Adaptation and validation of the Protein Screener 55+ to identify low protein intake among community dwelling older adults in New Zealand

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Abstract

Background

Recent research suggests at least 1.0g dietary protein/kg/day is required to maintain muscle mass strength and functionality in older age (Campbell, Trappe et al. 2001, Beasley, LaCroix et al. 2010, Houston, Tooze et al. 2017). The Protein Screener 55+ is a screening tool, developed in the Netherlands, to identify community dwelling older adults at risk of low protein intake (≤1.0g/kg adjusted body weight/day). In New Zealand there is currently no rapid method to screen for low protein intake in older adults. Therefore, the aim of this research was to adapt and validate the Protein Screener 55+ to detect low protein intake (≤1.0g/kg adjusted BW/day) in community dwelling older adults in New Zealand.

Methods

Protein intake was assessed among 367 community dwelling older adults aged 65 to 74 years using a 109-item food frequency questionnaire and a four-day food record. Univariate and multivariate logistic regression analysis was used to select food items which predicted protein intake ≤1.0g/kg adjusted body weight/day; based on amount (g/day) and frequency (over four weeks) from both the FFQ and the food record (to assess relative validity). A final restricted prediction model (screening tool) was developed and tested using a receiver operating characteristic (ROC) curve, to test the screening tool's discriminatory capacity for protein intake ≤1.0g/kg adjusted body weight/day. For ease of use, recoded frequency variables (from gram amounts of protein) were used for each protein predictor variable in the final tool.

Results

Participants were mostly female (63.9%), New Zealand European (94.3%) and of higher socioeconomic status (New Zealand Index of Multiple Deprivation score 1-4; 59.2%). Mean protein intake was 1.1± 0.4g per kg adjusted body weight per day and 152 (42%) had a protein intake ≤1.0g per kg of adjusted body weight per day. The final screening tool for predicting low protein intake based on frequency of intake included: beans; beef, mutton, lamb, pork; poultry; eggs; fish; milk and yoghurt. The area under the receiver operating characteristic curve was 0.835 (95% CI 0.794-0.876).

Conclusion

The adapted Protein Screener 55+ is a valid tool for detection of low protein intake among this group of community dwelling older adults in New Zealand. Further validation is needed to ensure applicability to the wider older adult population.

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List of Abbreviations

ANS	Adult Nutrition Survey
AUC	Area Under the Receiver Operator Characteristic Curve
BMI	Body Mass Index
BW	Body Weight
EAR	Estimated Average Requirement
FFQ	Food Frequency Questionnaire
LiLACS NZ	Life and Living in Advanced Age: a Cohort Study New Zealand
ROC	Receiver Operator Characteristic

Chapter 1. Introduction

1.1 Background

Dietary protein intake in older age is crucial for the maintenance of muscle mass, strength and functionality. Evidence suggests that intakes less than 1.0g/kg/day can lead to an increased risk of falls, fractures, institutionalisation, longer hospital admissions, and even premature death (Campbell, Trappe et al. 2001, Beasley, LaCroix et al. 2010, Bauer, Biolo et al. 2013, Houston, Tooze et al. 2017). Adequate dietary protein supports older adults to age successfully and independently, which is increasingly important as the proportion of the population who are over the age of 65 continues to grow (Statistics New Zealand 2016).

Currently in New Zealand, the estimated average requirement (EAR) for dietary protein is 0.68g/kg for men and 0.60g/kg for women aged 51-70 years and 0.86g/kg/day and 0.75g/kg/day for men and women over the age of 70 years respectively (National Health and Medical Research Council, Australian Government Department of Health and Ageing et al. 2006). More recently, it has been suggested that older adults have higher protein requirements. The Prevention in Older People-Assessment in Generalists' practices (PROT-Age) study group and the ESPEN expert group suggest 1.0-1.2g/kg/day to overcome anabolic resistance and preserve lean body mass and function in older age (Bauer, Biolo et al. 2013, Deutz, Bauer et al. 2014). The longitudinal Newcastle 85+ study found intakes of protein greater than 1.0g/kg/day were associated with 0.83 kg higher grip strength and better timed up-and-go performance after five years compared with those who had intakes less than 1.0g/kg/day (n=722) (Granic, Mendonça et al. 2018). A systematic review and meta-analysis of observational studies concluded that older adults with high protein intakes (>1.0g/kg/day) show higher mobility and lower limb physical functioning in comparison to those with relatively low protein intakes (<0.8g/kg/day) (Coelho-Júnior, Milano-Teixeira et al. 2018). Therefore, the evidence suggests that the current EAR for dietary protein in New Zealand may be inadequate for overcoming anabolic resistance and preserving mobility and functionality in older adults.

There has be minimal recent research to describe the protein intake of older adults in New Zealand. The 2008/09 NZ Adult Nutrition Survey (ANS) identified that 13.4% of men and 15.5% of women over the age of 70 did not meet the current EAR (University of Otago and Ministry of Health 2011). In a cohort of octogenarians participating in Life and Living in Advanced Age: a cohort study in New Zealand (LiLACS NZ) one third of men (33%) and 31% of women did not meet the EAR for protein for adults (Ram, Kerse et al. 2020). The median intake of protein among these octogenarians (n=574) was 0.87g/kg/day for women and 0.97g/kg/day for men which has been identified to be inadequate for preserving muscle mass and function in advanced age (Bauer, Biolo et al. 2013, Deutz, Bauer et al. 2014, Ram, Kerse et al. 2020).

The 2008/09 NZ Adult Nutrition Survey found the main sources of dietary protein for New Zealanders aged 15 years and over were bread (11.1% of all protein consumed), poultry (8.8%), milk (8.8%), beef (7.8%) and veal (7.8%) (University of Otago and Ministry of Health 2011). Among participants in the LiLACS NZ cohort, bread was also a main protein source for Māori and non-Māori women; whereas beef and veal were the main protein source for Māori and non-Māori men. Other key sources of dietary protein for women included milk, beef and veal, and seafood; and for men fish and seafood, milk, bread and poultry (Wham, Teh et al. 2016, Ram, Kerse et al. 2020).

To determine food and nutrient intake, dietary assessment methods such as multiple day food records and recalls are often used. However they can often be time consuming to administer and analyse, expensive and also require a trained individual to quantify and interpret the results (Gibson 2005, Ahmed and Haboubi 2010). To overcome these difficulties, short screening tools have been developed to measure dietary intakes of specific nutrients i.e. fat, or cholesterol (Rohrmann and Klein 2003, Taylor, Wong et al. 2003). Screening tools are quick to administer and are easier to analyse and interpret. Nutrition screening is a stepping stone to nutrition assessment; screening tools aim to identify ‘at risk’ individuals so they can then undergo a more detailed assessment of nutritional status and receive specific interventions to correct nutrition-related problems (Hamirudin, Charlton et al. 2016, Reber, Gomes et al. 2019).

1.2 Statement of problem

Currently, in New Zealand, there is no rapid way to screen older adults to detect low protein intake (≤1.0g/kg adjusted BW/day) in community dwelling older adults. Early identification of low protein intake may allow for early intervention before irrecoverable changes to functional status occur. Thus, a valid and effective screening tool is needed to identify individuals with low protein intake in this 'at risk' population group in New Zealand.

The Protein Screener 55+ (Pro 55+) is a short ten item questionnaire developed in the Netherlands that is able to distinguish between high or low protein intake in community dwelling older adults based on foods consumed, using a cut-off point of ≤1.0g protein/kg adjusted BW/day (Wijnhoven, Elstgeest et al. 2018). The Pro55+ fills the gap for a rapid method to identify low protein intake and overcomes many of the drawbacks seen with other dietary assessment methods.

The Pro55+ needs to be adapted for use in New Zealand. As intakes of protein foods differs between the Netherlands and New Zealand, the Pro55+ tool requires adaptation and validation to ensure accurate identification of low protein intake in community dwelling older adults in New Zealand.

1.3 Aims and objectives

Aim

To adapt and validate the Protein Screener 55+ developed in the Netherlands for use in New Zealand to detect low protein intake (≤1.0g/kg adjusted body weight/day) among community dwelling older adults, using a validated food frequency questionnaire.

Objectives

To establish protein intakes ≤1.0g/kg adjusted BW/day among community dwelling older adults aged 65 to 74 years old using a 109-item food frequency questionnaire.

To identify food sources that predict low protein intake (≤1.0g/kg adjusted BW/day) based on a 109-item food frequency questionnaire using univariate and multivariate logistic regression models.

To assess relative validity of models for predicting low protein intake (≤1.0g/kg adjusted BW/day) using a four-day food record and area under the receiver operator characteristic curves.

1.4 Structure of thesis

The thesis will be divided into the following chapters:

Chapter one is the introduction and provides background information to set the scene and the scope of the study, while also adding focus to the importance and purpose of the research question. Chapter two provides a thorough review of the current literature. This review defines low protein intake and the consequences of inadequate intake, while also detailing current protein status of older adults in New Zealand including food sources and distribution throughout the day. This review also defines protein screening tool requirements and addresses the lack of current appropriate tools for use in New Zealand. The third chapter is a research manuscript which provides a complete and concise presentation of the study in a journal formatted style. It includes an abstract, introduction, methods, results, discussion and conclusion. Formatting guidance for this manuscript was obtained from the Journal - Nutrition and Dietetics. Chapter four (the discussion/conclusion) provides a brief overview of the study, attainment of research aims and objectives, any impacts of the research, strengths and limitations of the research and recommendations for future research. Appendices provide additional detailed methodology, results not included in the manuscript, the final screening tool questionnaire and additional questionnaires used for the attainment of dietary data.

1.5 Research contributions

Researcher	Contributions to thesis
Brittany Malcolm	Main researcher and author. Statistical analysis and interpretation, writing, editing, and final preparation of the thesis.
Professor Carol Wham	Main academic supervisor of the Pro55+ validation study. Assistance with editing and final preparation of the thesis.
Associate Professor Kathryn Beck	Co-academic supervisor of the Pro55+ validation study. Principal investigator of the REACH study. Application for research ethics, development of the FFQ, assistance with data collection, statistical analysis and interpretation of the data for validation. Assistance with editing and final preparation of the thesis.
Karen Mumme	Assistance with data collection and 4DFR data entry, management and review of the FFQ data. Provided relevant data and statistical assistance for validation study.
Associate Professor Cathryn Conlon Associate Professor Pamala Von Hurst	Co-investigators of the main REACH study. Assistance with data collection, development of REACH study protocol
Owen Mugridge	Project co-ordinator of the REACH study. Participant recruitment and data collection.
Cassie Slade	Participant recruitment, data collection and assistance with entry of the 4DFR.
Nicola Gillies, Cherise Pendergrast, Angela Yu, Kimberly Brown and Harriet Guy	Assistance with data collection and data entry of the 4DFR.

Chapter 2. Literature review

2.1 The ageing population in New Zealand

The number of adults in New Zealand over the age of 65 is projected to double over the next 25 years; reaching between 1.3 and 1.5 million (Statistics New Zealand 2016, Statistics New Zealand 2020). This demographic shift has been progressing for some time, and is likely due to increased life expectancy, decreased fertility rates, and ageing of the 'baby boomer' generation (He, Goodkind et al. 2016, Clegg and Williams 2018). Figure 1. depicts population projections for the next 40 years, with an obvious peak shift towards those over 65 years. This population growth is predicted to produce significant demand on health care and disability support services in New Zealand (Ministry of Health 2002, Cornwall and Davey 2004, Dale 2017). Therefore, policy direction is required to decrease disease burden and the associated economic costs (Ministry of Health 2019).

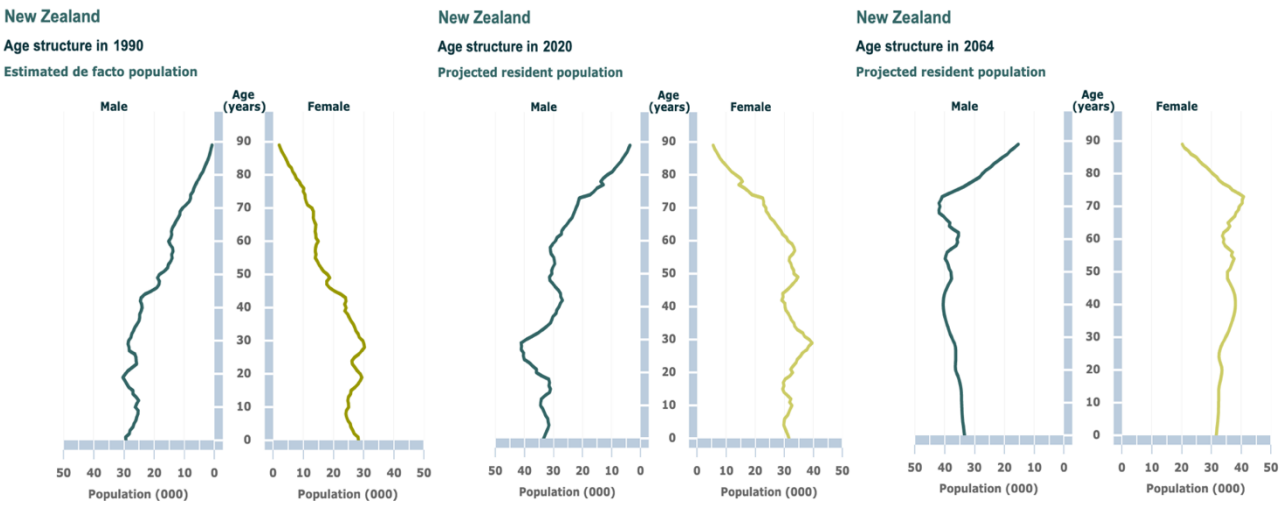

Figure 1. Population age structure projections from 1990 to 2068 (base 2016) (Statistics New Zealand 2017)

The number of people living with long term conditions who require care on a regular basis is expected to increase; people are living longer but are often not living longer in good health (Associate Minister of Health 2016). The prevalence of illness and

disability in older adults can be mitigated through investment into ‘successful ageing’. The New Zealand *Healthy Ageing Strategy*, published in 2016, promotes successful ageing through protecting the independence and quality of life of older adults by empowering them to be active, resilient and engaged (Associate Minister of Health 2016). Successful ageing is described as “the process of developing and maintaining the functional ability that enables wellbeing in older age” (World Health Organization 2015). Successful ageing aims to reduce the gap between total life expectancy and healthy life expectancy by maintaining autonomy and good health-related quality of life (Salomon, Wang et al. 2012, World Health Organization 2015, Associate Minister of Health 2016, Tesch-Römer and Wahl 2017).

‘Ageing in place’ is the term used to describe older adults continuing to live at home in their community with some level of independence, safety and comfort (Davey 2006). This tends to be preferred by older adults over moving into assisted living facilities and institutional care, as it enables them to uphold a sense of connection, autonomy and familiarity; which are deemed important for successful ageing (Dupuis-Blanchard, Neufeld et al. 2009, McKinnon and Escott-Stump 2011, Wiles, Leibing et al. 2012). The institutional care setting can often act as a structural and cultural barrier that impedes social interactions and personal autonomy (Bonifas, Simons et al. 2014, World Health Organization 2015). Services that enable older people to be supported to live in their own homes are provided by District Health Boards (Associate Minister of Health 2016). Provision of home support services give older people the ability to stay in their own homes and to receive the level of support they need to do so (Associate Minister of Health 2016).

Investing in healthy ageing strategies and resources will advance the quality of life and independence of older adults. It will help create environments and opportunities that empower people to remain in their homes as active members of their communities, which is invaluable for changing patterns of illness and institutionalisation commonly seen in older age.

2.2 Health of older people

A decline in health with age is commonly related to the physiological, physical and social changes that accumulate with ageing (Clegg, Young et al. 2013, World Health Organization 2015). These changes are loosely associated with chronological age and can be experienced very differently between individuals (Steves, Spector et al. 2012). Some people may experience good health in their older years while others may experience a significant reduction in physical and mental capacity, requiring care (World Health Organization 2015). A deterioration in health status with ageing may result in long term conditions like dementia, ischemic heart disease, cancer, depression, and frailty (Ministry of Health 2013, World Health Organization 2015). Currently, one in six older New Zealanders live with three or more long term conditions; however, it is estimated that half of health conditions experienced by older adults are avoidable through lifestyle changes (Associate Minister of Health 2016). Interventions that aim to improve the health status of older adults have the potential to reduce disease prevalence and facilitate successful ageing.

With advancing age older adults may experience social changes such as the narrowing of social networks, reduced participation in activities outside the home and more frequent bereavement (Charles and Carstensen 2010). Social isolation and subjective feelings of loneliness are commonly associated with risk of poor oral intake and malnutrition, therefore posing a significant risk to the health of older adults (Eskelinen, Hartikainen et al. 2016, Boulos, Salameh et al. 2017).

Physiological changes may also impact healthy eating such as decreased saliva production, loss of good dentition, delayed gastrointestinal motility, and decreased muscle mass (Malafarina, Uriz-Otano et al. 2013, Soenen, Rayner et al. 2016, Tieland, Trouwborst et al. 2018). The implications of this include reduced appetite, early satiety as well as impaired ability to chew food, self-feed and to procure and prepare food; all of which can ultimately result in reduced oral intake, risking the health of older adults (Giezenaar, Chapman et al. 2016, Robinson 2018).

The health status of older adults is most significantly impacted by changes in body composition that occur with ageing. Up to 13% of people over the age of 60, and half of people over the age of 80 are likely to experience chronic skeletal muscle mass loss (Von Haehling, Morley et al. 2010). Skeletal muscle loss is commonly concealed by a corresponding increase in adiposity (Roh and Choi 2020). Skeletal muscle mass is a principle component of body composition; muscle loss leads to a corresponding loss of muscle strength as well as functionality; termed sarcopenia (Chen, Lee et al. 2016, Dennison, Sayer et al. 2017).

Sarcopenia is directly linked to increased risk of falls, functional decline, loss of independence and early mortality; all of which counter successful ageing (Goodpaster, Park et al. 2006, Cruz-Jentoft, Baeyens et al. 2010, Morley, Abbatecola et al. 2011, Mustafa, Ellison et al. 2018, Cruz-Jentoft, Bahat et al. 2019). A systematic review and meta-analysis found sarcopenia to be a significant predictor of hospitalisation among older adults (n=2832) (Zhang, Zhang et al. 2018). The Concord Health and Ageing in Men Project (CHAMP) found a significant relationship between incidence of sarcopenia and activities of daily living disability (e.g. trouble bathing, grooming, and dressing), institutionalisation and mortality over a two year follow up in community dwelling Australian men over the age of 70 (n=1705) (Hirani, Blyth et al. 2015).

2.3 Nutritional health of older people

Nutrition is a basic need of life, and good nutrition is essential for good health (Ministry of Health 2013). The nutritional health status of older adults is impacted by normal changes of ageing; all of which may conspire against older people commonly leading to poor eating behaviours (Amarya, Singh et al. 2015).

Typical changes in eating behaviour result from older adults developing depression and loneliness, experiencing declines in taste and smell acuity, losing enjoyment of favourite foods and potential difficulties with food shopping and cooking (Malafarina, Uriz-Otano et al. 2013, Giezenaar, Chapman et al. 2016). As a result, older adults may chew food slower, consume smaller meals, and snack less often; collectively leading to reduced appetite, early satiety and reduced food intake (Brownie 2006, Giezenaar,

Chapman et al. 2016, Robinson 2018). Although food intake and basal metabolism generally decrease with age, macronutrient and micronutrient requirements remain unchanged or increase which directly impacts nutritional health of older adults (Zhu, Devine et al. 2010).

The nutritional health of older adults reported in the 2008/09 Adult Nutrition Survey (ANS) showed adults over the age of 70 consumed 48.3% of total energy from carbohydrate sources and 16.7% from protein compared to the Acceptable Macronutrient Distribution Range (AMDR); 45-65% for carbohydrate and 15-25% for protein. Fat contributed to 32% of total energy intake (AMDR is 20-35% total energy intake from fat). Protein intake as a percentage of total energy intake was similar for older (>70 years) and younger adults (31-50 years) (16.7% and 16.9% respectively) (University of Otago and Ministry of Health 2011). Participants in the Life and Living in Advanced Age: a Cohort Study in New Zealand aged 80 to 90 years consumed 45% of total energy intake from dietary carbohydrate, and 15.4% from dietary protein. The percentage of energy intake from dietary fat for LiLACS NZ octogenarians exceeded the AMDR upper range at 36.7% (Ram, Kerse et al. 2020).

The nutrient intakes of older adults in New Zealand is not well reported. The 2008/09 Adult Nutrition Survey found 92.8% of women and 86% of men over the age of 70 had inadequate intake of calcium. Selenium, zinc and vitamin B6 intake was inadequate for a large portion of women (78.6%, 89.7% and 53% respectively) as was selenium for men (63.8%) (University of Otago and Ministry of Health 2011). Among adults of advanced age in LiLACS NZ more than half of the 578 Māori and non-Māori participants had intakes below the estimated average requirement (EAR) for calcium, magnesium and selenium from food. Vitamin B6 intake was also low for Māori women, folate for all women, vitamin E for Māori women and all men, and zinc for all men (Wham, Teh et al. 2016).

With ageing, reduced consumption of food is likely to lead to the development of impaired nutritional status including macronutrient and micronutrient deficiencies, weight loss and malnutrition (Wham, Teh et al. 2016, Wham, Teh et al. 2016, Clegg and

Williams 2018). Malnutrition (characterised by low body mass and weight loss) may lead to increased morbidity and mortality and decreased quality of life (Cederholm, Bosaeus et al. 2015, Clegg and Williams 2018, Cruz-Jentoft, Bahat et al. 2019).

Among octogenarians participating in LiLACS NZ, 49% of Māori participants (n=255) and 38% of non-Māori (n=400) were at high nutrition risk using the Seniors in the community: Risk evaluation for Eating and Nutrition II tool (SCREEN II) (Wham, Teh et al. 2015). Among community dwelling adults in the Auckland region (median age of 79 years), 11% were at nutrition risk and a further one percent were malnourished using the Mini Nutrition Assessment-short form (n= 257) (Chatindiara, Williams et al. 2019).

The presence of poor nutritional health and malnutrition is likely to be largely underestimated in the population due to the lack of a single definition of nutritional status and inconsistency of measures of nutrition risk in screening tools (Donini, Savina et al. 2007, Phillips, Foley et al. 2010). More extensive research is needed to understand the full extent of poor nutritional status in New Zealand community-dwelling older adults, to prevent loss of functionality and reduced quality of life.

2.4 Dietary protein recommendations

Protein requirements are defined as the average daily dietary intake level that is sufficient to balance nitrogen loss from the body while ensuring growth and maintenance of fat-free mass (National Health and Medical Research Council, Australian Government Department of Health and Ageing et al. 2006, Joint FAO/WHO/UNU Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition 2007). The current estimated average requirement (EAR) for dietary protein to meet the requirements of half the healthy individuals differs depending on life stage and gender group. Older adults aged 51 to 70 years old are estimated to require 0.68g/kg/day for men and 0.60g/kg/day for women. For those over the age of 70 years the EAR is higher at 0.86g/kg/day for men and 0.75g/kg/day for women (National Health and Medical Research Council, Australian Government Department of Health and Ageing et al. 2006).

The New Zealand dietary protein EAR for older adults has been in place since 2006; at the time of implementation it was acknowledged that older adults are likely to require more dietary protein than younger adults. A meta-analysis by Rand, Pellett et al. (2003) estimated protein requirements in older adults are likely greater than current recommendations in adults over 50 years old due to a lower efficacy of utilisation. However, meta-analysis results from 19 studies (n=235) of older adults did not differ significantly from the estimated protein requirement of younger adults to achieve nitrogen balance (130 mg N/kg/day versus 104 mg N/kg/day), meaning overall protein recommendations from Rand, Pellett et al. (2003) do not differ based on age. A metabolic study conducted in America 2001, concluded that 0.8g/kg/day is not likely to be adequate to meet the protein needs of the vast majority of older people (n=10, aged 55-70) (Campbell, Trappe et al. 2001). Although robust data was unavailable at the time, the New Zealand dietary protein requirements show that adults over the age of 70 years likely require more dietary protein than their younger counterparts (National Health and Medical Research Council, Australian Government Department of Health and Ageing et al. 2006).

More recent investigations have solidified the idea that current dietary protein requirements are inadequate for older adults, as they do not consider the dietary protein needed to combat age related changes in metabolism, immunity and functionality (Clegg, Young et al. 2013, Mustafa, Ellison et al. 2018). The PROT-AGE study group and the ESPEN expert group recommend healthy, independent older adults should be consuming dietary protein in the range of 1.0-1.2g/kg/day to help mitigate the deleterious effect of low protein intake on successful ageing (Bauer, Biolo et al. 2013, Deutz, Bauer et al. 2014).

2.5 Consequences of low dietary protein intake

Low dietary protein intake relative to a person's needs is thought to contribute to an age-related decline in lean body mass, strength and loss of functionality. A large cohort from the Health, Ageing, and Body Composition (Health ABC) study (n=2066) found adults between 70 to 79 years who had low dietary protein intake (≤0.8g/kg/day) had 40% more lean tissue loss over a three year follow up than those with protein intake

greater than 1.2g/kg/day (Houston, Nicklas et al. 2008). A parallel-group randomised control trial conducted in New Zealand identified that consumption of a diet providing 1.6g protein/kg/day compared with the current guidelines (≤0.8g/kg/day) was found to have beneficial effects on trunk lean mass (+1.39 kg), appendicular lean mass (+0.11 kg) and knee extension peak power (+0.012 W) among community dwelling men over the age of 70 (n=29) (Mitchell, Milan et al. 2017).

A longitudinal study which investigated the associations between low protein intake (≤1.0g/kg/day) and decline in muscle strength and physical performance in the very old as part of the Newcastle 85+ study found intakes of protein less than 1.0g/kg/day were associated with 0.83 kg lower grip strength and worse timed up-and-go performance after five years (n=722) (Granic, Mendonça et al. 2018). A systematic review and meta-analysis of observational studies concluded that older adults with high protein intakes (>1.0g/kg/day) show higher mobility and lower limb physical functioning in comparison to those with relatively low protein intakes (≤0.8g/kg/day) (Coelho-Júnior, Milano-Teixeira et al. 2018). Other observational studies have reported the same associations between low protein intake (≤1.0g/kg) and decline in lean mass and physical strength (Thalacker-Mercer, Fleet et al. 2007, Isanejad, Mursu et al. 2015, Isanejad, Mursu et al. 2016, McLean, Mangano et al. 2016).

The relationship between dietary protein and changes in physical functioning over time was examined in participants over the age of 50 in the Framingham Offspring Study (n=1779). Those with a lower protein intake (≤0.8g/kg/day) were more likely to have a higher prevalence of disabilities and loss of independence at the end of the 12 year follow up period than those with protein intake greater than 1.0g/kg/day (Mustafa, Ellison et al. 2018). Over a 20 year period the relationship between low protein intake and reduced functional integrity (e.g. the ability to do house work) continued to exist, indicating insufficient dietary protein may affect functionality and independence over the rest of the life course (Hruby, Sahni et al. 2020).

The undesirable effects of low protein intake on muscle mass, strength and functionality is the grounding for the expert panel groups recommendations for healthy older adults

to consume at least 1.0-1.2g dietary protein/kg/day (Bauer, Biolo et al. 2013, Deutz, Bauer et al. 2014). Protein intake lower than this may compromise the health-related quality of life and independence of an older person; counteracting principles of successful ageing.

2.5 Dietary protein intake of older adults in New Zealand

Surveys have reported evidence to suggest that protein intakes are currently below 1.0g/kg/day among New Zealand older adults. The 2008/09 ANS found 13.4% of men and 15.5% of women over the age of 70 did not meet the (EAR) of 0.86g protein/kg/day and 0.75g/kg/day respectively (which itself is predicted to be an underestimation of protein needs) (University of Otago and Ministry of Health 2011). Mean intakes were 78g/day for men and 60g/day for women (University of Otago and Ministry of Health 2011)

Median protein intakes among octogenarians participating in the LiLACS NZ cohort were 0.93g/kg for Māori (n=216), which was similar to non-Māori (n=362) who had a median protein intake of 0.95g/kg/day. Although above the EAR, intakes are less than new expert panel recommendations for dietary protein of 1.0-1.2g/kg/day (Bauer, Biolo et al. 2013, Deutz, Bauer et al. 2014). In this longitudinal cohort study, 31% of women (n=207) and 33% of men (n=178) did not meet the EAR for protein (Ram, Kerse et al. 2020).

Australian older adults were also reported to have a low protein intake in the Australian National Nutrition Survey (2011/12) (O'Leary, Grech et al. 2020). It was found that 22.3% of men aged and 13.7% of women >70 years did not meet the Australian EAR for dietary protein (0.86g/kg/day for men and 0.75g/kg/day for women). However while the EAR for protein is 0.69g/kg/day for men and 0.60g/kg/day for women in those aged 51 to 70 years only 4.6% of men and 4.7% of women did not meet the EAR (O'Leary, Grech et al. 2020) indicative of protein intakes being more of a challenge in adults over 70 years.

Although protein intake among New Zealand community dwelling older adults is not extensively described, current evidence suggests protein intakes are likely to be inadequate. This finding is consistent with Australian older adult populations. Evidence of low protein intake may mean that lean mass, strength as well as physical functionality, as well as the successful ageing of this group is at risk.

2.6 Dietary protein sources

Animal-based proteins are generally considered more anabolic than plant based protein (Elmadfa and Meyer 2017, Berrazaga, Micard et al. 2019). They are better at stimulating muscle protein synthesis due to two main factors. Firstly, animal based protein has a higher digestibility compared to plant based protein, relating to difference in structure and reduced presence of anti-nutritional factors (Gilani, Tomé et al. 2015, Berrazaga, Micard et al. 2019). Secondly, animal protein commonly contains all eight essential amino acids required by the body, whereas plant protein tends to be low in specific essential amino acids such as leucine, or lysine; which limits the ability of the body to supply the amino acids required for muscle synthesis (Kim, Shin et al. 2018). Amino acids from eggs and milk have consistently been shown to be more effective in stimulating muscle protein synthesis than amino acids from wheat and soy (Yang, Churchward-Venne et al. 2012, van Vliet, Burd et al. 2015, Gorissen, Horstman et al. 2016).

The Food and Agriculture Organisation of the United Nations reported that worldwide, animal based proteins make up 41% of total protein compared to plant based proteins which contribute to 59% of all protein intake, despite research favouring animal based proteins for optimal stimulation of skeletal muscle anabolism (FAO UN Statistics Division 2009).

In New Zealand the 2008/09 ANS found bread to be the single largest contributor of protein in the diet for adults aged 71 and older (14.3% and 14.2% of all protein consumed for men and women respectively). This was followed by milk (11.2%), beef (9.7%), fish (6.5%) then poultry (5.6%) (University of Otago and Ministry of Health 2011). Among participants of the LiLACS NZ cohort, bread was the main protein

source for Māori women, for non-Māori women milk was the main protein source. Beef and veal were the largest contributors to protein intake for non-Māori men and fish and seafood for Māori men. Other main sources of dietary protein for women included beef, veal and poultry; for men, protein sources include bread, milk and poultry (Wham, Teh et al. 2016, Ram, Kerse et al. 2020). There is limited research identifying protein sources in New Zealand older adults.

2.7 Protein distribution at meals

The anabolic response to ingestion of dietary protein is not as efficient in older muscle, relating to the age-related resistance to anabolic stimuli (i.e. amino acids, resistance exercise) (Breen and Phillips 2011, Cardon-Thomas, Riviere et al. 2017). Anabolic resistance can be overcome to achieve optimal skeletal muscle accretion, when protein is consumed at regular intervals throughout the day rather than skewing distribution towards one meal (i.e. dinner) (Paddon-Jones and Rasmussen 2009, Cardon-Thomas, Riviere et al. 2017). A study of 25 to 65 year old adults (n=8) found that muscle protein synthesis rate was approximately 25% higher when protein was consumed evenly throughout the day versus when it was consumed skewed towards the evening meal (Mamerow, Mettler et al. 2014). The NuAge longitudinal study (n=1741) found that evenly distributed mealtime dietary protein intake, independent of total quantity, was associated with higher muscle strength score in both sexes and higher mobility score in men over a three year follow up (Farsijani, Morais et al. 2016). A dietary approach to preserve muscle mass and strength in ageing adults proposed that the ingestion of 25 to 30g of high quality dietary protein per meal will maximally stimulate muscle protein synthesis in older adults (Paddon-Jones and Rasmussen 2009). This hypothesis was strengthened by the findings of the 1999-2002 American National Health and Nutrition Examination Survey (NHANES) (n=1081) which found that consumption of one to two meals of 30-45g of dietary protein at each meal was associated with greater leg lean muscle mass and strength in older adults (Loenneke, Loprinzi et al. 2016).

In New Zealand, LiLACS NZ is the only study that has investigated the dietary protein distribution in older adults. In this octogenarian cohort, the majority of dietary protein was consumed at the dinner meal. Māori and non-Māori men (n=261) consumed a

median of 32.4g protein at dinner compared to only 13g for breakfast and 17.8g for lunch. Māori and non-Māori women (n=313) consumed a median of 23.3g protein at dinner compared to only 10.1g at breakfast and 14.5g for lunch (Ram, Kerse et al. 2020). This identifies an uneven distribution of dietary protein in New Zealand older adults which is often less than the recommended 25-30 grams per meal; and may be preventing optimal skeletal muscle maintenance for the prevention of functional decline.

2.8 Dietary assessment of protein

A nutritional assessment focussed on quantifying an individual's protein intake includes a detailed examination centring on overall dietary intake of protein; biochemical analysis, to determine tissue stores; and a clinical evaluation including client history, medications that may affect appetite, as well as a physical examination to explore the clinical signs of protein energy malnutrition (Gibson 2005, Mueller, Compher et al. 2011, Reber, Gomes et al. 2019). A nutritional assessment is needed to quantify nutritional status and is the basis of nutrition intervention (Charney 2008, Reber, Gomes et al. 2019).

Dietary protein intake can be quantified via a series of validated dietary assessment methods. The most common methods include: single or multiple pass twenty four hour food recalls, a dietary history, food frequency questionnaires (FFQ), or an estimated or weighed food record (Gibson 2005, Naska, Lagiou et al. 2017). A food record is deemed to be the gold standard dietary assessment method as it provides the most detailed information regarding the types of food and beverages consumed and therefore energy and nutrient intakes (Gibson 2005).

These dietary assessment methods pose several disadvantages for assessing an individual's protein intake; they are costly and time consuming to administer and analyse, and also require a skilled and trained individual to gather and quantify the data (Gibson 2005, Ahmed and Haboubi 2010). Undertaking a dietary assessment in older adults is particularly challenging relating to cognitive impairments often experienced in older age. Older adults may struggle to remember the foods they've eaten, they may not

be involved in food preparation impacting recall, they may also have disabilities that affect their ability to record dietary intake (Adamson, Collerton et al. 2009).

2.9 Screening for low dietary protein intake

Nutrition screening is a steppingstone to nutrition assessment. It involves comparing specific measurements from an individual with predetermined risk levels or "cut off" points. Nutrition screening can be undertaken on whole populations, specific subsets of the population who are thought to be 'at risk,' or on selected individuals (Gibson 2005). The purpose of nutritional screening is to identify 'at risk' individuals so they can then undergo a more detailed assessment of nutritional status and receive interventions to correct nutrition-related problems (Hamirudin, Charlton et al. 2016, Reber, Gomes et al. 2019).

Successful screening tools need to be practical and time efficient to administer as well as easy to interpret (Elia 2003, Kondrup, Allison et al. 2003, Phillips, Foley et al. 2010). To facilitate acceptance they need to be non-invasive and pose little risk to those being screened (Charney 2008). There needs to be an established evidence based link between the factor being screening and a significant public health concern (Elia 2003). Screening tools should be tested for validity prior to implementation, specifically with respect to age, gender, ethnicity and the particular setting of use (Elia 2003, Kondrup, Allison et al. 2003, Jones 2004).

Nutritional screening tools for use in the community setting have been identified as valuable preventative health care measures (Keller, McKenzie et al. 2001, Mueller, Compher et al. 2011). They allow for early treatment, which can often be more effective than treatment after the signs and symptoms of deficiency develop (i.e. loss of independence, institutionalisation, malnutrition) (Dwyer, Gahche et al. 2020). Other settings for screening include the hospital and rest homes, however nutrition screening in these settings is mostly targeted towards malnutrition, with the signs and symptoms typically far more advanced than those observed in adults in the community (Wham, Fraser et al. 2017).

The main nutritional concern identified by current validated nutrition screening tools is malnutrition risk or manifest malnutrition. These tools address risk factors for indicators of malnutrition such as, involuntary weight loss, poor appetite, or functional limitations (Phillips, Foley et al. 2010, Power, Mullally et al. 2018). One pitfall is that they do not investigate protein intake specifically. Dietary protein intake is known to be a preventable risk factor for the decline of muscle mass, strength and function in aging (Thalacker-Mercer, Fleet et al. 2007, Houston, Nicklas et al. 2008, de Souza Genaro and Martini 2010, Isanejad, Mursu et al. 2015, Isanejad, Mursu et al. 2016, McLean, Mangano et al. 2016). There is currently no rapid, easy to carry out, accurate and valid method to screen for low protein intake in New Zealand community-dwelling older adults.

2.10 Protein screening tools

The Protein Screener 55+ (Pro 55+) is a short food questionnaire which has been developed and validated for use in the Netherlands. It can distinguish between high or low protein intake in community dwelling older adults based on a cut off of 1.0g/kg adjusted body weight/day (1.0g/kg adjusted BW/day). The aim of the Pro55+ is to be able to quickly and accurately identify older adults at risk of the adverse effects of low protein intake in order to intervene in the early stages and prevent malnutrition and other impairments of nutritional status. The Pro 55+ is the first protein specific screening tool to be created and validated worldwide.

The Pro55+ is a short ten question screening tool. It determines low protein intake risk based on the consumption (amount on an average day or frequency in four weeks) of: slices of bread (number); glasses of milk (number); meat with warm meal (portion size; small, medium, large); cheese (amount and frequency); dairy products (like yoghurt) (frequency); egg(s) (frequency); pasta/noodles (frequency); fish (frequency); and nuts/peanuts (frequency) (Wijnhoven, Elstgeest et al. 2018).

The Pro55+ tool was originally developed using data from the Longitudinal Aging Study Amsterdam (LASA) study (n= 1348 men and women aged 56–101 years). Univariate and multivariate logistic regression analyses were used to identify food

sources that predicted low protein intake (≤1.0g/kg adjusted BW/day). Validity of the developed model was assessed using area under the receiver operator characteristic (ROC) curves and calibration slopes. Data from the HEalthy LIfe in an Urban Setting (HELIUS) study (n= 563 men and women aged 55–71 years) provided external validation of the developed Pro 55+ using the same validity assessment statistics (Wijnhoven, Elstgeest et al. 2018).

The final Dutch Pro55+ was deemed a valid tool for predicting low protein intake (≤1.0g/kg adjusted BW/day) based on an AUC of 0.889 (95% CI 0.870–0.907) (optimal AUC 1.00) and a calibration slope of 1.03 (optimal slope 1.00) (Wijnhoven, Elstgeest et al. 2018). The Pro55+ can be found in Appendix A. Figure 3. and can be completed online at https://proteinscreener.nl/#/.

Using screening tools without validation could lead to an inaccurate estimation of low protein intake in the New Zealand cohort, due to the difference in protein intake between the two populations. For example, according to the Dutch National Food Composition Survey 2010-2012 (n=739) the main protein sources of adults over 70 years in the Netherlands are meat and meat products (28%), dairy products (25%) and cereals and cereal products (20%); these findings did not differ when separated by gender (Ocké, Buurma-Rethans et al. 2013).

Although evidence of protein food sources consumed by older adults in New Zealand is minimal, LiLACS NZ found main dietary sources of protein differ to that of the Dutch population and differ between genders and ethnicities (n=578). Among Māori and non-Māori octogenarian women bread (13.2% of all dietary protein) and milk (11.5%) were the main dietary sources of protein. Beef and veal was the main source for non-Māori men (12.5%) and fish and seafood was the main source for Māori men (11%) (Wham, Teh et al. 2016, Ram, Kerse et al. 2020). This suggests the main sources of dietary protein differ between the two countries, and in New Zealand sources of protein have been identified to differ by gender and ethnicity.

The New Zealand Adult Nutrition Survey and the Dutch National Food Composition Survey identified other differences in dietary patterns and cultural foods that further the need to re-validate the Pro55+ tool. One of the most prevalent dietary patterns in the Dutch population is characterised by high consumption of fruit, vegetables, brown bread and low fat dairy (Geurts, van Bakel et al. 2017). Research identifying dietary patterns of New Zealanders, specifically older adults is limited, however the Adult Nutrition Survey found older adults (>70 years) were more likely to have three serves of fruit and two serves of vegetables a day, consume light or heavy grain bread, choose reduced fat milk and remove the excess fat from meat than their younger counterparts (University of Otago and Ministry of Health 2011). A more recent study conducted specifically in New Zealand older adults aged 65 to 74 years (n=367, 36% male) found a 'Mediterranean' dietary pattern (e.g. salad vegetables, avocado, nuts, oily fish, eggs and fruit) was more common in women, high physical activity and higher education status. A 'Western' dietary pattern (e.g. processed meats, sauces, cakes/biscuits, processed fish and vegetable oils) was positively associated with being male, having a higher alcohol intake, and living with others; where as a 'prudent' pattern (e.g. dried legumes, soy-based foods, wholegrains, carrots and spices) was passively associated with higher level of physical activity and lower alcohol intake (Mumme, Conlon et al. 2020).

Both New Zealand and the Netherlands are multicultural populations; culture and nationality influence food choices so it is expected that different ethnic dietary patterns will influence food choices differently in the two countries. Dutch culture is commonly inter-spread with Turkish, Moroccan and Surinamese traditional dietary patterns; commonly consumed foods from these backgrounds include roti (flat bread), chow mein, börek and poğaca (savoury pastries), ayran (yoghurt drink), couscous, tajine (stew), and lamb or mutton (Geurts, van Bakel et al. 2017). New Zealand food culture is different to that of the Netherlands and has changed over time. A traditional Māori dietary pattern was low in fat and high in fibre; this was then influenced by the Western colonisation and now this dietary pattern has changed to contain more high fat meats and refined carbohydrate (Rush, Hsi et al. 2010). Common foods include kaimoana (seafood), boil up (meat, puha and potato boiled together), hangi (food cooked slowly underground), kumara and meat. Western dietary patterns are also common in New

Zealand and typically include processed foods high in refined carbohydrates, foods include red meat, processed meat, dairy products eggs and refined grains (Cordain, Eaton et al. 2005).

With difference in dietary pattens and food intake between the New Zealand and Dutch population, the accurateness of the Pro55+ for use in New Zealand as it currently stands is limited as it is unlikely to contain the relevant food predictors of low protein intake in community dwelling New Zealand older adults. Therefore, the Pro55+ screening tool needs to be adapted and validated for use in New Zealand to detect low protein intake in community dwelling older adults.

2.11 Summary

The population of New Zealand is ageing, the number of adults over the age of 65 is expected to double in the next 25 years, as is the number of people living with long term conditions requiring care (Associate Minister of Health 2016, Statistics New Zealand 2016).

The health status and successful ageing of older adults is threatened by poor nutritional status, older adults typically experience both physiological and social changes that accumulate to negatively impact nutritional status (Amarya, Singh et al. 2015). Reduction in social networks, more frequent bereavement, decreased saliva production and delayed gastrointestinal motility result in early satiety, reduced hunger and overall decreased food consumption (Giezenaar, Chapman et al. 2016, Robinson 2018).

Nutrient requirements in older age do not decrease in response to the reduced intake of food or reduced physical activity typical of the ageing process (Zhu, Devine et al. 2010). In fact, dietary protein requirements increase with age making it harder for older adults to achieve good nutrition status. Currently older adults aged 51 to 70 years old are estimated to need 0.68g/kg/day for men and 0.60g/kg/day for women. For those over the age of 70 the EAR is higher at 0.86g/kg/day for men and 0.75g/kg/day for women (National Health and Medical Research Council, Australian Government Department of Health and Ageing et al. 2006). Recent research from the PROT-AGE

study group and the ESPEN expert group suggest that older adults should be consuming between 1-1.2g protein/kg/day to overcome anabolic resistance and prevent declines in lean body mass on top of achieving nitrogen balance (Bauer, Biolo et al. 2013, Deutz, Bauer et al. 2014).

Low protein intake (≤1.0g/kg/day) in older age has been identified as a risk factor which may lead to a decline in muscle mass and strength (Campbell, Trappe et al. 2001, Houston, Nicklas et al. 2008, Beasley, LaCroix et al. 2010, Granic, Mendonça et al. 2018). Observational studies have shown that a decline in mass and strength is associated with increased risk of disability and loss of independence over time (Houston, Tooze et al. 2017, Mustafa, Ellison et al. 2018).

There is minimal evidence to define the protein intake of older adults in New Zealand, however the LiLACS NZ cohort study (n= 578) found octogenarians typically did not meet the PROT-AGE study and the ESPEN expert group protein recommendations nor the current EAR. Of the LiLACS NZ cohort, 31% of women (n=207) and 33% of men (n=178) did not meet the EAR for protein compared with 15.5% of women and 13.4% of men over the age of 70 from the Adult Nutrition Survey (University of Otago and Ministry of Health 2011, Ram, Kerse et al. 2020).

Protein intake ≤1.0g/kg/day warrants early screening to safeguard the nutrition status of the growing older adult population group. It is considered more effective to identify ‘at risk’ individuals and intervene early to improve overall energy and protein intakes than it is to wait until overt malnutrition ensues; a screening tool is an effective way to do this (Mueller, Compher et al. 2011, Dwyer, Gahche et al. 2020).

To date, there are no validated nutrition screening tools to detect low protein intake (≤1.0g/kg/day) in healthy community dwelling older adults in New Zealand. The Pro55+ screening tool has been developed and validated for use in community dwelling older adults in the Netherlands. As dietary patterns and food intake differ between the Netherlands and New Zealand populations, the Pro55+ needs to be adapted and

validated to ensure it can accurately detect low protein intake in community dwelling older adults in New Zealand.

Chapter 3. Manuscript

Adaptation and validation of the Protein Screener 55+ to identify low protein intake among community dwelling older adults in New Zealand

3.1 Abstract

Aim

To adapt and validate the Protein Screener 55+ developed in the Netherlands for use in New Zealand to detect intake of ≤1.0g protein/kg adjusted body weight/day among community dwelling older adults.

Methods

Protein intake was assessed among 367 community dwelling older adults aged 65 to 74 years using a 109-item food frequency questionnaire and a four-day food record. Univariate and multivariate logistic regression analysis was used to select food items which predicted protein intake ≤1.0g/kg adjusted body weight/day; based on amount (g/day) and frequency (over four weeks) from both the FFQ and the food record (to assess relative validity). A final restricted prediction model (screening tool) was developed and tested using a receiver operating characteristic (ROC) curve, to test the screening tool's discriminatory capacity for protein intake ≤1.0g/kg adjusted body weight/day. For ease of use recoded frequency variables (from gram amounts of protein) were used for each protein predictor variable in the final tool.

Results

Among 367 participants (63.9% female; mean age 69.7 ± 2.6) mean ± SD protein intake was 1.1g ± 0.4 per kg adjusted body weight per day. Forty-two percent (35.5% female) had a protein intake ≤1.0g per kg of adjusted body weight per day (n=152). The final screening tool for predicting low protein intake based on frequency of intake included: beans; beef, mutton, lamb, pork; poultry; eggs; fish; milk and yoghurt. The area under the receiver operating characteristic curve was 0.835 (95% CI 0.794-0.876).

Conclusions

The adapted Protein Screener 55+ is a valid tool for detection of low protein intake among this group of community dwelling older adults in New Zealand. Further validation is needed to ensure applicability to the wider older adult population.

Keywords

aged, independent living, protein, New Zealand, ROC curve, surveys and questionnaires

3.2 Introduction

Consumption of adequate dietary protein in older adults is important to preserve muscle mass, strength and prevent disability (Campbell, Trappe et al. 2001, Houston, Tooze et al. 2017, Coelho-Júnior, Milano-Teixeira et al. 2018, Granic, Mendonça et al. 2018). Loss of independence as a result of a decline in muscle mass, strength and function may lead to institutionalisation, frailty, and even premature death (Paddon-Jones and Rasmussen 2009, Bauer, Biolo et al. 2013, Mitchell, Milan et al. 2017).

The Framingham Offspring Study (n=1779) found those with a lower protein intake (<0.8g/kg/day) in older age (50 years or older) were more likely to have a higher prevalence of disabilities and loss of independence than those with protein intake greater than 1.0g/kg/day at the end of the 12 year follow up period (Mustafa, Ellison et al. 2018). A systematic review and meta-analysis of seven observational studies concluded that older adults with relatively low protein intakes (≤0.8g/kg/day) had lower mobility and lower limb physical functioning in comparison to those with high protein intakes (>1.0g/kg/day) (Coelho-Júnior, Milano-Teixeira et al. 2018).

The current New Zealand and Australian estimated average requirement (EAR) of dietary protein for men is 0.86g/kg/day and 0.75g/kg/day for women over 70 years (National Health and Medical Research Council, Australian Government Department of Health and Ageing et al. 2006). These recommendations have not been updated in 14 years; since then the PROT-AGE study group and the ESPEN expert group both

suggest older adults average daily protein intake should be in the range of 1.0-1.2g/kg/day to achieve nitrogen balance but to also maintain and regain lean body mass and function (Bauer, Biolo et al. 2013, Deutz, Bauer et al. 2014).

Findings from the 2008/09 Adult Nutrition Survey showed 13.4% of men and 15.5% of women over 70 years did not meet the EAR for protein with mean intakes of 78g and 60g per day respectively (University of Otago and Ministry of Health 2011). Among adults of advanced age the median weight-adjusted protein intake for Māori and non-Māori men was reported to be 1.05 and 0.98g/kg/d respectively, and for Māori and non-Māori women, 0.87 and 0.91g/kg/day respectively (Wham, Teh et al. 2016). Evidence suggests older adults in New Zealand may not consume an adequate dietary protein intake to overcome anabolic resistance and reduce the loss of muscle mass.

Currently, protein intake is determined through dietary assessment methods which need to be undertaken by a trained individual and are time consuming to conduct and analyse e.g. food records, 24-hour recalls, diet history or food frequency questionnaires (Kondrup, Allison et al. 2003, Gibson 2005, Phillips, Foley et al. 2010). To remedy this, nutrition screening tools have been developed which allow for the early detection and correction of nutritional concerns (Dwyer, Gahche et al. 2020).

The Protein Screener 55+ (Pro 55+) is a short ten item questionnaire developed and validated for use in the Netherlands (Wijnhoven, Elstgeest et al. 2018). The Pro 55+ is able to distinguish between high or low protein intake based on a cut off of ≤1.0g protein/kg adjusted body weight/day in community dwelling older adults and is able to quickly and accurately identify those at risk of inadequate dietary protein intake. Dietary patterns and food intake differ between the Netherlands and New Zealand populations consequently, the Pro55+ needs to be adapted and validated for use in New Zealand.

Therefore, the aim of this study was to adapt and validate the protein screener 55+ (Pro55^{+}) for use in New Zealand to detect intake of ≤1.0g protein/kg adjusted BW/day among community dwelling older adults.

3.3 Methods

Study procedure and participants

Participants were part of the Researching Eating, Activity and Cognitive Health (REACH) cross sectional study, described elsewhere (Mumme, von Hurst et al. 2019). Briefly, adults aged 65-74 years were recruited (over 12 months in 2018 and 2019), exclusion criteria included diagnosis of dementia or any condition(s) that may impair cognitive function (e.g. stroke or traumatic head or brain injury), or the experience of an event in the last two years which may impact dietary intake and cognitive function (such as the death or illness of a family member). Ethical approval for the REACH study was granted by the Massey University Human Ethics Committee: Southern A, Application 17/69; all participants provided written informed consent.

Data collection

Participants visited the Human Nutrition Research Unit at Massey University on one occasion for data collection as part of the wider REACH study. At this appointment anthropometric measurements were taken, and relevant questionnaires were completed for socio-demographic and lifestyle characteristics, level of physical activity and dietary intake.

Anthropometric measures including height (cm) and weight (kg) were taken according to the International Society for the Advancement of Kinanthropometry (ISAK) protocols, using a stadiometer (cm) and Tanita Electronic Scales (kg) (Marfell-Jones, Stewart et al. 2012). Body mass index (BMI) was calculated as body weight (kg) divided by body height squared (m^2).

Socio-demographic factors included age, sex, ethnicity, education, and socio-economic deprivation status. Lifestyle characteristics included smoking history and living arrangements. Ethnicity was established as either New Zealand European, Māori, Pasifika, Chinese, Indian, Middle Eastern/Latin American/African or other; participants could select all that were applicable. For statistical analysis the ethnic group selected first was used and participants were categorised as New Zealand European, Asian or Māori/Pasifika. Education level was based on the highest education level and categorised into three groups: secondary, post-secondary, or university degree. Socio-economic deprivation was assessed using street address based on the New Zealand Index of Multiple Deprivation (IMD) (Exeter, Zhao et al. 2017). The IMD is categorised into the following: 1-4 (least deprived), 5-7, 8-10 (most deprived). Smoking status was categorised into yes or no, including former smokers. Current living arrangement was separated into living alone or living with others.

Physical activity information was collected through the written International Physical Activity Questionnaire (IPAQ) and categorised as low, moderate and high (Craig, Marshall et al. 2003). High physical activity equates to approximately one or more hours of activity per day of at least a moderate intensity activity level, moderate physical activity equates to the equivalent of half an hour of at least moderate intensity on most days, and low physical activity is anything less than the definition of moderate physical activity.

Dietary intake data was collected through two methods. Firstly, a self-administered 109-item food frequency questionnaire (FFQ) was used to assess food intake over the previous month and was administered using SurveyMonkey with a researcher present to answer questions. The FFQ was adapted from Beck, Kruger et al. (2012) to include additional food items and serving sizes as well as changes to frequency response time. Information on the frequency of food consumption (over four weeks) was obtained. For the second dietary assessment method participants completed an estimated four-day food record over consecutive days, including at least one weekend day. Participants viewed an instructional video explaining how to record details of all foods and beverages including the type, brand and cooking methods, including estimate of food

quantities through the aid of food photographs, household measures, and measuring scales. Food records were returned to the researchers through pre-paid post.

Analysis of dietary data

To quantify the frequency of food intake over the four-week period for statistical analysis, numeric values were allocated to each frequency interval from the original FFQ (e.g. two to three times per week equals ten times per month) (Appendix B.2. Table 3). To quantify the amount of food intake each 109 food item from the FFQ was allocated a specific corresponding amount (e.g. chicken/duck, ½ cup or palm size) which was matched to the Concise New Zealand Food Composition Tables 12th edition (e.g. one cup of diced chicken is 143g) (Sivakumaran, Huffman et al. 2017) (Appendix B.2. Table 4). Where there was more than one possible food for an item (e.g. beef, lamb, mutton or pork), the average weight from all listed items from the Food Composition tables was used. The gram amount for each food was then multiplied by the numeric value of frequency to give grams per month and then further divided by 28 to give grams per day over four weeks. This gave FFQ data in frequency of consumption and amount consumed (g/day) over four weeks.

To ensure the adapted Pro55+ tool contained relevant protein predictor variables, the 109 FFQ food items were further collapsed into 38 food groups based on the main sources of dietary protein identified in the 2008/09 Adult Nutrition Survey. Decisions were made by the main researcher and research supervisors (Appendix B.2. Table 4) (University of Otago and Ministry of Health 2011).

Data from the four-day food record were entered by trained nutritionists into FoodWorks10 (a nutrient analysis programme), all entries were quality checked by a New Zealand Registered Dietitian (Xyris Pty Ltd 2019). Foodworks is based on the Concise New Zealand Food Composition Tables 12th edition and was used to analyse nutrient intake (Sivakumaran, Huffman et al. 2017). Foods recorded in the four-day food record were allocated to the corresponding 109 groupings and serving sizes from the FFQ. The 109 food items were further collapsed into 38 food groups as in the FFQ,

the amount of each food item consumed was added together and was then divided by four to give an average grams per day (Appendix B.2. Table 4).

Using the FFQ data, protein, carbohydrate and fat intake was expressed as the percentage of total energy intake (% TEI). Protein intake was also expressed in grams per day and grams per kilogram of adjusted body weight per day (g/kg adjusted BW/day). Adjusted body weight was used because in overweight people 'extra weight' is usually adipose tissue, while underweight people require extra protein to build muscle tissue (Wijnhoven, Elstgeest et al. 2018). For those with a BMI <23.9 kg/m^2 (classified as underweight) an adjusted body weight was calculated using a BMI of 24 kg/m^2, and those with a BMI >31 kg/m^2 (classified as overweight) an adjusted body weight was calculated using a BMI of 30.9 kg/m^2 (Winter, MacInnis et al. 2014).

Protein intake from the FFQ was classified as either normal/high (>1.0g/kg adjusted BW/day) or low (≤1.0g/kg adjusted BW/day) based on the increasing consensus of evidence suggesting that protein intake >1.0g/kg/day is protective of muscle mass, strength and functionality (Campbell, Trappe et al. 2001, Houston, Nicklas et al. 2008, Beasley, LaCroix et al. 2010, Bauer, Biolo et al. 2013, Deutz, Bauer et al. 2014).

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 27 (IBM Corp 2020). Descriptive analyses were completed for the total study population, those with protein intake ≤1.0g/kg adjusted BW/day and those with protein intake >1.0g/kg adjusted BW/day for sociodemographic, health related and dietary intake variables from the FFQ. Variables were tested for normality using the Kolmogorov-Smirnov and Shapiro-Wilk tests and normality plots (Field 2013). All numeric variables were normally distributed and were expressed as mean ± standard deviation (SD); categorical data were presented as frequencies. Differences between protein intake ≤1.0g/kg adjusted BW/day and >1.0g/kg adjusted BW/day were determined using independent T-test for normally distributed nominal data and chi-square tests for categorical data; a *p*-value of ≤ 0.05 was considered statistically significant.

The association between amount consumed (g/day) and frequency of consumption (over four weeks) of all 38 food groups from the FFQ and low protein intake (≤1.0g/kg adjusted BW/day) was analysed using separate univariate logistic regression analyses (enter method). The dependent, dichotomous variable was protein intake in g/kg adjusted BW/day; the predictor variables were the 38 food groups. A *p*-value of ≤ 0.001 was considered statistically significant, indicating inclusion of the predictor variable in the next step, the multivariable analysis model.

Following this, separate reduced multivariate regression models for amount consumed (g/day) and frequency of consumption (over four weeks) were built using the backward step-wise selection approach with variables from the univariate logistic regression that were significant predictors of low protein intake (≤1.0g/kg adjusted BW/day). Firstly, variables with the highest *p*-value were removed in the model with $p \leq 0.05$ considered statistically significant. Secondly, variables that were not considered important protein contributing foods according to the 2008/09 Adult Nutrition Survey were removed to include minimum questions in the final model; decisions were made by the main researcher and research supervisors (University of Otago and Ministry of Health 2011). At each of these two restriction steps, the discriminative capacity of the models was analysed using the area under the receiver operating characteristic curve (AUC). An AUC of 0.5 represents worthless discriminatory capacity and 1, perfect discriminatory capacity; when the AUC is greater than 0.8 the discrimination of the model is considered good (Wijnhoven, Elstgeest et al. 2018). Adjusted body weight was added to the final restricted models so that performance would be independent of body weight.

Next, the relative validity of the two FFQ restricted regression models was tested to allow for incorporation of the most accurate predictor variables into the final New Zealand Pro 55+ screening tool. Relative validity comparison was made to the four-day food record. Univariate and multivariate logistic regression analyses and subsequent restriction steps were conducted for the four-day food record, following the methodology above. AUC discriminatory capacity testing was also conducted, and adjusted body weight was added to the final restricted model.

Based on results from the two FFQ and one four-day food record logistic regression analyses, the final model was selected aiming to ensure inclusion of the least amount of protein predictor variables with the best discriminatory capacity (AUC). To create a user-friendly tool, frequency categories rather than amount (g/day) categories, were used for each protein predictor variable. Amount of food consumed data (g/day) from the FFQ was recoded into either three frequency categories: ≤ once/week (low intake, reference category), two to six times per week (medium intake) and ≥ once/day (high intake) or two frequency categories: ≤ once/week (low intake, reference category) or ≥ twice/week (high intake). Logistic regression analysis using the enter method was used with protein intake (≤1.0g protein/kg adjusted BW/day) as the dependent dichotomous variable. Adjusted body weight and frequency categories (medium and high intakes) for each food group were entered into the model as predictor variables. Discriminatory capacity of the final useable tool was tested using AUC.

3.4 Results

A total of 367 men and women completed the 109-item FFQ. Sociodemographic, health and dietary characteristics of the participants are provided in Table 1. Most participants were women (63.9%) of NZ European descent (94.3%) and from areas with low socioeconomic deprivation (59.2%).

The mean ±SD BMI of participants was 26.3± 4.5 kg/m^2, with significant differences between those with ≤1.0g protein/kg versus >1.0g protein/kg adjusted BW/day (27.4 kg/m^2 and 25.5 kg/m^2 respectively) (p<0.001). Mean protein intake as a percentage of total energy intake differed significantly between those with ≤1.0g versus >1.0g protein/kg adjusted BW/day from (17% and 18.8% respectively) (p<0.001) (Table 1.). Protein intake ≤1.0g/kg adjusted BW/day was reported in 41.5% of participants (54.6% females; 45.4% males) who completed the FFQ (Appendix C.1. Table 5). The mean intake of protein per kg of adjusted body weight was 1.0 ± 0.3 for men and 1.2 ± 0.4 for women (Appendix C.1. Table 6).

Table 1. Participant sociodemographic, health and dietary characteristics by low versus adequate protein intake.

	Total Population	Protein intake ≤ 1.0g/kg adjusted BW/day	Protein intake >1.0g/kg adjusted BW/day	P-value ◊
N (%)	367	153 (41.6)	214 (58.4)	
Age (years)	69.7 ± 2.6	69.9 ± 2.6	69.6 ± 2.5	0.322
Gender				
Female	234 (63.9)	83 (54.6)	151 (70.6)	0.002*
Male	132 (36.1)	69 (45.4)	63 (29.4)	
Ethnicity n (%)				
European	345 (94.3)	142 (93.4)	203 (94.9)	0.465
Māori/Pasifika	10 (2.7)	6 (3.9)	4 (1.9)	
Asian	11 (3.0)	4 (2.6)	7 (3.3)	
Education n (%)				
Secondary	82 (22.4)	34 (22.4)	48 (22.4)	0.554
Post-secondary	148 (40.4)	66 (43.4)	82 (38.3)	
University	136 (37.2)	52 (34.2)	84 (39.3)	
Living arrangement n (%)				
With others	258 (70.5)	104 (68.8)	154 (72.0)	0.564
Alone	108 (29.5)	48 (31.6)	60 (28.0)	
Physical activity n (%)				
Low	31 (8.7)	17 (11.3)	14 (6.8)	0.257
Moderate	122 (34.2)	47 (31.1)	75 (36.4)	
High	204 (57.1)	87 (57.6)	117 (56.8)	
Socio-economic deprivation (IMD score) n (%)				
1-4 (least)	212 (59.2)	83 (55.0)	129 (62.3)	0.162
5-7	146 (40.8)	68 (45.0)	78 (37.7)	
8-10 (most)	0 (0)	0 (0)	0 (0)	
Smoking status n (%)				
Yes	75 (20.9)	117 (77.5)	166 (80.2)	0.534
No/former	283 (79.1)	34 (22.5)	41 (19.8)	

Height (cm)	167.4 ± 9.1	169.1 ± 9.5	166.1 ± 8.6	0.002*
Body weight (kg)	73.8 ± 15.0	78.6 ± 15.3	70.4 ± 13.8	<0.001*
BMI (kg/m^2)	26.3 ± 4.5	27.4 ± 4.3	25.5 ± 4.5	<0.001*
Energy (kj/day) [1]	7578.5 ± 2129.4	6111.8 ± 1560.3	8618.4 ± 1853.9	<0.001*
Protein g/day[1]	80.79 ± 24.81	60.58 ± 14.16	95.14 ± 20.36	<0.001*
Protein (g/kg adjusted BW/day) [1]	1.1 ± 0.4	0.8 ± 0.2	1.4 ± 0.3	<0.001*
Protein (% kj) [1]	18.0 ± 3.1	17.0 ± 3.0	18.8 ± 3.0	<0.001*
Carbohydrate (% kj) [1]	39.6 ± 6.1	40.2 ± 6.3	39.3 ± 5.9	0.172
Fat (% kj) [1]	36.9 ± 5.5	36.7 ± 6.1	36.9 ± 5.0	0.718

Number (percentage, %). Mean ± SD. ◊ Differences between protein intake ≤ 1.0g/kg adjusted BW/day and protein intake >1.0g/kg adjusted BW/day participants (Independent samples T-test, Chi-square test). *P-value ≤0.05 considered significant. [1] Data gathered from 109-item REACH food frequency questionnaire. BMI, body mass index. BW, body weight.

A total of 330 men and women completed the four-day food record. Sociodemographic and dietary characteristics are shown in Appendix C.1 Table 7. There were no significant differences between participant characteristics of those who completed the food frequency questionnaire and those who completed the four-day food record.

Results from the FFQ univariable logistic regression models are shown in Appendix C.1. Table 8. Of the 38 food group variables tested; 18 variables on amount (g/day) and ten variables on frequency (over four weeks) were deemed significant protein intake predictor variables based on their association with protein ≤1.0g/kg adjusted BW/day (p ≤0.001). The AUC for the model on amount (g/day) was 0.954 (95% CI 0.933-0.974) and for frequency (over four weeks) was 0.887 (95% CI 0.854-0.921).

Results from the multivariate backwards stepwise logistic regression are shown in Appendix C.1. Table 9. The model on amount (g/day) was reduced from 18 to 13 variables (AUC 0.981, 95% CI 0.935-0.975) and frequency (over four weeks) from ten to nine variables (AUC 0.887, 95% CI 0.854-0.921). Variables included in the

multivariate analysis are shown in Appendix C.2. After removal of variables with *P*-value >0.05 and insignificant protein contributing foods the restricted model on amount contained eight variables (AUC 0.936, 95% CI 0.913-0.960) and frequency contained seven variables (AUC 0.883, 95% CI 0.849-0.918) (Appendix C.1. Table 10). Insignificant protein foods removed to create restricted models are shown in Appendix C.2.

Relative validity of the FFQ models were assessed using the four-day food record data that was completed by 330 REACH participants. Univariate logistic regression was completed for all 38 variables and six variables were deemed significant predictors of low protein intake (AUC 0.761, 95% CI 0.710-0.813) (Appendix C.1. Table 8). The model was not reduced through multivariate backwards stepwise regression (AUC 0.761, 95% CI 0.710-0.813) (Appendix C.1. Table 9). However, three further variables were omitted from the multivariate model relating to removal of variables with a *P*-value >0.05 or insignificant protein contributing foods. The restricted model contained four variables (AUC 0.736, 95% CI 0.682-0.791) (Appendix C.1. Table 10). Insignificant protein foods removed to create restricted model are shown in Appendix C.2.

The final restricted multivariate model selected for identifying significant protein predictor food variables was based on the amount (g/day) of food consumed from the FFQ and included eight food group items consumed in the last month: beans; beef, mutton, lamb, pork; poultry; eggs; fish; milk; nuts and yoghurt consumed. The AUC of the final model was 0.967 (95% CI 0.952-0.982) after controlling for adjusted body weight. All eight significant protein predictor foods and corresponding regression coefficients after converting back to frequency categories are shown in Table 2.

The final useable screening tool selected for detecting low protein intake (≤1.0g/kg adjusted BW/day) in community dwelling older adults consisted of seven significant variables (Table 2.); beans, beef, chicken, eggs, fish, milk, and yoghurt. Nuts was removed from the final restricted multivariate model and is not included in the final useable screening tool; further logistic regression analysis rendered nuts to be

statistically insignificant when classified into frequency categories (medium intake compared to low intake *p*-value 0.494 and high intake compared to low intake *p*-value 0.280). The exponentiation of the β coefficient shows the odds ratio of each food item compared to the reference category. For example, for people who consume half a cup of yoghurt once or more than once per day, the odds are reduced by a half for protein ≤1.0g/kg adjusted BW/day compared to those who have yoghurt once or less than once per week.

Table 2. Final model for prediction of protein intake ≤1.0g/kg adjusted BW/day

	Recoded answer categories	β [2]	S.E.	Wald	Sig. [3]	Exp (β) [4]
Constant		5.868	0.831	49.825	0.000	353.679
Food group items and reference serving size[1] (consumed in past four weeks)						
Beans, ½ cup	≤1/week	Reference category				
	≥2/week	-1.643	0.535	9.444	0.002	0.193
Beef, mutton, lamb or pork, palm size or ½ cup	≤1/week	Reference category				
	2-6 /week	-1.643	0.318	26.771	0.000	0.193
	≥1/day	-2.395	0.735	10.607	0.001	0.091
Chicken, turkey or duck, palm size or ½ cup	≤1/week	Reference category				
	≥2/week	-1.497	0.465	10.380	0.001	0.224
Egg, 1 whole	≤1/week	Reference category				
	2-6/week	-0.498	0.298	2.792	0.095	0.608
	≥1/day	-1.047	0.288	3.193	0.000	0.351
Fish, palm size or ½ cup	≤1/week	Reference category				
	2-6 /week	-1.345	0.300	20.092	0.000	0.260
	≥1/day	-1.897	0.619	9.396	0.002	0.150
Milk, 1 cup	≤1/week	Reference category				
	2-6/week	-0.817	0.592	1.906	0.167	0.442
	≥1/day	-1.223	0.250	23.878	0.000	0.294
Nuts 1 Tbsp. Nut butter 1 tsp.	≤1/week	Reference category				
	2-6 /week	-0.223	0.325	0.469	0.494	0.800
	≥1/day	-0.194	0.180	1.169	0.280	0.824
Yoghurt, ½ cup	≤1/week	Reference category				
	2-6 /week	-0.631	0.337	3.496	0.062	0.532
	≥1/day	-0.637	0.175	13.326	0.000	0.529

[1] The original food items from the 109 REACH FFQ were collapsed into 38 food groups. [2] β, unstandardised regression coefficient, the negative sign means that a higher amount/frequency of intake is associated with lower odds of intake ≤1.0g/kg adjusted body weight/day. [3] *P*-value ≤0.05 considered significant. [4] Exp (β), exponentiation of

the B coefficient, an odds ratio. kg, kilogram; S.E., standard error; Tbsp, tablespoon; tsp, teaspoon; Wald, Wald statistic.

The significance of the final model was retested using enter method logistic regression, all variables showed statistical significance *p*-value ≤0.05. The discriminatory capacity of the final model was tested, the AUC was 0.835 (95% CI 0.794-0.876) (Figure 2.)

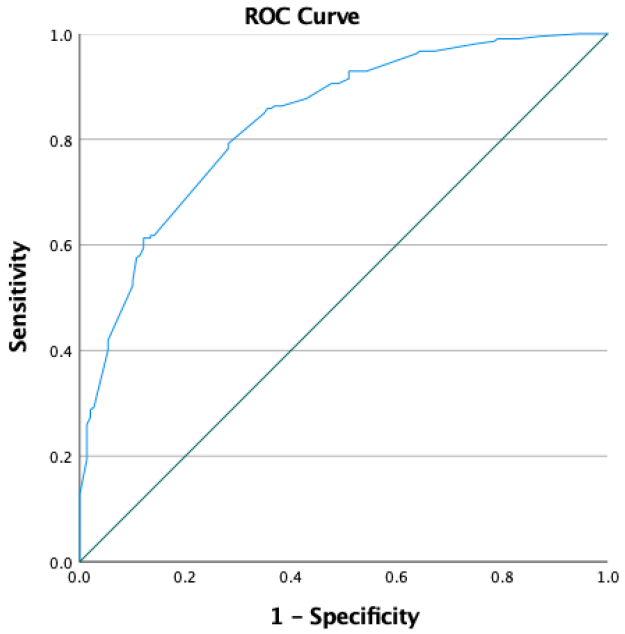

Figure 2. Receiver-operating characteristic curve of the final model for predicting low protein intake ≤1.0g/kg adjusted BW/day.

3.5 Discussion

This study is the first to validate a short food questionnaire to screen for low protein intake in community living older adults in New Zealand. Eight protein predictor foods were identified by logistic regression and relative validity analyses (comparing with the food record), however the final useable tool contains seven questionnaire items to identify protein intake ≤1.0g/kg adjusted BW/day (Appendix C.3. Table 11). Discriminatory foods consumed over four weeks include: beans (e.g. black beans, kidney beans); beef, mutton, lamb or pork; chicken; eggs; fish (e.g. tuna, salmon, hoki, snapper); milk (e.g. cow's milk or plant milk); and yoghurt. The seven foods were

recoded into frequency categories for ease of use as a screening tool. For beef, eggs, fish, milk and yoghurt frequency categories were ≤ once/week (low intake, reference category), two to six times per week (medium intake) or ≥ once/day (high intake). Answers for beans and chicken were recoded into two categories: either ≤ once/week (low intake, reference category) or ≥ twice/week (high intake). High and medium frequency intake were combined for beans and chicken due to very few people consuming these food items ≥ once/day.

The seven food predictors we identified differ to the ten food predictors identified in the Netherland protein screening tool (Pro55+). Specifically, the adapted New Zealand Pro55+ tool does not include bread, nuts or pasta and noodles but does incorporate beans; furthermore meat or dairy foods have not been combined into one category as in the Netherland Pro 55+. The variations in significant protein predictor foods demonstrate key variations in dietary intake between the two populations. Previous studies in the Netherlands, suggest meat and meat products contribute less to daily dietary protein intake than in New Zealand (28% of all dietary protein in the Netherlands versus 31% in New Zealand); however bread and cereal products contribute slightly higher dietary protein (20% in the Netherlands versus 17.2% in New Zealand) (Ocké, Buurma-Rethans et al. 2013, Ram, Kerse et al. 2020).

The main dietary sources of protein identified among Dutch older adults (70 years and older) were meat and meat products (28%), dairy products (25%) and cereals and cereal products (including bread) (20%) (Ocké, Buurma-Rethans et al. 2013). By comparison the main dietary sources of protein identified among New Zealand older adults were poultry (5.6%), milk (11.2%), beef (9.7%), and fish (6.5%) in the 2008/09 Adult Nutrition Survey, and among octogenarians beef and veal (10.7%), milk (10.7%), fish and seafood (7.4%), and poultry (6.9%) as identified by LiLACS NZ (University of Otago and Ministry of Health 2011, Ram, Kerse et al. 2020). These significant protein predictors are reflected in the final model for detecting low protein intake in both the Dutch and New Zealand versions of the Pro55+. This provide assurance the protein predictors in the current study are relevant based on previous observations which have identified differences in protein sources among older populations.

Distinguishing between cases of low and adequate protein intake using the adapted Pro55+ is based on the amount of food consumed rather than the frequency of consumption. The discriminatory capacity of the model based on amount of food consumed was higher than that of frequency of consumption after controlling for adjusted body weight (AUC 0.967 versus AUC 0.932 respectively), indicating the amount of food consumed (g/day in four weeks) is a more accurate screening classification. Logistic regression analysis from the four-day food records confirmed the amount of food consumed (g/day) and the eight identified protein predictor foods provided the most appropriate screening classification; whereby the least number of variables were included with the highest discriminative capacity (AUC 0.967 amount FFQ versus AUC 0.811 amount four-day food record).

To create a user-friendly screening tool, the amount of food consumed (g/day) of the eight protein predictors was recoded into two/three frequency categories. Further logistic regression analysis rendered nuts to be statistically insignificant (medium intake compared to low intake *p*-value 0.494 and high intake compared to low intake *p*-value 0.280) and nuts were therefore removed from the final model. The newly adapted screening tool showed good relative validity and discriminatory capacity. The AUC of the final adapted model was 0.835 (95% CI 0.794-0.876); close to 1.0 indicating the screening tool shows high probability to predict low protein intake in an individual when the outcome is indeed low protein intake (true positive) (Krzanowski and Hand 2009). Therefore, the adapted screening tool has good ability to correctly discriminate between cases of low protein intake (≤1.0g/kg adjusted BW/day) and adequate protein intake (>1.0g/kg adjusted body weight/day) based on intake of the seven food groups identified.

A cut off of ≤1.0/kg adjusted body weight/day was used in this screening tool to define low protein intake based on evidence that intakes of ≤1.0g protein/kg/day leads to increased risk of institutionalisation, longer hospital stays, and reduced quality of life; secondary to a significant decline in lean mass and strength (Campbell, Trappe et al. 2001, Beasley, LaCroix et al. 2010, Granic, Mendonça et al. 2018, Mustafa, Ellison et

al. 2018, Hruby, Sahni et al. 2020). In adults of advanced age, protein intake less than 1.0g/kg/day has been associated with lower grip strength and timed up-and-go performance at a five year follow up in the Newcastle 85+ study (Granic, Mendonça et al. 2018). Similarly, participants in the Health ABC study with low intake (≤1.0g protein/kg/day) were at greater risk of developing a mobility limitation over a six year follow up (e.g. trouble walking one-quarter mile or climbing ten steps without rest) (Houston, Tooze et al. 2017).

Conversely, consumption of dietary protein above 1.0g/kg/day has shown to have minimal beneficial effect. A meta-analysis of seven longitudinal observational studies found no significant difference in mobility and lower limb physical functioning between intakes of 1.0g/kg/day and 1.2g/kg/day in older adults (Coelho-Júnior, Milano-Teixeira et al. 2018).

A strength of this study was an even proportion of participants with low and adequate dietary protein and the use of dietary data gathered through two different methods (food frequency questionnaire and food records). This allowed comparison between the two methods to ensure the most appropriate predictor variables were included in the final protein screening tool model. Dietary records are more valid tools for determining intake than other dietary assessment methods such as food frequency questionnaires or diet recalls, therefore act as a good method for comparison of dietary data gathered between different assessment methods (Kowalkowska, Slowinska et al. 2013). The final prediction model included three of the five reduced multivariate model predictor variables for four-day food record in amount (g/day). The adapted screening tool of the current study has a better discriminative capacity than the four-day food record (amount g/day) multivariate model; the AUC of the final model 0.835, 95% CI 0.794-0.876, compared to four day food record AUC 0.811, 95% CI 0.765-0.858.

The limitations of retrospective dietary assessment in this age group may include underreporting and reliance on memory. However misreporting and underreporting in dietary assessment is more likely to affect reporting of discretionary foods over protein rich foods (Macdiarmid and Blundell 1998). Further analysis should be undertaken to

ensure over, and under-reporters are excluded in determining the final model, this was conducted for the FFQ data however not for the food record data analysis.

The current study sample was largely female (63.9%), New Zealand European (93.4%), aged between 65 to 74 years and may not be representative of all community dwelling older adults in New Zealand. The 2018 census found 83.3% of the population aged 65 to 74 are of New Zealand European ethnicity and 51.3% are female (Statistics New Zealand 2020). It is possible that the food group variables for predicting low protein intake in community dwelling older adults may differ from the current study. In particular food and protein intake can differ among those in advanced age (University of Otago and Ministry of Health 2011, Giezenaar, Chapman et al. 2016, Wham, Teh et al. 2016, Robinson 2018). Therefore, the Pro55+ New Zealand may be limited for adults over 75 years as it was validated in those aged 65 to 74.

The Adult Nutrition Survey found bread to be the key source of protein for adults over the age of 70 (14.3% for men and 14.2% for women) (University of Otago and Ministry of Health 2011). Bread was also a key source of protein among Māori women of advanced age in LiLACS NZ (12.4% of protein comes from bread) (Wham, Teh et al. 2016). We found bread was not a significant predictor of protein intake which may reflect an alteration of dietary patterns over the past 12 years, especially among women. In more recent years women have tended to consume less bread than men as wholegrains, sugars and refined wheat products are perceived as harmful to health (Clarke and Best 2017). In a meta-analysis which investigated the efficacy of the low-carbohydrate diet for weight loss the majority of studies had over 60% of female participants, which may indicate women are more likely to be participants in the low carbohydrate fad than men (Bueno, de Melo et al. 2013). Given that the majority of participants in the current study were women, the low carbohydrate health fad may be applicable and explain why bread was not a significant predictor of low protein intake. The applicability of the Pro 55+ New Zealand may need to be considered if this health fad were to diminish in the near future.

The validated Pro 55+ New Zealand can be used to detect older adults at risk of the adverse effects of low protein intake before overt malnutrition or sarcopenia ensues. The screening tool shows good discriminative ability to detect protein intake ≤1.0g/kg adjusted body weight/day, thus can be used to validly screen for protein intake ≤1.0g/kg adjusted body weight/day in community dwelling older adults in New Zealand.

Chapter 4: Conclusion and recommendations

4.1 Research outcomes

This study successfully validated a screening tool to identify low protein intake ≤1.0g/kg adjusted body weight/day in community dwelling older adults in New Zealand. Univariate and multivariate analyses was used to create a reduced prediction model to detect low protein intake. The final model contains seven food items, including beans; beef, mutton, lamb, pork; poultry; eggs; fish and yoghurt. The frequency categories are as follows: three frequency categories for beef, eggs, fish, milk and yoghurt; ≤ once/week (low intake, reference category), two to six times per week (medium intake) or ≥ once/day (high intake), and two frequency categories for beans and chicken; ≤ once/week (low intake, reference category) or ≥ twice/week (high intake). The AUC of the adapted tool was 0.835 (95% CI 0.794-0.876) indicating good validity to distinguish between cases of low and adequate protein intake in this population group.

Nutritional screening tools for use in the community setting have been identified as valuable preventative health care measures (Keller, McKenzie et al. 2001). Having a tool that enables the screening of older adults to identify low protein intake will allow for early nutritional assessment and intervention in the community to help older adults maintain their independence.

This protein screening tool has the potential to reduce the negative health consequences associated with low protein intake. Evidence suggests that if adequate protein status can be upkept throughout ageing, older adults will be better able to maintain their functionality, independence, and health related quality of life (Vellas, Hunt et al. 1997, Mustafa, Ellison et al. 2018). Independence and quality of life are crucial for facilitating successful ageing, which becomes even more pertinent as the proportion of older adults over the age of 65 continues to increase.

The adapted New Zealand Pro55+ screening tool (Appendix C.3. Table 11) can now be used to determine the intakes of the predictor foods identified by this study. Low

frequency of intake of these seven foods indicate risk of protein intake ≤1.0g/kg adjusted body weight/day and therefore identifies older adults that need further nutritional assessment and appropriate intervention to prevent declines in muscle mass, strength and functionality.

4.2 Strengths

This study is the first to validate a screening tool to assess protein intake in older New Zealanders. The large sample size of the REACH study is a strength of this study (Collins, Ogundimu et al. 2016). The study also had a relatively even split between participants who had low dietary protein (≤1.0g/kg adjusted body weight/day) and high/adequate dietary protein (>1.0g/kg adjusted BW/day). Having two different methods of gathering dietary information (food frequency questionnaire and four-day food record) enabled better selection of the most accurate predictor variables for the final model as it allowed for cross reference to a more gold-standard dietary assessment method (food records).

4.3 Limitations

The Protein Screener 55+ study sample differs from the make-up of the general population of older adults in terms of gender, ethnicity and socioeconomic status which limits the applicability of the tool to other sample groups. The 2018 census found both people of New Zealand European ethnicity and females make us a smaller percentage of the total population of adults aged 65 to 74 compared to this study sample. While those aged 65 to 74 make up only 57% of the total population of adults over the age of 65 (Statistics New Zealand 2020). Hence, the study population includes representation from just over half of the older adults of New Zealand. If the study were to include octogenarians or adults in more advanced age, it could be possible that the predictor variables change as intakes in these groups may differ compared to younger older adults.

4.4 Recommendations

Following on from the current study it is recommended that this screening tool be further investigated in a representative sample of New Zealand community dwelling older adults to ensure further confidence in the tool to detect low protein intake in community dwelling older adults of New Zealand. Specifically, a sample with a more even distribution of male to females, a wider selection of ethnicities and incorporation of different socioeconomic groupings, in accordance with recent census data. Further investigations to test validity could be against food records or 24-hour nitrogen balance studies.

To ensure the applicability of the Pro55+ New Zealand, further studies should be undertaken among older adults in a wider age range, socioeconomic status, ethnicities, and geographical locations to determine protein intake (including sources and amounts). It should also be reiterated that this tool is not an assessment of protein status, it is a screening tool, and interventions to correct nutritional inadequacies should not be based on this screening tool alone. However, the screening tool can work alongside nutrition assessment and intervention to prevent declines in muscle mass and strength and loss of functionality seen with protein intakes less than 1.0g/kg/day.

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Appendix A. Protein Screener 55+ (Pro 55+)

Final model for prediction of protein intake ≤1.0 g/kg adjusted BW/d in community-dwelling men and women aged 55+ years from the development sample (n = 1319[1]) and re-calibrated regression coefficients based on application of the model in the validation sample.

(Food) questions	Recoded answer categories[2]	β[3]	Se	W	P-value	Shrunk β[4]
Constant		19.361	1.700	129.74	0.000	17.812
Adjusted body weight, kg[5]		0.106	0.011	99.565	0.000	0.0974
Slices of bread on average day in last 4 weeeks	<3 slices	reference category				
	3 slices	-0.326	0.197	2.75	0.098	-0.300
	4 slices	-1.175	0.219	28.75	0.000	-1.081
	≥5 slices	-2.750	0.358	59.13	0.000	-2.530
Glasses of milk on average day in last 4 weeks	<1 glass	reference category				
	1 glass	-0.344	0.179	3.69	0.055	-0.316
	≥2 glasses	-1.681	0.254	43.80	0.000	-1.547
Portion size meat warm meal on average day in last 4 weeks	Small portion	reference category				
	Medium portion	-1.326	0.219	36.81	0.000	-1.220
	Large portion	-3.074	0.277	123.08	0.000	-2.828
Consumption frequency dairy product in 4 last weeks	Continuous scale: <1 d/wk—7 d/wk	-0.175	0.030	34.56	0.000	-0.161
Consumption frequency egg(s) in 4 weeks	<1 d/wk	reference category				
	1 d/wk	-0.256	0.203	1.59	0.208	-0.236
	2 d/wk	-0.636	0.226	7.92	0.005	-0.585
	≥3 d/wk	-1.480	0.262	31.89	0.000	-1.361
Consumption frequency pasta/noodles in 4 weeks	≤1 d/4 wk	Reference category				
	2–3 d/4 wk	-0.432	0.228	3.59	0.058	-0.397
	1 d/wk	-0.713	0.220	10.52	0.001	-0.656
	≥2 d/wk	-1.409	0.269	27.54	0.000	-1.296
Consumption frequencyfish in 4 weeks	≤1 d/4 wk	reference category				
	2–3 d/4 wk	-0.454	0.230	3.88	0.049	-0.236
	1 d/wk	-0.757	0.215	12.45	0.000	-0.585
	≥2 d/wk	-1.100	0.251	19.24	0.000	-1.361
Consumption frequencynuts/peanuts in 4 weeks	Not in 4 wk	reference category				
	1–3 d/4 wk	-0.393	0.216	3.33	0.068	-0.362
	≥1 d/wk	-0.888	0.202	19.31	0.000	-0.817
Consumption frequency bread, bun, rusk, cracker, etc. with cheese or cheese spread in 4 weeks	Continuous scale: <1 d/wk—7 d/wk	-0.177	0.033	28.77	0.000	-0.163
Slices of bread, bun, rusk, cracker, etc. with cheese or cheese spread on average day in last 4 weeks	≤1 slice	reference category				
	2 slice	-0.654	0.179	13.39	0.000	-0.602
	≥3 slice	-1.214	0.283	18.47	0.000	-1.117

[1]29 participants are not included in final multivariable model because of missing values on one or more questions

[2]The original answer categories were recoded into 8 categories (analyzed as continuous variable) or into 2–4 categories, depending on the distribution of the answers

[3]β, unstandardized regression coefficient, the minus sign means that a higher amount/frequency of food intake is associated with a lower log odds on protein intake < 1.0 g/kg adjusted BW/d

[4]Shrunk β = shrunken unstandardized regression coefficients, based on linear shrinkage factor of 0.92 estimated by validation of regression equation in validation sample.

[5]Adjusted BMI was calculated for those with a BMI >25 kg/m2 (age ≤70 y) or >27 kg/m2 (age >70 y) by applying the body weight corresponding to a BMI of respectively 25 or 27 kg/m2. For those with a BMI <18.5 kg/m2 (age ≤70 y) or <22.0 kg/m2 (age >70 y) body weight corresponding to a BMI of respectively 18.5 or 22 kg/m2 was applied [12]. d, day; se, standard error; W, Wald statistic; wk, week

Figure 3. Final model from the Protein Screener 55+ for predicting low protein intake (≤1.0g/kg adjusted BW/day) in community dwelling older adults in the Netherlands.

Appendix B. Supplementary methods

B.1 Selection of study sample

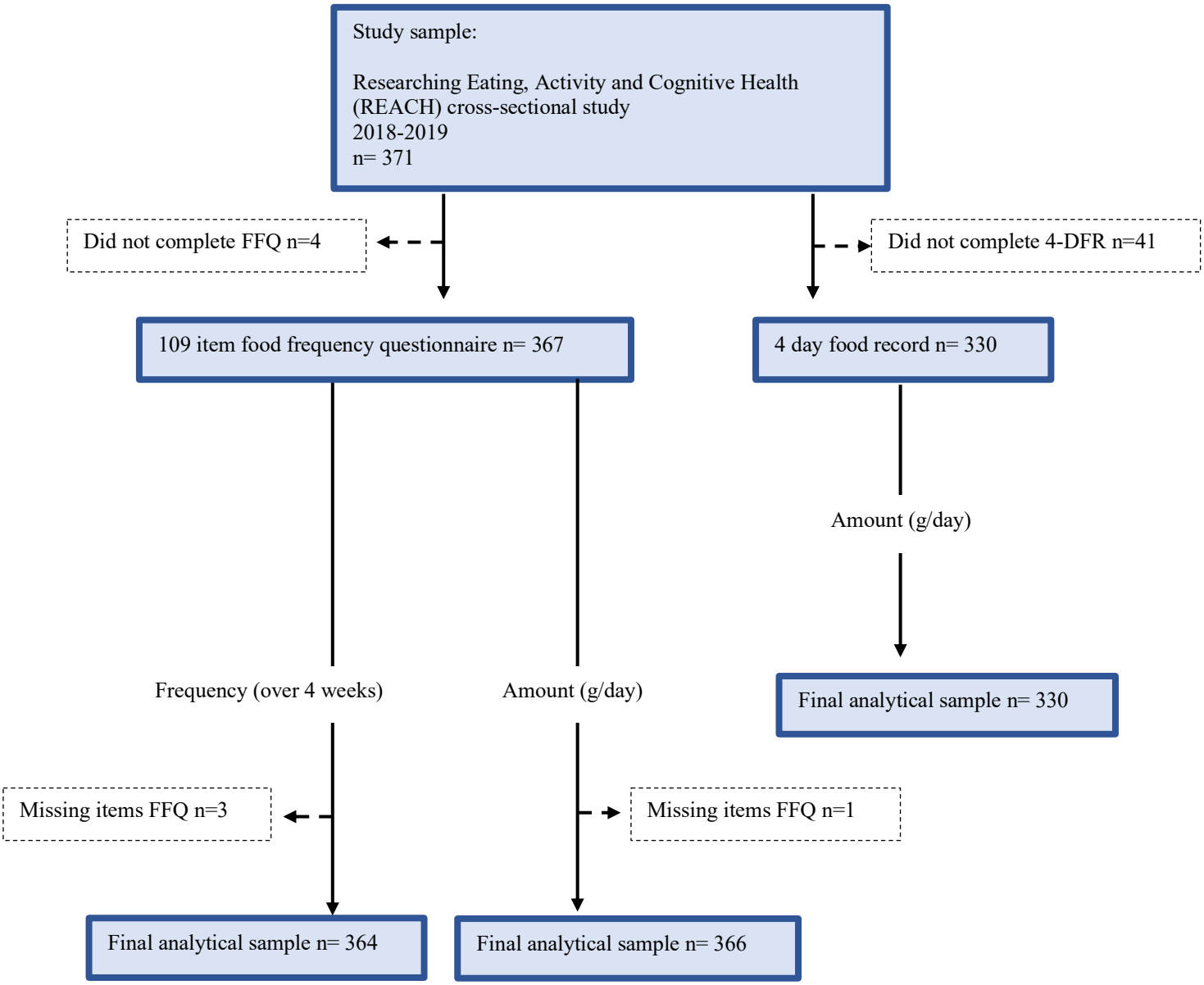

Figure 4. Flow chart describing the selection of the study sample for statistical validation.

B.2 Food frequency questionnaire data handling continued

Table 3. Numeric allocation of frequency of intake

Question in FFQ	Number of times food was consumed in four weeks
Never/not this month but sometimes	0
One to three times per month	2
Once per week	4
Two to three times per week	10
Four to six times per week	20
Once per day	28
Two to three times per day	70
Four to five times per day	126

Table 4. Allocation of serving amounts (g) to foods from original REACH 109 question FFQ and four-day food record using the New Zealand Food Composition Tables[1].

Original REACH FFQ questions and 4-day food record categories	Corresponding food from FOODfiles[1]	FOODfiles[1] Serving	FOODfiles[1] serving (g)	Combined food group variables used in statistical analysis
Beer, lager, cider (all varieties) [1 can or bottle]	Beer, mid-strength (4% alcohol by volume)	100mL	100.6	**Alcohol**
Red wine [1 small glass]	Wine, red, (13.5% alcohol by volume), Pinot Noir	1 small glass (100mL, 1.1 standard drinks)	99.4	
White wine [1 small glass]	Wine, white, dry, (12% alcohol by volume), Sauvignon Blanc	1 small glass (100mL, 1.0 standard drinks)	99.2	
Port, sherry, liquors [1 small glass]	Sherry, medium	1 small glass (100mL, 1.5 standard drinks)	100	
Spirits e.g. gin, brandy, whiskey, vodka [1 shot or 30ml]	Spirit, 70 proof	1 shot (30mL, 0.9 standard drinks)	28.5	
Ready to drink alcoholic beverages [1 bottle or can]	Rum or whiskey & regular cola, 10% alcohol by volume, pub or home-mixed	1 tall glass (250 mL, 2.0 standard drinks)	252	
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]	Bean, mixed beans, canned in brine, drained	1 cup (250mL)	216.9	**Beans (canned or dried)**
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]	Beef, hindquarter skirt steak, separable lean, braised	No specific serving size	100	**Beef, lamb, hogget, mutton, pork, veal**
Biscuits, plain [2 biscuits]	Biscuit, Arrowroot	1 biscuit	8	**Biscuits**

Biscuits, chocolate or cream filled [2 biscuits]	Biscuit, with cream filling	1 biscuit	15	
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, roti, rewena bread [1 medium slice or 1/2 medium roll]	Bread, wheat, white, prepacked, upper North Island	1 medium slice (12.4 x 10.7 x 1.0cm)	28.7	**Bread**
Whole meal or wheat meal bread and rolls including sliced and specialty breads [1 medium slice or 1/2 medium roll]	Bread roll or bun, wholemeal, fortified	1 bun medium (8.2cm diameter)	54.4	
Whole grain or multi grain bread and rolls including sliced and specialty breads [1 medium slice or 1/2 medium roll]	Bread, mixed grain, light, sliced, prepacked	1 slice sandwich (11.4 x 10.3 x 1.1cm)	31.7	
Bran based cereals, muesli, porridges – e.g. rolled oats, oat bran, oat meal, All Bran, Sultana bran [1/2 cup]	Sultana Bran, Kellogg's, fortified	1 cup (250mL)	45	**Breakfast cereals**
Weetbix, cornflakes or rice bubbles [2 weetbix or 1/2 cup]	Weet-Bix, Sanitarium, fortified	1 biscuit (8.4 x 4.2 x 1.9cm)	16.8	
Sweetened cereals e.g. Nutrigrain, Fruit Loops, Honey Puffs, Frosties, Milo cereal, CocoPops [1/2 cup]	Nutri-Grain, Kellogg's, fortified	1 cup (250mL)	36.8	
Other breakfast cereals e.g. Special K, Light and tasty [1/2 cup]	Light 'n' Tasty, Sanitarium, fortified	1 cup (250mL)	78.5	
Pancakes, waffles, sweet buns, scones, sweet muffins, fruit bread, croissants, doughnuts, brioche [1 serve]	Muffin, blueberry	1 muffin (6.0cm top diameter x 4.0cm height)	60	**Cakes and desserts**
Cakes, slices, pastries [1 medium serve]	Cake, fruitcake	1 slice (7.5 x 5.0 x 1.5cm)	40	

Non-milk based puddings e.g. pavlova, sweet pastries, fruit pies, trifle [1 medium serve]	Pudding, sponge, fruit, steamed	No specific serving size	100	
Cheese e.g. Cheddar, Colby, Edam, Tasty, blue vein, camembert, parmesan, gouda, feta, mozzarella, brie, processed [2 slices]	Cheese, edam	1 cube (2cm)	8	**Cheese**
Cottage cheese, ricotta cheese [1 Tbsp]	Cheese, cottage	1 tablespoon (15mL)	16.7	
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]	Chicken, breast, lean & fat, roasted	1 cup diced (250mL)	143	**Poultry**
Couscous, polenta, congee, Bulgur wheat, quinoa e.g. tabbouleh [1/2 cup cooked]	Couscous, white wheat, cooked in water, not drained, no salt or fat added	1 cup (250mL)	143.2	**Couscous, polenta, congee, Bulgur wheat, quinoa**
Eggs – boiled, poached, raw [1 egg]	Egg, chicken, white & yolk, poached	1 egg (size 5, 51.3g)	47	**Eggs**
Eggs - fried, scrambled, egg based dishes including quiche, soufflés, frittatas, omelettes [1 egg]	Egg, chicken, white & yolk, fried in vegetable oil	1 egg (size 5, 51.3g)	39.9	
Cream, sour cream, cream cheese, cheese spreads [1 Tbsp]	Cheese, cream	1 tablespoon (15mL)	14.4	**Fats**
Butter, ghee [1 tsp]	Butter, salted	1 teaspoon (5mL)	4.6	
Margarine [1 tsp]	Margarine, canola, monounsaturated, 70% fat	1 teaspoon (5mL)	4.8	
Vegetable oils [1 tsp]	Oil, vegetable, blend, salad & cooking	1 tablespoon (15mL)	14	
Coconut cream [1 Tbsp]	Coconut, cream, premium	1 cup (250mL)	255.2	
Coconut oil [1 Tbsp]	Coconut oil	1 tablespoon (15mL)	13.8	
White sauce, cheese sauce, gravies [1 Tbsp]	Gravy, dry powder mix, assorted flavours, prepared with water	1 cup (250mL)	251.4	

Creamy dressings e.g. mayonnaise, tartar, thousand island, ranch dressing [1 Tbsp]	Dressing, potato salad, Eta	1 tablespoon (15mL)	15	
Albacore tuna, salmon, sardines, herring, kahawai, swordfish, carp, dogfish, gemfish, Alfonsino, rudderfish, anchovies [Palm size or 1/2 cup]	Salmon, king, fillet, skin & bones removed, fresh, baked without fat, no salt added, New Zealand	1 fillet (14.7 x 6.6 x 2.8cm)	199.2	**Fish**
Mackerel, snapper, oreo, barracouta, trevally, dory, trout, eel [Palm size or 1/2 cup]	Snapper, flesh, baked	1 cup flaked (250mL)	144	
Tuna (canned), hoki, gurnard, hake, kingfish, cod, tarakihi, groper, flounder [Palm size or 1/2 cup]	Tarakihi, flesh, baked	1 cup flaked (250mL)	144	
Hot chocolate, drinking chocolate, Cocoa, Ovaltine, Nesquik, Milo [1 cup]	Energy food drink, powder, malted barley and chocolate, Milo, Nestle, fortified	No specific serving size	100	**Flavoured drinks and sugar sweetened beverages**
Low calorie cordials [1 glass]	Diet cordials	No specific serving size	100	
Cordials including syrups, powders e.g. Raro [1 glass]	Juice concentrate, Lemon & Barley Syrup, Barkers, fortified	100mL	112	
Fruit and vegetable juices (all varieties) [1 glass]	Juice, apple and orange, unsweetened, Fresh Up, fortified	1 cup (250mL)	262	
Sports drinks e.g. Powerade [1 glass]	Sports drink, ready to drink, Powerade	1 cup (250mL)	255.4	
Energy drinks e.g. Red Bull, V [1 glass]	Energy drink, assorted flavours, V, Frucor, fortified	1 cup (250mL)	258.2	
Diet soft/fizzy drinks e.g. Sprite Zero, Diet Coke, Coke Zero [1 glass]	Soft drink, carbonated, lemon flavour, artificially sweetened	100mL	100.6	
Soft/fizzy drinks e.g. Sprite, Coke [1 glass]	Soft drink, cola flavour, sugar-sweetened, caffeinated	100mL	103.3	
Apples, pears, nashi pears [1 medium]	Apple, flesh & skin, raw, combined varieties	1 fruit (7.0cm diameter)	163.2	**Fruit**
Banana [1 medium]	Banana, yellow, ripened, raw	1 fruit medium (19-20cm long)	110.8	

Citrus fruits e.g. orange, tangelo, tangerine, mandarin, grapefruit, lemon, lime [1 medium or 2 small]	Orange, flesh, raw, USA	1 fruit (7.3cm diameter)	149.4	
Stone fruit e.g. apricots, nectarines, peaches, plums, lychees [1 medium or 2 small]	Apricot, flesh & skin, raw	1 apricot	54	
Avocado [1/4 avocado]	Avocado, flesh, raw	1 cup mashed (250mL)	237.7	
Olives [4 olives]	Olive, in brine	1 olive	2.8	
Strawberries, blackberries, cherries, blueberries, boysenberries, loganberries, cranberries, gooseberries, raspberries (fresh, frozen, canned) [1/2 cup]	Blueberry, raw	1 cup (250mL)	156.9	
Dried fruit e.g. sultanas, raisins, currants, figs, apricots, prunes, dates [2 Tbsp]	Raisin, seedless	20 raisins	8.7	
All other fruit e.g. feijoa, persimmon, tamarillo, kiwifruit, grapes, mango, melon, watermelon, pawpaw, papaya, pineapple, rhubarb [1 medium or 1/2 cup]	Kiwifruit, Zespri Green (Hayward) Kiwifruit, Zespri, raw	1 cup mashed (250mL)	256.5	
Liver, kidney, other offal (including pate) [1/2 cup]	Lamb, offal, lambs fry, fried	No specific serving size	100	**Liver, kidney and other offal**
Marmite, vegemite [1 tsp]	Spread, yeast extract, Marmite, Sanitarium, fortified	1 teaspoon (5mL)	5.8	**Marmite and vegemite**
Cow's milk including milk as a drink, milk added to drinks (e.g. milky coffees), milk added to cereal [1 cup]	Milk, cow, standard 3.3% fat, fluid	1 cup (250mL)	258	**Milk**
Soy milk, coconut milk, rice milk, almond milk [1 cup]	Soy milk, So Good Regular Soy Milk, Sanitarium, fortified	1 cup (250mL)	255	

Nuts e.g. peanuts, mixed nuts, macadamias, pecan, hazelnuts, brazil nuts, walnuts, cashews, pistachios, almonds [1 Tbsp]	Nut, mixed, salted	1 cup (250mL)	150	**Nuts**
Nut butters or spreads e.g. peanut butter, almond butter, pesto [1 tsp]	Peanut butter, smooth & crunchy, salt added, no sugar added	1 teaspoon (5mL)	6.1	
White pasta, noodles e.g. spaghetti, canned spaghetti, vermicelli, egg noodles, rice noodles, instant noodles [1/2 cup cooked]	Pasta, white wheat flour, assorted shapes, regular, boiled, drained, no salt added	1 cup penne (250mL)	100.4	**Pasta**
Whole meal pasta, noodles [1/2 cup cooked]	Pasta, wholemeal wheat flour, assorted shapes, boiled, drained, no salt added	1 cup spirals or penne (250ml)	111.2	
Peas and lentils e.g. chickpeas, hummus, falafels, split peas, cow peas, dahl [1/2 cup]	Chickpea, cooked	1 cup (250mL)	173	**Peas and lentils**
Potato e.g. boiled, mashed, baked, jacket, instant, roasted [1 medium or 1/2 cup]	Potato, flesh, floury, boiled, drained, mashed, no salt added	1 cup mashed (250mL)	246.2	**Potato, kumara, taro and other root vegetables**
Hot potato chips, French fries, wedges [1/2 cup]	Fries, potato, straight cut, Independent Shops	10 fries	89	
Kumara, taro, green banana, cassava e.g. boiled, mashed, baked, roasted [1 medium or 1/2 cup]	Kumara, flesh, boiled, drained, no salt added	1 cup whole (250mL)	346.6	
Other root vegetables e.g. yams, parsnip, swedes, beetroot, turnips [1 medium or 1/2 cup]	Beetroot, canned in water, sliced, drained	1 slice (0.5 x 4.6cm diameter)	10.2	
Crumbed fish e.g. patties, cakes, fingers, nuggets [1 patty/cake or 2 fingers/nuggets]	Fish, fillet, crumbed, frozen, fried	1 fillet	65	**Processed fish**
Fish fried in batter (from fish & chips shop) [1 piece of palm size fish]	Fish, battered, deep fried, Independent Shops	1 piece	146	
Vegetarian sausages / meat, vegetarian burger patty, textured	Sausage, vegetarian style, fried, no added fat [2]	No specific serving size	100	**Processed meats**

vegetable protein [1 sausage or 1 patty]				
Corn beef (canned), boil up, pork bones, lamb flaps, povi masima [Palm size or 1/2 cup]	Beef, corned silverside, shaved & sliced, deli	1 cup (250mL)	163.8	
Ham, bacon, luncheon sausage, salami, pastrami, other processed meat [2 medium slices]	Ham, sliced	1 slice (10.0 x 10.0 x 0.3cm)	29	
Meat pies, sausage rolls [1 meat pie or 2 sausage rolls]	Pie, mince, individual size, ready to eat, commercial	1 pie	171	
Sausages, frankfurters, cheerios, hot dogs [1 medium sausage]	Sausage, assorted meats & flavours, grilled	1 sausage	78	
White rice [1/2 cup cooked]	Rice, white, polished, boiled	1 cup (250mL)	144	**Rice**
Brown rice [1/2 cup cooked]	Rice, brown, boiled	1 cup (250mL)	206	
Light dressings e.g. French and Italian dressing, balsamic vinegar [1 Tbsp]	Dressing, French, Kraft	1 tablespoon (15mL)	15	**Sauces**
Tomato sauce, barbeque sauce, sweet chilli sauce [1 Tbsp]	Sauce, tomato, ketchup	1 teaspoon (5mL)	6.3	
Pickles, chutney, mustard [1 Tbsp]	Pickle, sweet	1 tablespoon (15mL)	17	
Crackers e.g. crisp bread, water crackers, rice cakes, cream crackers, Cruskits, Mealmates, vitawheat [2 medium crackers]	Cracker, wheat, Supreme, Arnott's & Somerset, Huntley & Palmers	1 cracker	8	**Savoury snacks**
Muesli or cereal bar (all varieties) [1 bar]	Muesli bar, fruit & nut	1 bar	45	
Potato crisps [1/2 cup]	Potato chip or crisp, plain, salted, fried in assorted oils	10 chips or crisps	22.3	
Seeds e.g. pumpkin seeds, sunflower seeds, pinenuts, sesame seeds, tahini [1 Tbsp]	Seed, sunflower, kernel, dried	1 tablespoon ground (15mL)	6.9	**Seeds**

Shellfish e.g. cockles, kina, oysters, paua, scallops, shrimp/prawn, pipi, roe [1/2 cup]	Scallop, raw	1 scallop	14	**Shellfish**
Green mussels, squid [1/2 cup]	Mussel, green, meat, marinated, assorted flavoured, drained, ready to eat, Sealord	1 mussel	16	
Soup, homemade or canned [1 cup]	Soup, vegetable, canned	1 cup (250mL)	257	**Soup**
Spices e.g. turmeric, ginger, cinnamon [1 tsp]	Spice, cinnamon, ground	1 teaspoon (5mL)	2.6	**Spices**
Sugar (all varieties) added by you to food / drinks [1 tsp]	Sugar, caster	1 teaspoon (5mL)	4.8	**Sugar and confectionary**
Jam, marmalade, honey, syrups, sweet spreads or preserves [1 tsp]	Jam, berry fruit	1 tablespoon (15mL)	15.6	
Sweets, lollies [5-6 lollies]	Lollies, Minties, Pascall	1 mintie	7	
Chocolate (all other varieties) [4 squares]	Chocolate, milk chocolate, Dairy Milk, Cadbury	1 chunky bar	51.3	
Smoothies, milk shakes (made from milk, yoghurt, ice cream), milk shakes, flavoured milk [1 cup]	Milk, cow, chocolate flavour, fluid, ultra-high temperature processed	1 cup (250mL)	264	**Sweetened dairy**
Milk based puddings e.g. rice pudding, custard, semolina, instant puddings, dairy food [1/2 cup]	Dessert, assorted flavours, dairy food	1 cup (250mL)	258	
Ice cream [1/2 cup]	Ice cream, vanilla, standard	1 cup (250mL)	143	
Coffee (all varieties) [1 cup]	Coffee beverage, instant, dry powder with water & milk standard 3.3% fat	1 cup (250mL)	250	**Tea and coffee**
Tea [1 cup]	Tea beverage, black	1 cup (250mL)	255	
Herbal tea, fruit tea [1 cup]	Tea beverage, herbal, brewed	1 cup (250mL)	250	
Tofu, soybeans, tempeh [1/2 cup]	Tofu, soybean curd, regular, firm, simmered or pouched, no salt added	1 piece	33.2	**Tofu, soybeans and tempeh**

Carrots [1 medium or 1/2 cup]	Carrot, flesh, fresh, steamed	1 cup sliced 0.5cm thick (250mL)	135.7	**Vegetables**
Peas, green [1/2 cup]	Pea, green, frozen, boiled, drained, no salt added	1 cup (250mL)	180.6	
Green beans, broad beans, runner beans [1/2 cup]	Bean, green runner or dwarf, seeds with pod, fresh, steamed	1 cup sliced (250mL)	122.8	
Broccoli, cauliflower, brussel sprouts, cabbage (all varieties) [1/2 cup]	Broccoli, boiled, drained, no salt added	1 cup (250mL)	164	
Salad vegetables e.g. lettuce, cucumber, celery, sprouts [1/2 cup]	Salad, Mesclun, leaves, raw	1 cup (250mL)	37.5	
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]	Spinach, English, boiled, drained, no salt added	1 cup (250mL)	150	
Tomatoes (all varieties) [1 medium or 1/2 cup]	Tomato, whole, raw	1 medium whole (6.6cm diameter)	123	
All other vegetables e.g. corn, pumpkin, mushrooms, capsicum, peppers, courgette, zucchini, gerkins, marrow, squash, asparagus, radish, eggplant, artichoke [1/2 cup]	Mushroom, raw	1 cup chopped (250mL)	68	
Onions, leeks, garlic [1 Tbsp]	Onion, flesh, boiled, drained, no salt added	1 onion	50	
Water including tap, bottled or sparkling water [1 glass]	Water, tap	1 cup (250mL)	250	**Water**
Yoghurt [1/2 cup]	Yoghurt, premium, assorted fruits	1 cup (250mL)	261.1	**Yoghurt**

[1] Concise New Zealand Food Composition Tables 12th edition, 2016 (Sivakumaran, Huffman et al. 2017).

[2] Australian Food Composition Database (Commonwealth of Australia and Food Standards Australia New Zealand 2019).

Appendix C. Supplementary results

C1: Additional tables

Table 5. Frequency of low protein intake (≤1.0g/kg adjusted BW/day) among study population of men and women.

	Low protein intake (≤1.0 g protein/kg ABW/day)	
	109 item FFQ n=366	**Four-day food record** n= 330
Total study population n (%)	152 (41.5)	122 (37.1)
Male n (%)	69 (52.3)	39 (34.2)
Female n (%)	83 (35.5)	82 (38.3)

Table 6. Mean protein intake per kg of adjusted body weight per day for men and women.

	Protein intake (g/kg adjusted BW/day)	
	109 item FFQ n=366	**Four-day food record** n= 330
Male mean ± SD	1.0 ± 0.3	1.2 ± 0.3
Female mean ± SD	1.2 ± 0.4	1.1 ± 0.3

Table 7. Participant sociodemographic, health and dietary characteristics by food frequency questionnaire and four-day food record completion.

	FFQ	**Four- DRF**	**P-value** ◊
Age (years)	69.7 ± 2.6	68.9 ± 2.4	0.656
Gender			
Female	234 (63.9)	215 (65.3)	0.753
Male	132 (36.1)	114 (34.7)	
Ethnicity n (%)			
European	345 (94.3)	312 (94.8)	0.616
Māori/Pasifika	10 (2.7)	8 (2.4)	
Asian	11 (3.0)	9 (2.7)	

Education n (%)			
Secondary	82 (22.4)	73 (22.1)	0.107
Post-secondary	148 (40.4)	138 (41.2)	
University	136 (37.2)	118 (33.4)	
Living arrangement n (%)			
With others	258 (70.5)	229 (69.6)	0.381
Alone	108 (29.5)	100 (30.4)	
Physical activity n (%)			
Low	31 (8.7)	26 (7.9)	0.246
Moderate	122 (34.2)	109 (32.1)	
High	204 (57.1)	187 (56.8)	
Socio-economic deprivation (IMD score) n (%)			
1-4 (least)	212 (59.2)	194 (59.0)	0.236
5-7	146 (40.8)	129 (41.0)	
8-10 (most)	0 (0)	0 (0)	
Energy (kj/day)	7578.5 ± 2129.4	7793.9 ± 2009.7	0.834
Protein (g/kg adjusted BW/day)	1.1 ± 0.4	1.09 ± 0.38	0.771
Protein (% kj)	18.0 ± 3.1	18.1 ± 4.0	0.210
Carbohydrate (% kj)	39.6 ± 6.1	39.3 ± 5.6	0.500
Fat (% kj)	36.9 ± 5.5	37.8 ± 0.4	0.922

Number (percentage, %). Mean ± SD. ◊ Differences between food frequency questionnaire participants and four-day food record participants (Independent samples T-test, Chi-square test). *P-value ≤0.05 considered significant. BW, body weight. FFQ, food frequency questionnaire. Four- DFR, four day food record. kJ, kilojoule

Table 8. Univariate logistic regression model for predicting protein intake ≤1.0g/kg adjusted BW/day

Combined food group variables	P-value		
	FFQ amount (g/day)[1]	FFQ frequency (in 4 weeks)[1]	Food record[2]
Alcohol	0.763	0.665	0.769
Beans (canned or dried)	0.005*	0.010*	0.876
Beef, lamb, hogget, mutton, pork, veal	<0.001*	<0.001*	0.001*
Biscuits	0.009*	0.753	0.315
Bread	0.085	0.322	0.052
Breakfast cereals	<0.001*	<0.001*	<0.001*
Cakes and desserts	0.364	0.344	0.095
Cheese	0.003*	0.003*	0.001*
Chicken, turkey or duck	0.001*	0.006*	0.007*
Couscous, polenta, congee, Bulgur wheat, quinoa	0.082	0.903	0.477
Eggs	<0.001*	0.986	0.055
Fats	0.073	0.275	0.145
Fish	<0.001*	0.907	0.174
Flavoured drinks and sugar sweetened beverages	0.147	0.396	0.978
Fruit	0.003*	0.096	0.094
Liver, kidney and other offal	0.210	0.488	0.278
Marmite and vegemite	0.020	0.024	0.148
Milk	<0.001*	<0.001*	0.010*
Nuts	0.001*	0.001*	0.027
Pasta	0.031	0.085	0.629
Peas and lentils	0.129	0.161	0.813
Potato, kumara, taro and other root vegetables	0.002*	0.221	0.195
Processed fish	0.864	0.389	0.696
Processed meats	0.143	0.823	0.861
Rice	0.180	0.503	0.020
Sauces	0.007*	0.006*	0.863
Savoury snacks	0.009*	0.021	0.927
Seeds	0.006*	0.006*	0.912
Shellfish	0.047	0.343	0.279
Soup	0.342	0.432	0.198
Spices	0.107	0.400	0.134
Sugar and confectionary	0.067	0.354	0.858
Sweetened dairy	0.083	0.034	0.894
Tea and coffee	<0.001*	0.093	0.333
Tofu, soybeans and tempeh	0.330	0.435	0.153
Vegetables	<0.001*	0.072	<0.001*
Water	0.447	0.206	0.197
Yoghurt	<0.001*	<0.001*	0.026

**P*-value ≤0.01 is statistically significant. [1] Data from FFQ. [2] Data from four-day food record.

Table 9. Multivariate regression models predicting protein intake ≤ 1.0g/kg adjusted BW/day.

Food frequency questionnaire[1]				Food record[2]	
Amount (g/day)	P-value	**Frequency (in 4 weeks)**	P-value	**Amount (g/day)**	P-value
Beans	<0.001*	Beans	0.002*	Beef, lamb, mutton, pork	<0.001*
Beef, mutton, lamb and pork	<0.001*	Beef, mutton, lamb and pork	<0.001*	Breakfast cereals	0.001*
Breakfast cereals	0.024*	Breakfast cereals	0.049	Cheese	0.001*
Cheese	0.076	Cheese	<0.001*	Chicken, turkey and duck	0.005*
Chicken, turkey and duck	0.004*	Chicken, turkey and duck	0.013*	Milk	0.062
Eggs	0.016*	Milk	<0.001*	Vegetables	0.001*
Fish	<0.001*	Nuts	0.005*		
Milk	<0.001*	Sauces	0.008*		
Nuts	0.004*	Yoghurt	<0.001*		
Savoury snacks	0.050*				
Tea and coffee	0.039*				
Vegetables	0.044*				
Yoghurt	<0.001*				

*P-value ≤0.05 is statistically significant. [1] Data from FFQ. [2] Data from four-day food record.

Table 10. Restricted multivariate regression models for predicting protein intake ≤ 1.0g/kg adjusted BW/day.

Food frequency questionnaire[1]				Food record[2]	
Amount (g/day)	P-value	**Frequency (in 4 weeks)**	P-value	**Amount (g/day)**	P-value
Beans	<0.001*	Beans	0.002*	Beef, lamb, mutton, pork	<0.001*
Beef, mutton, lamb and pork	<0.001*	Beef, mutton, lamb and pork	<0.001*	Breakfast cereals	0.001*
Chicken, turkey and duck	0.004*	Cheese	<0.001*	Cheese	0.001*
Eggs	0.016*	Chicken, turkey and duck	0.013*	Chicken, turkey and duck	0.005*
Fish	<0.001*	Milk	<0.001*		
Milk	<0.001*	Nuts	0.005*		
Nuts	0.004*	Yoghurt	<0.001*		
Yoghurt	<0.001*				

*P-value ≤0.05 is statistically significant. [1] Data from FFQ. [2] Data from four-day food record.

C.2 Multivariate analysis results continued

Variables included in the multivariate analyses are:

- FFQ amount (g/day): beans; beef, mutton, lamb and pork; biscuits; breakfast cereals; cheese; chicken, turkey, duck; eggs; fish; fruit; milk; nuts; potato, kumara, taro, root vegetables; sauces; savoury snacks; seeds; tea, coffee; vegetables; yoghurt.
- FFQ frequency (over four weeks): beans; beef, mutton, lamb and pork; breakfast cereals; cheese; chicken, turkey, duck; milk; nuts; sauces; seeds; yoghurt
- Four-day food record amount (g/day): beef, mutton, lamb and pork; breakfast cereals; cheese; chicken, turkey, duck; milk; vegetables

Insignificant protein contributing variables removed from multivariate models are as follows:

- FFQ amount (g/day): savoury snacks; tea, coffee; vegetables
- FFQ frequency (over four weeks): breakfast cereals; sauces
- Four-day food record amount (g/day): breakfast cereals; vegetables

C.3 Final adapted Protein Screener 55+ New Zealand

Table 11. Final adapted Pro 55+ screening tool for identifying community dwelling older adults at risk of low protein intake (≤ 1.0g/kg adjusted BW/day) in New Zealand.

What is your age in years? _____

What is your body weight in kg? _____

What is your height in centimetres? _____

Please answer by ticking the box which best describes how often you ate a particular food or drink in the past month. **In the past month I have had this food...**

Food	Example	Serving size	≤1/week	2-6 times per week	≥ 1/day
Beef, mutton, lamb or pork	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.	½ cup or palm size			
Egg	Fried, scrambled, boiled, poached, raw, egg-based dishes including quiche, soufflés, frittatas, omelettes.	1 whole			
Fish	Tuna (canned), hoki, gurnard, hake, kingfish, cod, tarakihi, groper, flounder, mackerel, snapper, oreo, barracouta, trevally, dory, trout, eel, albacore tuna, salmon, sardines, herring, kahawai, swordfish, carp, dogfish, gemfish, Alfonsino, rudderfish, anchovies.	½ cup or palm size			
Milk	Cow's milk, soy milk, coconut milk, rice milk, almond milk, including milk as a drink, milk added to drinks (e.g. milky coffees), milk added to cereal.	1 cup			
Yoghurt	Dairy, soy, coconut	½ cup			
			≤1/week	≥2/week	
Beans	Canned or dried e.g. black beans, butter beans, haricot beans, kidney beans, cannellini beans, refried beans, baked beans, chilli beans. Not including legumes	½ cup			
Chicken	Chicken, turkey or duck e.g. roast, steak, fried, steamed, BBQ, casserole, stew, stir fry, curry, mince dishes, frozen dinners	½ cup or palm size			