bottle]	Alcoholic beverages	
Red wine [1 small glass]		
White wine [1 small glass]		
Port, sherry, liquors [1 small glass]		
Spirits e.g., gin, brandy, whiskey, vodka [1 shot or 30ml]		
Ready to drink alcoholic beverages [1 bottle or can]		
Light dressings e.g., French and Italian dressing, balsamic vinegar [1 Tbsp]	Sauces and spices	
Tomato sauce, barbeque sauce, sweet chilli sauce [1 Tbsp]		
Pickles, chutney, mustard [1 Tbsp]		
Spices e.g., turmeric, ginger, cinnamon [1 tsp]		
White sauce, cheese sauce, gravies [1 Tbsp]		
Soup, homemade or canned [1 cup]	Soups, bouillon	
Tofu, soybeans, tempeh	Soya products	
Vegetarian sausages / meat, vegetarian burger patty, textured vegetable protein [1 sausage or 1 patty]		

Soy milk, coconut milk, rice milk, almond milk [1 cup]	Plant-based milk, cream	
Beef, lamb, hogget, mutton, pork, veal e.g., roast, steak, fried, chops, schnitzel, silverside, casserole, stew, stir fry, curry, BBQ, hamburger meat, mince dishes, frozen dinners [Palm size or 1/2 cup]	Red meat	**Meat and fish**
Liver, kidney, other offal (including pate) [1/2 cup]		
Chicken, turkey or duck e.g., roast, steak, fried, steamed, BBQ, casserole, stew, stir fry, curry, mince dishes, frozen dinners [Palm size or 1/2 cup]	Poultry	
Corn beef (canned), boil up, pork bones, lamb flaps, povi masima [Palm size or 1/2 cup]	Processed meat	
Ham, bacon, luncheon sausage, salami, pastrami, other processed meat [2 medium slices]		
Meat pies, sausage rolls [1 meat pie or 2 sausage rolls]		
Sausages, frankfurters, cheerios, hot dogs [1 medium sausage]		
Albacore tuna, salmon, sardines, herring, kahawai, swordfish, carp, dogfish, gemfish, Alfonsino, rudderfish, anchovies [Palm size or 1/2 cup]	Fish and shellfish	
Mackerel, snapper, oreo, barracouta, trevally, dory, trout, eel [Palm size or 1/2 cup]		
Tuna (canned), hoki, gurnard, hake, kingfish, cod, tarakihi, groper, flounder [Palm size or 1/2 cup]		
Crumbed fish e.g., patties, cakes, fingers, nuggets [1 patty/cake or 2 fingers/nuggets]		
Fish fried in batter (from fish & chips shop) [1 piece of palm size fish]		
Shellfish e.g., cockles, kina, oysters, paua, scallops, shrimp/prawn, pipi, roe [1/2 cup]		
Green mussels, squid [1/2 cup]		
Cream, sour cream, cream cheese, cheese spreads [1 Tbsp]		
Butter, ghee [1 tsp]		
Creamy dressings e.g., mayonnaise, tartar, thousand island, ranch dressing [1 Tbsp]		

Port, sherry, liquors [1 small glass]		
Spirits e.g., gin, brandy, whiskey, vodka [1 shot or 30ml]		
Ready to drink alcoholic beverages [1 bottle or can]		
Cow's milk including milk as a drink	Milk	**Dairy and eggs**
Milk added to hot chocolate, drinking chocolate, Cocoa, Ovaltine, Nesquik, Milo [1 cup]	Milk beverages	
Milk added to smoothies, milk shakes	Milk from coffee, tea and creamers	
Milk based pudding e.g., rice pudding, custard, semolina, instant puddings, dairy food [1/2 cup]	Creamy desserts, puddings (milk based)	
milk added to drinks (e.g., milky coffees, tea)	Milk from coffee, tea and creamers	
Yoghurt	Yoghurt	
Cheese e.g., Cheddar, Colby, Edam, Tasty, blue vein, camembert, parmesan, gouda, feta, mozzarella, brie, processed [2 slices]	Cheese	
Cottage cheese, ricotta cheese [1 Tbsp]		
Eggs – boiled, poached, raw [1 egg]		
Eggs - fried, scrambled, egg based dishes including quiche, soufflés, frittatas, omelettes [1 egg]	Eggs and eggs products	