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**Protein intake, distribution, sources, adequacy and determinants
in Māori and Non-Māori Octogenarians: Life and Living in
Advanced Age: A Cohort Study in New Zealand (LiLACS NZ).**

A thesis presented in partial fulfilment of the requirements for the degree of Master of
Science in Nutrition and Dietetics

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Abstract

Background: Protein intake and its food sources is important to prevent age related loss of muscle mass and strength. An even protein intake distribution throughout the day has been suggested as necessary to stimulate muscle protein synthesis. This study examined the prevalence and determinants of inadequate protein intake, primary food sources and mealtime distribution of protein in Māori and non-Māori of advanced age.

Aim: To examine the intake, food sources, distribution, and adequacy of protein among Māori and non-Māori participants in Life and Living in Advanced Age: A Cohort Study in New Zealand.

Methods: Dietary intake was assessed in 214 Māori and 360 non-Māori octogenarians with a repeat 24-hour multiple pass recall. Adequate protein intake was determined using the estimated average requirements (EAR) cut-off points of ≤ 0.75 g/kg for women and ≤ 0.86 g/kg for men. Contribution (%) of the primary food groups to protein intake were assessed. Protein intake distribution was determined for the three main meals. A logistic regression model was built to predict meeting the EAR for protein intake per adjusted body weight per day.

Results: Total median (IQR) protein intake for women was 0.87 (0.68-1.12) g/kg and for men was 0.97 (0.78-1.21) g/kg. The EAR for protein was not met by a third of both women (30.9%) and men (33.3%). Main food group contributors to protein were beef/veal, fish/seafood, milk, bread and differed by gender and ethnicity. For women and men respectively protein distribution (g/meal) was lowest at breakfast (10.1 and 13.0), followed by lunch (14.5 and 17.8) and dinner (23.3 and 34.2). Being a woman and having depressive symptoms were associated with consuming less total protein. Controlling for all other variables the odds of meeting the EAR for protein was higher in participants who had their own teeth or partial dentures (P=0.036).

Conclusions: Among advanced age Māori and non-Māori these findings highlight the prevalence of low protein intake, uneven mealtime protein distribution and importance of dentition for adequate protein intake.

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Abbreviations

AMDR	Acceptable Macronutrient Distribution Range
BMI	Body Mass Index (kg/m ²)
EAR	Estimated Average Requirement
g/kg/d	Grams per kilogram per day
g/day	gram per day
g/meal	gram per meal
KCAL	Kilocalorie
LiLACS NZ	Life and Living in Advanced Age: A Cohort Study in NZ
NZANS	The New Zealand Adult Nutrition Survey
NRV	Nutrient Reference Value
NZ Dep score	<i>New Zealand Deprivation Index</i> : Based on an index that ranges from 1 to 10
RDI	Recommended Daily Intake
WHO	World Health Organisation
24-hr MPR	24-hour Multiple Pass Recall

Glossary

Māori	The indigenous population of New Zealand
Octogenarian	Persons aged from 80 to 89 years old
Kōha	Donation
Kāi	Food
Kāimoana	Seafood
Marae	Meeting house
Edentulism	Without natural teeth

1. Introduction

Emerging evidence shows that older adults will become the largest age group in New Zealand by 2050, with 1.14 million people expected to reach the age of 65 (Stats NZ., 2015). Currently New Zealand's health and social support structures are not in place to cope with this change in demographics which will impact heavily on health, retirement plans and living arrangements in the future (Ministry of Health, 2016c). The District Health Boards in New Zealand collectively spend almost \$1 billion on older adults although they only make up 15% of the total population (Ministry of Health, 2016a). This same trend is apparent in most developed OECD populations worldwide (World Health Organization, 2017).

Older adults are a unique population group who have increased nutrient needs due to physiological changes with ageing (Houston DK. et al., 2008; Ministry of Health, 2013a). When muscle mass and strength are compromised, physical function and independence may diminish (Houston DK. et al., 2008). This may lead to adults in advanced age being at a higher risk of falls and fractures therefore experiencing poorer health outcomes (Ministry of Health, 2013a). As a result, older adults may be hospitalized earlier and combined with decreased immune function, they may be unable to regain their independent life. In order to prepare and adjust to this unavoidable change it is important to understand the determinants of protein intake and how prevalent protein intake is in community dwelling older adults.

Very little is known about the relationship between protein intake, distribution, and their determinants in Māori octogenarians. This is due to the smaller number of Māori aged 80 years old and over compared to the non-Māori population in New Zealand, therefore a larger age range is required to estimate adequate protein intake (Ministry of Health, 2018b). However, studies have shown when protein intake is expressed as gram per kilogram of body weight, Māori are less likely to meet the adequate cut-off points (Wham C., Teh R., et al., 2015). The New Zealand Adult Nutrition Survey (2008/09) showed that 13.4% of men and 15.5% of women over 71 years of age had inadequate protein intake, including 8.0% of Māori men over the age of 51 (University of Otago. & Ministry of Health, 2011).

Low protein intake is commonly found in the very old as shown in the findings of the Newcastle 85+ Study where 28% of the participants did not meet the protein requirements (Mendonça N. et al., 2018). Higher protein intakes are needed to positively affect anabolism and protein synthesis to maintain muscle mass and strength and physical function in older people. Although, the New Zealand Adult Nutrition Survey (2008/09) showed 13.4% of men and 15.5% of women, 71 years and over, did not meet the EAR for protein, it is an estimate and not enough information has been gathered specifically on octogenarians (University of Otago. & Ministry of Health, 2011).

There is limited research to understand on why protein intake is low in some individuals at advanced age and not in others. This and the pressure of an increase in population of adults 80 years and over could lead to adverse effects such as an increase in falls and fractures leading to dependency, higher rates of hospitalization and less optimal quality of life (Ministry of Health, 2013a). For example, a study showed that malnutrition was positively associated with the likelihood of being in institutionalized care for older adults and less likely for older adults to live in the community (Tieland M., Borgonjen-Van den Berg KJ., van Loon LJ., & de Groot LC, 2012). Therefore, it is crucial to investigate the determinants and find the reasons why inadequate protein intake occurs and more importantly how to reduce these determinants to increase protein intake and improve health outcomes in the elderly.

1.1. Aims and objectives

Aim:

This cross-sectional study will investigate the prevalence and the determinants of low protein intake, main protein food sources and the distribution of protein intake among Māori and non-Māori octogenarians participating in Life and Living in Advanced Age: A Cohort Study in New Zealand (LiLACS NZ).

Objectives:

- 1.** To undertake a secondary analysis of dietary intake assessed by 24-hour Multiple Pass Recalls on two non-consecutive days to determine the prevalence of low protein intake based on the Estimated Average Requirements (EAR) for adults over 70 years, protein distribution during the day and food groups contributing to low and adequate protein intake.
- 2.** To examine the determinants of low protein intake in both Māori and non-Māori participants using multivariate linear regression to explore sociodemographic, health and physical predictors of protein intake.

1.2. Structure of thesis

This thesis contains four chapters with references and appendices attached at the end of the document. Chapter one includes the introduction which details the gaps in the literature surrounding octogenarians and protein intake. The aim and objectives of this study are also included. Chapter two consists of a literature review based on older adults, their patterns of protein intake and the potential determinants of protein intake. Chapter three contains the research study manuscript with the following sub-headings; abstract, introduction, methods and materials, results, discussion, and conclusion. The research manuscript was written for publication in the *Nutrients* Journal. Chapter four includes the strengths and limitations of this study and recommendations for increasing protein intake among older adults based on this research. Chapter five contains the appendices of supplementary materials that did not make the final copy of this thesis but helps to paint a detailed overview of the study. It includes the supporting results of participants and the Recommended Daily Intake (RDI) for protein, statistical analysis based on the RDI and food sources of protein.

1.3. Researcher's contributions

Table A. Researcher's contributions

Anishka Ram	Master's student: <ul style="list-style-type: none">- Literature review- Statistical analysis for protein determinants- Manuscript
Professor Carol Wham	Supervisor: Life and Living in Advanced Age: A Cohort Study in New Zealand (LiLACS NZ) <ul style="list-style-type: none">- Thesis guidance and assistance- Research manuscript guidance and assistance
Simon Moyes	Senior Data Analyst: <ul style="list-style-type: none">- Statistical analysis for multivariable and regression models- Finalizing statistical analysis chapter
Professor Ngaire Kerse	Principal Investigator LiLACS NZ: <ul style="list-style-type: none">- Statistical analysis overview- Research manuscript overview

2. Literature Review

2.1. Ageing in New Zealand

The New Zealand population is ageing, with older adults over the age of 85 the fastest growing age group. By 2050, those aged 85 plus years will comprise a fifth (22%) of adults over 65 years (Ministry of Social Development, 2016a; Super Seniors, Accessed 2018). Along with the increase in the population of advanced aged adults it is expected that life expectancy for women will rise from 79.5 years old to 83.2 years and for men increase from 74.3 to 79.5 years (Ministry of Social Development, 2016b). With advanced age a progressive decline in health may occur and limited physical function and dependence may result from long term illnesses which can place a burden on existing healthcare and disability services (Ministry of Health, 2013b). The New Zealand Healthy Ageing Strategy (2016) aim to support older people with high and complex needs to help and encourage older adults to age in place (Ministry of Health, 2016b). A Ministry of Health report showed that women lived on average 16.7 years with a disability that required carers and extra assistance compared to men who lived 14.3 years with a disability (Ministry of Social Development, 2016b).

A key strategy is to increase access to health care services to support individuals who are frail, lonely and vulnerable, regardless of their financial situation, placing the older adult and their values at the centre of treatments (Ministry of Health, 2016b). Support services available to older adults with disabilities that hinder daily activities include assistance with personal cares, chores, equipment and access to carers, provided a needs assessment has been completed beforehand (Ministry of Health, 2011). A carer may be a relative, friends or an agency that provides a support person to assist with grocery shopping and meal preparation (Ministry of Health, 2011). Most individuals aged 85 years and over with a disability have help available with groceries and chores (47%) and 10% need help with personal cares (Te Pou, 2011). The Strategy also aims for transport services and regional boards to work together and increase transport options for older adults to better access facilities and reduce social isolation (Ministry of Health, 2016b).

2.2. Health of Older Adults

Although ageing is a natural process it is linked to many age-related diseases such as heart disease, high risk of stroke, sarcopenia, osteoporosis, depression and dementia that contribute to disabilities (Ministry of Health, 2018b). An increase in age is a strong indicator for heart disease therefore, adults aged 80 years and above are more likely than any other age group to be diagnosed with heart disease (Ministry of Health, 2018a). High body mass index (BMI), hypertension, type 2 diabetes, smoking, drinking and excessive intake of sugar, sodium and saturated fat are known risk factors of cardiovascular disease (Ministry of Health, 2018a).

Octogenarians are at a significant risk of stroke with an estimated 33.5% of older adults hospitalised for the illness (Smithard DG, 2017). Individuals who have suffered a stroke may be more likely to develop dysphagia (difficulty in swallowing), frailty, poor oral hygiene, and become at high risk of malnutrition and dehydration (Holland G. et al., 2011; Smithard DG, 2017). Malnutrition can result from inadequate intake of nutrient dense foods particularly those that are hard to chew such as meat, nuts and fruits and vegetables (Smithard DG, 2017). Swallowing difficulty may also be exacerbated by sarcopenia, a significant decrease in

muscle mass, strength and physical function with ageing (& Paddon-Jones D. & Rasmussen BB, 2009). Individuals with dysphagia, may require the texture of foods to be softened to assist with swallowing and may reduce or avoid foods, therefore leading to a decrease in dietary intake and protein quality (Steele CM. et al., 2015).

About 50% of all octogenarians' experience sarcopenia and if left undetected and untreated, sarcopenia may lead to a decline in physical function and possibly disability (Visser M. et al., 2002). Studies have shown increased physical activity, combined with adequate protein intake, can reverse the loss of muscle mass and increase muscle attenuation (Deutz NE. et al., 2014). Although older adults may find it difficult to incorporate physical activity in their everyday lives, it is important reduce the loss of muscle mass, strength, and physical function. Consuming an adequate amount of protein is associated with slowing down the effects of sarcopenia by effectively activating muscle protein synthesis and promoting anabolism (Cardon-Thomas D., Riviere T., Tiegies Z., & Greig C, 2017).

Osteoporosis, as a result of low bone mass and bone tissue, may lead to an increased risk of frailty and fractures which tends to be more common in women than men (Chen P., Li Z., & Hu Y, 2016). Bone health can deteriorate as a result of a low protein intake and little or no weight bearing exercise (Wengreen HJ. et al., 2004). Osteoporosis may lead to earlier hospitalisation, chronic pain and significantly restrict physical function (Chen P. et al., 2016). A positive relationship between dietary protein intake, muscle and bone health has been proposed whereby an increase in protein may allow better absorption of calcium for osteoblast activity and formation of the bone matrix (Cifuentes M. et al., 2003).

Depression may be prevalent in octogenarians and is due to long term feelings of low mood and leading to a loss of interest in activities which ultimately can affect all aspects of daily living (Atlas A., Kerse N., Rolleston A., Teh R., & Bacon C, 2017). Many risk factors may lead to depression in older adults and having hypertension earlier in life is associated with a greater chance of being depressed in advanced age (Siennicki-Lantz A., André-Petersson L., Wollmer P., & Elmståhl S, 2013). Older adults with chronic illnesses who take many medications may also develop depression more easily and may lead to less control over their own health (Ministry of Health, 2013a). Being around loved ones encourages a positive attitude however many older adults live alone (Lyyra TM., Törmäkangas TM., Read S., Rantanen T., & Berg S, 2006). Those living alone may experience isolation and are less likely to seek support from people they trust because they feel as though they are a burden. Older adults who live alone may lack motivation to prepare and eat meals, may often skip meals, consume less food and have a poor appetite (Ministry of Health, 2013a). Adults of advanced age with depressive symptoms are at higher risk of malnutrition (Wham CA. et al., 2015).

Oral health is of concern because older adults who experience tooth loss and pain may not be able to consume a variety of foods. As a result of edentulism (removal of all teeth), the enjoyment of food reduces as the texture of foods change (Lee JS. et al., 2004). Many older adults with dentures complain of pain while chewing and are forced to make changes to their food preferences (Lee JS. et al., 2004). They also report having poor appetite and may develop a poor health status due to ill-fitting dentures (Lee JS. et al., 2004). Changing the texture of food and replacing hard to chew foods such as meat to reduce pain can lead to a lower protein intake and difficulty in meeting protein requirements (Hutton B., Feine J., & Morais J, 2002). The Makila study reported that 51% of older adults with teeth ate meat

whereas it was consumed by only 26% of edentate older adults (Hutton B. et al., 2002). Factors that may lead to edentulism include not visiting the dentist, poor oral hygiene, a lower education, increased intake of foods high in sugar causing tooth decay and having a disability (Lee JS. et al., 2004). Older adults who depend on carers may find it hard to make and attend dentist appointments and may need extra assistance in this area (Avlund K., Holm-Pedersen P., & Schroll M, 2001). In New Zealand it is common to see older adults only seek dental advice when there is an issue and many do not visit the dentist regularly (CBG Health Research, 2015).

It is not uncommon for older people to live on their own. Women are more likely to live on their own until they reach the age of 85, men (80%) tend to live alone between the age of 70 to 74 whereas, around a third of older adults with a disability reside on their own (Te Pou, 2011). Unfortunately, older adults are likely to experience loneliness and low social support as family relations may change or they may move away, loss of interest in social activities or have reduced physical function (Atkinson, Salmond, & Crampton, 2014).

Adults in advanced age may take part in voluntary activities to provide knowledge and teach skills to younger adults (Te Pou, 2011) and older Māori with an active role in cultural practices may have prominent positions such as kaumatua or kuia who lead and support their own iwi or marae. They may receive a kōha (donation) for their time that may be of monetary value. However, a large proportion of Māori and non-Māori aged 65 years and over have pension only income which is lower than the average New Zealand income (Te Pou, 2011). Annual pension rates are estimated to be between \$21,000- \$25,000 if older adults live alone or \$16,000- \$19,000 if they live with a partner (Ministry of Social Development, 2016b).

Individuals who reside in regions of low socioeconomic status may be identified by the New Zealand deprivation score (NZDep score) (Atkinson et al., 2014). A deprivation score is provided for each mesh block in NZ which are the smallest geographical areas determined by geocoding, the process of transforming addresses on a map into mesh blocks. The socioeconomic level of a region is based on eight categories including, income level, employment status, access to telecommunications and transportation, receive support, education and qualification levels, home ownership and living space (Atkinson et al., 2014). The NZDep score ranges from 1 (region being the least deprived), to a score of 10 (regions that are the most deprived) (Atkinson et al., 2014). Older adults who receive a pension and other forms of income are more likely to have a NZDep score between 1 to 4, whereas older adults with pension only income tend to receive a score between 8 to 10.

Food insecurity has been reported among older adults with reduced income, those not owning the house they reside in and being of an ethnic minority (Deeming C, 2011; Vargas VP., Alvarado SO., & Atalah ES, 2013). Food insecurity is the inability to access fresh, safe foods and in socially acceptable ways and could possibly lead to poor cognitive function, higher chance of having depression and poor physical function (Vilar-Compte M., Gaitán-Rossi P., & Pérez-Escamilla R, 2017). Barriers to accessing foods in advanced age are lack of transport as older adults may not drive anymore plus the need to pay for transport or wait for others to provide transport (Vilar-Compte M. et al., 2017). Disabilities or poor physical function may also limit access to food as octogenarians may not live near fresh, inexpensive markets or be able to travel without medical assistance and equipment (Vilar-Compte M. et al., 2017).

A significant number of older adults have chronic illnesses and require special diets that may include foods that are relatively expensive; this may lead to feelings of anxiety when facing

food insecurity (Vilar-Compte M. et al., 2017). Although providing and delivering prepared meals to older adults can reduce food insecurity some do not accept meals as a result of the stigma towards food assistance (Oemichen M. & Smith C, 2016). Men are more likely to be food insecure due to the inability to cook meals as preparing and cooking meals has traditionally been the role of women (Vilar-Compte M. et al., 2017).

2.3. Health of Older Māori

Māori aged 80 years old and over make up just under 4,000 people and numbers are expected to increase to almost 12,000 by 2026 (Anna Kellett, 2017). Overall, life expectancy for Māori women is 73.2 years and 69.0 years for Māori men and non-Māori men and women can live up to 75.5 years and 75.7 years old respectively, without any form of disability (Ministry of Social Development, 2016b). As a result of experiencing health disparities and living in areas of high socioeconomic deprivation Māori individuals may be at a greater risk of having poorer health outcomes and greater mortality risk (Ministry of Social Development, 2016b). Māori are 1.5 times more likely to be hospitalised due to CVD, are 1.7 times more likely to be obese and nearly 4.7 times more likely to be diagnosed with diabetes compared to non-Māori (Ministry of Health, 2014).

Although younger Māori adults have greater muscle mass than non-Māori adults, ethnicity may not be an indicator of sarcopenia in advanced age (Hao R. & Guo H, 2012; Rush E., Freitas I., & Plank L, 2009). Māori octogenarians are more likely to be frail at a younger age than non-Māori (Atlas A. et al., 2017). A three-year study reported that fewer Māori older adults (47%) experienced falls compared to older non-Māori (57%) (Atlas A. et al., 2017). As a Māori population have a lower life expectancy they do not suffer high rates of diseases such as osteoporosis, colorectal cancer and dementia (Ministry of Health, 2019). However, Māori are at a higher risk of being diagnosed with COPD, lung cancer and diabetes earlier in life than non-Māori which can significantly affect the outcome of their health in advanced age (Ministry of Health, 2019).

2.4. Nutrition Status of Older Adults

Social, physical, environmental, and individual factors all contribute to the nutrition status of older adults (Ministry of Health, 2016b). These factors may include the cost and access to nutrient dense foods, cultural aspects of eating such as availability of traditional foods and use of cooking practices, living alone and the individual's health status and food preferences (Ministry of Health, 2016b; Vilar-Compte M. et al., 2017). Older adults must cope with the natural ageing process which includes a gradual decrease in the rate of the body's metabolism, absorption and storage of nutrients that may contribute to poor nutritional status (Ministry of Health, 2018b).

Total energy intake and output gradually declines in advanced age as a result of a drop in basal metabolic rate, lean body mass and total water in the body (Greenlund LJS. & Nair KS, 2003). Energy requirements are determined by gender, physical activity levels and body weight of the individual. Energy requirements for the very old are difficult to establish due to the heterogeneity of individuals in this group who may or may not have disabilities, therefore it is more beneficial to create total energy requirements specific to the individual's needs (NHMRC, 2006).

Achieving a good nutritional status is important to promote and maintain a healthy body weight, muscle mass, strength and physical function (Houston DK. et al., 2008). Older adults who consume adequate amounts of energy through regular meals and snacks are able to counter the catabolism and anabolic resistance that occurs in the ageing body (Deutz NE. et al., 2014). Due to the reduction in the rate of metabolism and production of new cells, combined with the presence of multiple chronic diseases, older adults must replenish their energy stores by either maintaining or increasing the amount of energy they consume throughout the day. A higher energy intake may be beneficial to counter physiological changes they may experience due to chronic illness, gastrointestinal issues, poor oral health and being under or overweight.

The combination of higher energy requirements and inadequate energy intake may negatively impact the health and well-being of the very old. This can manifest in low muscle mass and may contribute to malnutrition (Bauer J. et al., 2013). It is well-known that adults in advanced age tend to reduce their energy intake as a result of poor appetite, not feeling hungry or ignoring hunger signals because of side effects of medications (Ministry of Health, 2016b). Inadequate energy and macronutrient intake may also be common due to changes in taste and smell that may lead to limiting particular foods (Wham C. & Yaxley A, 2020). Older adults tire easily and may not want to cook meals which could result in consumption of foods that whilst convenient are high in fat, salt, sugar and energy but not nutritionally complete (Chernoff R, 2004). Most importantly, inadequate intake of food energy may compromise overall protein intake.

2.5. Protein Requirements for Older Adults

Nutrient requirements for older adults are provided as Nutrient Reference Values (NRV's) for Australians and New Zealanders where requirements for older adults are aggregated for adults 70 years and over (NHMRC, 2006). Protein requirements for older adults ≥ 70 years are derived from data from nitrogen balance studies in younger adult males (Rand WM., Pellett PL., & Young VR, 2003). The estimated average requirement (EAR) is the average amount of daily protein intake necessary to reduce the likelihood of deficiency in 50% of the population and is adjusted for body weight with cut off points of 0.86g/kg/day and 0.75g/kg/day for New Zealand and Australian men and women, respectively (NHMRC, 2006; Rand WM. et al., 2003). The EAR for protein for adults ≥ 70 years was increased by 25% of younger adults, however the data supporting this increase are limited (Campbell WW., Trappe TA., Wolfe RR., & Evans WJ, 2001). The recommended daily intake (RDI) for protein is also used as a reference for protein requirements in New Zealand and Australia, which accounts for the needs of 97 to 98% of the population (0.94g/kg/day for women and 1.07g/kg/day for men (NHMRC, 2006). A study of six female octogenarians, aged between 80-87 years, used an indicator amino acid oxidation (IAAO) technique instead of the traditional nitrogen balance technique and found their dietary protein requirement was 29% higher than the EAR (0.66g/kg/day) for Americans (Rand WM. et al., 2003; Tang M. et al., 2014). This study clearly showed that adults in advanced age must have higher protein requirements that are specific to their needs in order to retain physical function and continue to reside in the community.

Internationally, the recommended daily allowance (RDA), for protein expressed as 0.8g/kg/day, is the cut off point for both men and women aged 19 years and over (World

Health Organization, 2007) but has been challenged as too low to meet the protein needs of many older adults. International study groups have reviewed dietary protein needs with ageing, the PROT-AGE study group and the European Society for Clinical Nutrition and Metabolism (ESPEN) concur that protein requirements need to be increased and recommend intakes of between 1.0-1.2g/kg/day of protein for healthy older adults (≥ 65 years) to maintain muscle mass, accommodate age-related changes and overcome catabolic conditions such as sarcopenia, osteoporosis and reduced immune function (Bauer J. et al., 2013; Deutz NE. et al., 2014). The NuAge study of participants, aged from 67 to 84 years, found that consumption of a higher amount of protein (men with an average intake of 1.04g/kg/day and women with an average intake of 1.05g/kg/day) allowed adults of advanced age to better retain their muscle mass over a two year period (Farsijani S. et al., 2017; Mamerow MM. et al., 2014). Findings from the Health ABC Study showed men and women aged 70 to 79 years consumed an average protein intake of 0.8g/kg/day in the lowest quintile (Quintile 1) and 1.2g/kg/day in the highest quintile (Quintile 5) with those in the highest quintile losing 40% less muscle mass compared to the group that had the lowest protein intake, over a three year period (Houston DK. et al., 2008). From these findings it was suggested protein requirements should be increased to 1.1g/kg/day to protect older adults from risk of malnutrition, decreased muscle mass, strength and physical function (Houston DK. et al., 2008). There are few studies of adults in advanced age however an exception is the Newcastle 85+ Study in the UK where among community dwelling octogenarians (aged ≥ 85 years) detailed dietary assessment using 2x24hour multiple pass recalls found 28% of participants had a low protein intake using the RDA cut-off point of <0.8 g/kg/day (Mendonça N. et al., 2018). This latter study suggests a third of advanced aged older adults may consume an inadequate amount of protein. Investigations have reported the main reasons older adults have an inadequate protein intake include chewing difficulties (with foods such as meat), having no teeth or dentures that do not fit properly and cause pain when eating, fear of eating fat and increasing saturated fat from meat and meat products, change in taste preferences and increased satiety or limited access to food (Bauer J. et al., 2013; Paddon-Jones D., Short KR., Campbell WW., Volpi E., & Wolfe RR, 2008).

Expressed as percent contribution to energy the acceptable macronutrient distribution range (AMDR) for protein ranges from 15 to 25% (NHMRC, 2006). In the Newcastle 85+ study it was observed that participants with low protein intake had an AMDR of 13% whereas those that had adequate protein intake had 15.8% of their energy derived from protein (Mendonça N. et al., 2018). The NHANES (2003/04) survey found that men aged 71 years and over consumed 13.2% of energy from protein at the lowest (5th) percentile while those in the highest (95th) percentile had an AMDR of 18.9%; women in the same age group had an AMDR of 12.5% in the lowest percentile and 19.4% in the highest percentile (Fulgoni III VL, 2008). A study on Dutch older adults (mean age 77.2 years) compared dietary protein intake and found 15% of energy was derived from protein in community dwelling older adults whereas frail and institutionalized older adults had an AMDR of 16% (Tieland M., Van Loon L., & de Groot L, 2015).

In a previous analysis of LiLACS NZ it was observed that Maori octogenarians who were hospitalised due to infection, consumed on average 15.3% of protein compared to those who were hospitalised where observed protein intake was 17.2% of energy (Wham C., Baggett F., et al., 2015). As protein intake adjusted for energy in hospitalised individuals were within the

recommended AMDR range (15-20%); this finding also suggests that protein requirements may need to be increased for adults in advanced age (Wham C., Baggett F., et al., 2015). As mentioned above, older adults experience a range of issues that contribute to not meeting the cut-off points for adequate protein intake. It also has been observed that women have reduced energy intake and are more likely to not be able to reach the AMDR for protein compared to older men (Mendonça N. et al., 2018; Zhu K. et al., 2010).

2.6. Animal vs. Plant Protein Intake

Protein may differ in quality among foods derived from animal and plant sources. Sources of protein differ in biological value, rate of digestion and quality of protein such as the amount of essential amino acids (EAA) available in the food source, for example animal sources of protein have significantly higher biological value compared to plant proteins (Tome D, 2012). Protein from plant sources score between 0.57 to 0.71 on the protein digestibility corrected amino acids (PDCAAS) score, suggesting that the rate of digestion of plant proteins is relatively slow; whereas the PDCAAS score ranges from 0.92 to 1.00 for animal protein which are more rapidly digested (Pereira PM. & Vicente AF, 2013). Plant based sources do not have enough of the EAA such as lysine and methionine compared to animal sources which contain all of the EAA (Katsanos CS., Kobayashi H., Sheffield-Moore M., Aarsland A., & Wolfe RR, 2005). However, some vegetables and grains contain a high amount of EAA, and it is possible for older adults to increase the amount of protein in their diet by combining a range of non-animal derived sources at every meal (Chernoff R, 2004).

Among participants aged 70 to 79 years old, in the Health ABC Study, it was found animal protein sources were more efficient in increasing muscle protein synthesis and maintaining muscle mass, in women (Houston DK. et al., 2008). The Framingham Offspring Cohort study showed that individuals with a higher amount of protein (from plant or animal sources) had a smaller reduction in grip strength over six years; and those who consumed large amounts of animal protein but no plant protein, were more likely to have a lower loss of grip strength (McLean RR., Mangano KM., Hannan MT., Kiel DP., & Sahni S, 2015). Also, among Chinese older adults it was observed those who consumed large amounts of protein from plant sources had a lower muscle mass after four years (Chan R., Leung J., Woo J., & Kwok T, 2014). These findings suggest that consumption of animal protein sources is important for older adults to maintain muscle mass and strength.

2.7. Protein Distribution

To fully stimulate muscle protein synthesis it has been suggested older adults require a minimum of 30g of total protein at each meal (& Paddon-Jones D. & Rasmussen BB, 2009). This response is determined by leucine which serves as a critical signal for triggering initiation of muscle protein synthesis. Evidence suggests that protein intake spread evenly across the day may have a greater impact on maintaining lean muscle mass and can lead to an estimated 25% increase in muscle strength and negatively affect muscle mass in the very old (Farsijani S. et al., 2017; Mamerow MM. et al., 2014). It is common to find almost half of the daily protein intake being consumed at dinner while lower protein is consumed at breakfast and lunch due to these meals being smaller (Volpi E. et al., 2013). Due to an ingestion of uneven protein intake, it is difficult to effectively stimulate the muscle and any minor changes could be reversed. A study showed the effects of a large consumption of whey

protein taken at once; while this led to a significant increase in muscle protein synthesis the effects did not last longer than 90 minutes and the effects returned to baseline (Atherton PJ. et al., 2010).

There seems to be a dose-response relationship between dietary protein intake and protein synthesis. A study comparing two cohorts, those who were younger (mean age 35 years) and older (mean age 68 years) found that consumption of over 30g of protein, and less than 25g at each meal did not show any significant effects on the muscle (Symons TB., Sheffield-Moore M., Wolfe RR., & Paddon-Jones D, 2009). This is due to the fact that the body cannot store large amounts of dietary protein and therefore be able to stimulate muscle protein synthesis later in the day or when required, as the effects may only last for three hours (Volpi E. et al., 2013).

Overall, protein intake can be increased by consuming more protein from high quality sources and evenly spreading the amount of protein throughout the day to reduce the loss and maintain muscle mass, strength, and physical function. Therefore, this study will investigate protein intake in New Zealand octogenarians by exploring protein sources, distribution, and the determinants associated with inadequate protein intake.

3. Research Study Manuscript- *Nutrients* Journal

Protein intake, distribution, sources, adequacy and determinants in Māori and Non-Māori Octogenarians: Life and Living in Advanced Age: A Cohort Study in New Zealand (LiLACS NZ).

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Abstract

Background: Protein intake, food sources and distribution are important to prevent age related loss of muscle mass and strength.

Methods: The prevalence and determinants of inadequate protein intake, food sources and mealtime distribution were examined in 214 Māori and 360 non-Māori of advanced age with a 2x 24-h multiple pass recall. Contribution (%) of food groups to protein was assessed. Low protein intake was defined as ≤ 0.75 g/kg for women and ≤ 0.86 g/kg for men. A logistic regression model was built to explore predictors of low protein intake.

Results: A third of both women (30.9%) and men (33.3%) had a low protein intake. Main food group contributors were beef/veal, fish/seafood, milk, bread and differed by gender and ethnicity. For women and men respectively protein distribution (g/meal) was lowest at breakfast (10.1 and 13.0), followed by lunch (14.5 and 17.8) and dinner (23.3 and 34.2). Being a woman ($p=0.003$) and having depressive symptoms ($p=0.029$) were associated with consuming less protein. In adjusted models the odds of adequate protein intake were higher in participants with their own teeth or partial dentures ($p=0.036$).

Conclusion: Findings highlight the prevalence of low protein intake, uneven mealtime protein distribution and importance of dentition for adequate protein intake among adults in advanced age.

Keywords: older adults; protein intake; protein distribution; food sources; LiLACS NZ

Introduction

By 2051, the 85 years and over age group are expected to make up almost a quarter of the 65+ population (Stats NZ, 2018). The very old experience more co-morbidities and disabilities as they get older and can potentially place a high burden on health care costs and services (Ministry of Health, 2018b). We previously determined half (49%) of Māori and 38% of non-Māori octogenarians were at high risk of malnutrition (Wham C., Teh R., et al., 2015).

Those who are malnourished may have an inadequate protein intake which could contribute to possessing lower muscle mass and strength (& Paddon-Jones D. & Rasmussen BB, 2009). Older adults have a lower rate of anabolism and are more likely to suffer from less than optimal immune functions compared to younger adults, therefore protein intake must be increased (Bauer J. et al., 2013). Adequate protein intake is important for optimal synthesis with protein intakes below 0.75g/kg/d for women and 0.86g/kg/d for men, can lead to decreased physical function and can contribute to sarcopenia which is the result of a loss of muscle mass and physical function (Visser M. et al., 2002).

Protein can be obtained from both plant and animal sources; however, they differ greatly in protein quality, rate of digestion and biological value that affect the rate of skeletal muscle protein synthesis (Phillips SM., Tang JE., & Moore DR, 2009). For example, animal sources such as meat, eggs and seafood have almost all the essential amino acids, higher biological value and can be digested quicker than plant sources such as vegetables and grains (Tome D, 2012). Bread is typically the main source of protein in the very old followed by milk and poultry in women and beef/veal and fish and seafood in men (Wham C., Teh R., et al., 2015).

The distribution of protein intake may also influence the maintenance of lean muscle mass. A key strategy to increase the rate of protein synthesis is to evenly spread protein throughout the day, by aiming to have between 25 to 30g of protein in each main meal (Mamerow MM. et al., 2014). Among adults of advanced age, in the Newcastle 85+ study, it was observed that protein intake was skewed towards the end of the day. Protein intake was lowest in the morning especially at breakfast whereas, the largest amount of protein was consumed during the evening meal (Mendonça N. et al., 2018).

Therefore, the aim of this study was to investigate the intake, food sources, distribution, and adequacy of protein among Māori and non-Māori participants in Life and Living in Advanced Age: A Cohort Study in New Zealand.

Materials and Methods

2.1. Life and Living in Advanced Age: A Cohort Study in New Zealand (LiLACS NZ)

We conducted a cross-sectional study using data from the baseline and follow-up assessment of Life and Living in Advanced Age, A Cohort Study in New Zealand (LiLACS NZ), a longitudinal study of the very old in New Zealand (Hayman KJ. et al., 2012). Eligibility criteria were Māori aged 70 to 80 years and non-Māori aged 85 years living within the Bay of Plenty and Lakes District Health Board regions. Younger Māori participants were recruited as the gap in life expectancy between Māori and non-Māori was 8.2 years for men and 8.8 years for women (Ministry of Social Development, 2008). Details of the recruitment process are described elsewhere (Hayman KJ. et al., 2012).

At baseline in 2010, 671 participants completed a comprehensive questionnaire, conducted by trained interviewers and a health assessment by a trained nurse (Dyall L. et al., 2013). Participant age, demographic, and health characteristics were established. Rates of participation varied across questionnaires and assessments.

A 12-month follow-up visit was undertaken in 2011 and included a physical assessment including weight, BMI, fat mass and muscle mass as well as grip strength and level of physical activity. A detailed dietary assessment using the 24-hour multiple pass recall (24h MPR) on two separate days of the week was offered as part of that stage of the study.

2.2. Socio-economic, health and physical factors

At baseline, whether the participants had practical help available was established. Participants responded either yes/no to: “When you need some extra help, can you count on anyone to help with daily tasks such as grocery shopping, cooking, house cleaning, telephoning, give you a ride?” Current living arrangement was categorised as living alone (yes/no). The NZ deprivation (NZDep) index obtained from the Ministry of Health was used as an indication of sociodemographic deprivation. The index was contrasted from geo-coded addresses and included eight dimensions of material and social deprivation reflecting lack of income, employment, communication, transport, educational qualifications, home ownership and living space (Atkinson et al., 2014). At follow-up participants responded yes/no to whether they received a pension only income.

Health factors were established at baseline. Oral health was established by whether the participants wore dentures, had trouble with biting and chewing or with swallowing problems. Depression was assessed by the 15-item Geriatric Depression Screening Scale (GDS-15) (Montorio I. & Izal M, 1996), a reliable and valid self-rating depression screening scale developed specifically for older people (Yesavage J. & Sheikh J, 1986). A higher score indicates more depressive symptoms, with a cut-off of 5 or more considered to indicate significant depressive symptoms (Almeida OP. & Almeida SA, 1999; Conradsson M. et al., 2013). The Short Form health score 12 (SF-12) (Fleishman JA., Selim AJ., & Kazis L, 2000) was used to provide a view of health related quality of life of the participants based on their perceived experience, knowledge and awareness of their personal, physical, mental and emotional status. The scale presents two summary scores: physical and mental health related QoL. The maximum score is 100; any score lower than 40 is indicative of perception of poor health and above 60 is indicative of reasonable and better health (Montazeri A., Vahdaninia M., Mousavi SJ., & Omidvari S, 2009).

At follow-up the participants height was measured with a SECA 213 free-standing stadiometer two times unless the variance between both figures were greater than 1cm then a third attempt was recorded (Hayman KJ. et al., 2012). Weight was estimated using a Tanita digital measuring scale (BC-545, Tanita Corporation) and height and weight were used to calculate body mass index (BMI) using the formula kg/m^2 . Measurement of fat mass and muscle mass were estimated by bioimpedance using the Tanita scale (Inner Scan Body Composition Monitor, BC-541, Tanita Corporation, Japan). Grip strength was estimated using a Takei digital handgrip dynamometer-Grip D. The average value of three readings from the strongest hand was recorded (Hayman KJ. et al., 2012). A Physical Activity Scale for the Elderly (PASE), validated in community living adults (Washburn RA., McAuley E., Katula J., Mihalko SL., & Boileau RA, 1999) was used to assess physical activity. PASE consists of ten items used to identify leisure, household and occupational related activity and duration of each activity over

a one-week period. The total PASE score was derived by multiplying the duration of each activity (hours/week) or participation (yes/no) by the empirically derived item weights and summing over all activities. Also, the following information was collected, age and pension only income was ascertained using the question “What are your sources of income?” The answers were categorised into either yes/no.

2.3. Dietary Assessment

As part of the follow-up assessment, 214 Māori and 306 non-Māori participants completed a 24-hour MPR on two separate days of the week. The validity of the 24h MPR is detailed elsewhere (Adamson AJ. et al., 2009; Nelson, Dick, Holmes, Thomas, & Dowler, 2002). Interviewers were instructed to record the weight of the food either by reading food labels or estimating the portion size with household utensils. The “Photographic Atlas of Food Portion Sizes”, previously applied in the Newcastle 85+ study was adapted for the new Zealand diet and used to assess the portion of foods when an item could not be quantified (Nelson, Atkinson, & Meyer, 1997; Robinson F., Morritz W., McGuinness P., & Hackett AF, 1997). All data from the 24h MPR was analysed by FOODFiles, a comprehensive database of foods and drinks.

Protein intake was assessed using the NZ NRV’s (NHMRC, 2006) and estimated average requirements (EAR) in grams per kilogram adjusted for body weight per day, to determine the adequacy in men (0.86g/kg/d) and women (0.75g/kg/d). Protein distribution was assessed across breakfast, lunch, and dinner in grams per meal.

All foods from the 24h MPR were allocated into one of thirty-three food items, used in the New Zealand Adult Nutrition Survey (NZANS) (2008/09), that contributed to protein intake (University of Otago. & Ministry of Health, 2011). The top fifteen food items that contributed the most protein to the participants’ diet was reported. Supplements were not included.

2.4. Statistical Analysis

The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to check for normality for variables (age, has practical help available, living alone, socio-economic deprivation, pension only income, wears dentures, difficulty biting and chewing, swallowing difficulties, depression, SF-12 physical health, SF-12 mental health, body mass index (BMI), weight, grip strength, PASE score, fat mass percentage and muscle mass percentage). Categorical data are presented as percentages and corresponding sample size. Normally distributed data were expressed as a mean and standard deviation (\pm SD); non-Gaussian distributed variables were expressed as medians and interquartile ranges (IQR). The Mann-Whitney U test and independent T-test were used to determine differences between Māori and non-Māori, men and women for age, SF-12 physical health, SF-12 mental health, BMI, weight, grip strength, PASE score, fat mass percentage, muscle mass percentage, energy, protein (energy %), protein (g/d), protein (g/kg/d), protein distribution (breakfast, lunch and dinner) and chi-square tests were used to test differences for categorical data (practical help available, living alone, socio-economic deprivation, pension only income, wears dentures, difficulty biting and chewing, swallowing difficulties, depression, total protein, acceptable macronutrient distribution range (AMDR), and low and adequate protein based on EAR). Protein from food groups were

presented as a mean (\pm SD) and contribution to protein intake was expressed as a percentage (%) and adjusted for body weight (g/kg/d). A p -value of <0.001 was statistically significant.

A reduced multivariate regression model was built to predict protein intake in g/kg/d. Age, gender and ethnicity were included in the model other potential predictors included practical support, depression, living alone, NZDep, wears dentures, chewing problems, swallowing difficulties, SF-12 physical and mental health scores, and pension only income. Variables with the highest p -value were removed in the model and $p \leq 0.2$ was considered statistically significant. A logistic regression model was constructed to predict meeting the EAR for adequate protein intake (g/kg/d). The IBM SPSS Statistics 23 program was used to conduct the statistical analysis.

2.5. Ethics Approval

The study was granted an ethics approval by the Northern Regional Ethics Committee of New Zealand in December 2009 (NTX/09/09/088) (Hayman KJ. et al., 2012).

Results

3.1. Participants Characteristics

The sociodemographic health and physical characteristics of Māori and non-Māori men and women are illustrated in Table 1. The median age for Māori was 83 years (IQR 81-85), slightly younger than non-Māori with a median age of 86 years (IQR 85-86) ($p < 0.001$). More women (51% Māori, 65% non-Māori) than men (25% Māori, 36% non-Māori) lived alone ($p = 0.001$). Also, more women (54% Māori, 54% non-Māori) had dull mouth dentures compared to men (42% Māori, 40% non-Māori) ($p = 0.011$). Rates of participation varied across the assessments both at baseline and at 12 months follow-up.

3.2. Dietary Assessment 24h Multiple Pass Recall (MPR)

There were 214 (37%) Māori and 360 (63%) non-Māori participants who completed the 12 months follow-up 2x24h MPR dietary assessment and were measured for body weights. Table 2 shows the energy, protein intake, distribution and requirements for Māori and non-Māori men and women. Women (1466kcal/d (IQR 1219-1755)) were more likely to consume less total energy than men (1875kcal/d (IQR 1537-2247)) ($p < 0.001$). Protein intake per day per kilogram of body weight was also significantly lower for women (58.6g/d), (0.9g/kg/d) compared to men (74.6g/d), (1.0g/kg/d) ($p \leq 0.001$). Furthermore, women were more likely to consume less protein (g/meal) than men, at breakfast (10.1g, 13.0g), lunch (14.5g, 17.8g) and dinner (23.3g, 34.2g) ($p < 0.001$), respectively. On average women and men consumed 15.5% and 15.8% of their energy from protein, respectively ($p = 0.003$). More participants met the EAR for protein intake for both women (69%) and men (67%). Māori (66% women, 72% men) were more likely to meet the AMDR for protein than non-Māori (56% women, 56% men) ($p = 0.003$).

3.3. Top Food Group Contributors to Protein Intake

Tables 3a and 3b shows consumption of adequate protein (g/kg/d) and protein intake (%) of the top 15 food groups based on EAR for women and men. Overall, the largest contributors of protein intake, for Māori and non-Māori were beef/veal, bread, and milk. The top three contributors of protein for Māori women were beef/veal (12.9%), milk (11.0%) and bread (10.5%), for non-Māori women, milk (12.2%), bread (11.3%) and beef/veal (10.0%); for Māori men, fish/seafood (11.2%), poultry (10.4%) and beef/veal (9.5%). And for non-Māori men, beef/veal (13.9%), bread (11.0%) and milk (10.0%).

3.4. Determinants of Protein Intake (g/kg/d)

Controlling for age and ethnicity, a reduced multivariate regression model was built to predict determinants of protein intake (Table 4). For Māori and non-Māori participants being a woman ($p=0.003$) and having depressive symptoms ($p=0.029$) was associated with consuming less protein.

3.5. Logistic Regression Model to Predict Meeting the EAR for Protein (g/kg/d)

Table 5 shows a logistic regression model to predict meeting the EAR for protein in grams per kilogram per day for women (≤ 0.75 g/kg/d) and men (≤ 0.86 g/kg/d), after controlling for age and ethnicity. Māori and non-Māori participants with no dentures (their own teeth) or partial dentures were more likely to meet the EAR ($p=0.036$).

Table 1. Sociodemographic, health and physical characteristics of Māori and non-Māori men and women.

	Māori			Non-Māori			P-value ¥ (ethnic group)	P value ◇ (sex)
	Women	Men	Total Māori	Women	Men	Total non-Māori		
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)		
Number	124 (57.9)	90 (42.1)	214 (37.3)	189 (52.5)	171 (47.5)	360 (62.7)		
Age (years) (Median, IQR)	84 (81-86)	82 (81-85)	83 (81-85)	86 (85-86)	86 (85-86)	86 (85-86)	P<0.001*	0.452
Has practical help available								
No	7 (6.6)	3 (4)	10 (5.5)	21 (11.5)	16 (9.5)	37 (10.5)	0.053	0.449
Yes	99 (93.4)	72 (96)	171 (94.5)	162 (88.5)	152 (90.5)	314 (89.5)		
Living alone								
No	52 (49.1)	56 (74.7)	108 (59.7)	64 (35)	106 (63.9)	170 (48.7)	0.017	P<0.001*
Yes	54 (50.9)	19 (25.3)	73 (40.3)	119 (65)	60 (36.1)	179 (51.3)		
Socio-economic deprivation (NZDep score)								
1-4 (least)	18 (14.5)	13 (14.4)	31 (14.5)	36 (19)	50 (29.2)	86 (23.9)	P<0.001*	0.016
5-7	24 (19.4)	26 (28.9)	50 (23.4)	77 (40.7)	70 (40.9)	147 (40.8)		
8-10 (most)	82 (66.1)	51 (56.7)	133 (62.1)	76 (40.2)	51 (29.8)	127 (35.3)		
Pension only income								
No	54 (57.4)	34 (51.5)	88 (55.0)	118 (69.4)	112 (74.7)	230 (71.9)	P<0.001*	0.574
Yes	40 (42.6)	32 (48.5)	72 (45.0)	52 (30.6)	38 (25.3)	90 (28.1)		
Wears dentures								
No dentures	18 (18.9)	17 (26.6)	35 (22.0)	32 (18.9)	37 (24.5)	69 (21.6)	0.903	0.011
Partial	26 (27.4)	20 (31.3)	46 (28.9)	45 (26.6)	54 (35.8)	99 (30.9)		
Full mouth	51 (53.7)	27 (42.2)	78 (49.1)	92 (54.4)	60 (39.7)	152 (47.5)		
Difficulty biting and chewing								
Never, rarely	76 (80)	52 (81.3)	128 (80.5)	137 (80.6)	127 (84.7)	264 (82.5)	0.593	0.356
Sometimes, often, always	19 (20)	12 (18.8)	31 (19.5)	33 (19.4)	23 (15.3)	56 (17.5)		
Swallowing difficulties								
Never, rarely	76 (80.9)	53 (91.4)	129 (84.9)	135 (88.8)	136 (90.1)	271 (89.4)	0.158	0.129
Sometimes, often, always	18 (19.1)	5 (8.6)	23 (15.1)	17 (11.2)	15 (9.9)	32 (10.6)		
Depression (GDS-15)								
0-4 (Not depressed)	87 (82.1)	51 (68)	138 (76.2)	149 (81.9)	146 (86.9)	295 (84.3)	0.024	0.796
5-15 (Depressed)	19 (17.9)	24 (32)	43 (23.8)	33 (18.1)	22 (13.1)	55 (15.7)		
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)		
SF-12 Physical health	45.2 (37.0, 52.5)	46.8 (32.3, 52.4)	45.6 (35.4, 52.5)	43.0 (30.3, 51.4)	46.7 (38.1, 52.3)	44.8 (34.8, 52.1)	0.548	0.093
SF -12 Mental health	55.3 (47.7, 59.0)	54.5 (45.6, 58.9)	54.7 (47.1, 59.0)	57.1 (51.6, 60.7)	56.3 (51.7, 59.2)	56.7 (51.7, 60.1)	0.024	0.325
Anthropometry- Physical assessment								
BMI (kg/m ²)	28.7 (24.0, 31.6)	28.0 (25.5, 32.1)	28.5 (24.7, 31.8)	26.5 (23.7, 30.2)	26.2 (24.1, 28.5)	26.3 (24.0, 29.3)	P<0.001*	0.992
Weight (kg)	66.9 (58.8, 79.9)	77.9 (68.7, 87.6)	71.9 (63.4, 84.5)	63.8 (57.4, 72.1)	75.1 (70.0, 82.2)	70.9 (61.9, 78.4)	0.024	P<0.001*
Grip strength (kg)	20.0 (17.4, 23.0)	29.9 (25.1, 35.0)	22.6 (18.3, 28.7)	18.4 (15.1, 21.3)	30.4 (25.6, 33.9)	22.6 (17.5, 30.0)	0.866	P<0.001*
PASE score	77 (34, 124)	96 (51, 144)	83 (47, 138)	70 (36, 112)	86 (39, 127)	75 (36, 119)	0.109	0.012
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)		
Fat mass (%)	37.6 (± 7.3)	29.5 (± 10.1)	34.4 (± 9.4)	37.9 (± 6.1)	28.0 (± 6.1)	33.0 (± 7.9)	0.100	0.806
Muscle mass (%)	59.0 (± 7.0)	67.7 (± 7.4)	62.5 (± 8.3)	58.4 (± 7.6)	68.4 (± 5.8)	63.3 (± 8.4)	0.612	0.233

Variables are from Waves 1 & 2 questionnaires. Number (percentage, %). Median (25th-75th percentiles). Mean (± SD). ¥Differences between Māori and non-Māori participants (Mann-Whitney U Test, Chi-square Test, Independent samples T-test). ◇Differences between men and women (Mann-Whitney U Test, Chi-square Test, Independent samples T-test) *P-value <0.001 considered significant.

Table 2. Energy, protein intake, distribution and requirements for Māori and non-Māori men and women.

	Women			Men			P-value ¥ (ethnic group)	P-value ◇ (sex)
	Maori	Non-Māori	Total	Maori	Non-Māori	Total		
(n, %)	124 (39.6%)	189 (60.4%)	313 (54.5%)	90 (34.5%)	171 (65.5%)	261 (45.5%)		
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)		
Energy (kcal)	1433 (1147, 1724)	1499 (1267, 1793)	1466 (1219, 1755)	1747 (1444, 2164)	1890 (1604, 2295)	1875 (1537, 2247)	0.001	P <0.001*
Protein (% energy)	16.2 (13.8, 19.8)	15.3 (13.4, 17.7)	15.5 (13.6, 18.1)	16.3 (14.6, 18.7)	15.5 (13.3, 17.8)	15.8 (13.6, 17.9)	0.003	0.709
Total protein AMDR (% energy)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)		
≤14%	42 (33.9)	84 (44.4)	126 (40.3)	26 (28.9)	76 (44.4)	102 (39.1)	0.003	0.774
15% and above	82 (66.1)	105 (55.6)	187 (59.7)	64 (71.1)	95 (55.6)	159 (60.9)		
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)		
Protein (g)	55.4 (46.2, 72.3)	59.8 (48.0, 68.7)	58.6 (47.0, 70.8)	72.9 (53.4, 93.5)	75.5 (61.9, 88.0)	74.6 (59.6, 89.7)	0.270	P <0.001*
Protein (g/kg)	0.86 (0.64-1.11)	0.90 (0.73-1.12)	0.87 (0.68-1.12)	0.97 (0.73-1.35)	0.97 (0.84-1.18)	0.97 (0.78-1.21)	0.176	0.001
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)		
Protein distribution (g/meal)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)		
Breakfast	9.7 (7.2, 14.4)	10.4 (7.0, 13.3)	10.1 (7.1, 13.7)	11.9 (8.7, 17.9)	13.1 (10.2, 17.9)	13.0 (9.4, 17.9)	0.324	P <0.001*
Lunch	14.6 (9.3, 23.4)	14.4 (10.4, 20.9)	14.5 (10.0, 21.6)	16.8 (10.7, 24.5)	18.3 (12.9, 25.1)	17.8 (12.2, 25.0)	0.381	P <0.001*
Dinner	23.3 (13.7, 30.3)	23.2 (14.3, 32.6)	23.3 (14.2, 31.4)	35.2 (20.1, 54.1)	33.5 (21.9, 45.6)	34.2 (21.0, 46.4)	0.873	P <0.001*
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)		
¹ EAR Low Protein								
Women ≤0.75g/kg	42 (36.2)	50 (27.5)	92 (30.9)	-	-	-	0.015	
Men ≤0.86g/kg	-	-	-	36 (42.9)	48 (28.6)	84 (33.3)		
¹ EAR Adequate Protein								
Women >0.75g/kg	74 (63.8)	132 (72.5)	206 (69.1)	-	-	-	0.037	
Men >0.86g/kg	-	-	-	48 (57.1)	120 (71.4)	168 (66.7)		

Variables are from Wave 2 questionnaire. Median (25th-75th percentile). Number (n) (percentage, %). ¹Low protein and Adequate protein based on Estimated Average Requirements (EAR), for over 70 years old Women 0.75g/kg/day, Men 0.86g/kg/day.

¥Differences between Māori and non-Māori participants (Mann-Whitney U Test, Chi-square Test, Independent samples T-test).

◇Differences between men and women (Mann-Whitney U Test, Chi-square Test, Independent samples T-test). *P-value <0.001 considered significant.

Table 3a. Top 15 food group intake for Māori and non-Māori women low and adequate protein intake based on the Estimated Average Requirement (EAR)

Food Groups	Māori Women				Food Groups	Non-Māori Women			
	Contribution to protein intake (%)		Protein intake (g/kg/d)			Contribution to protein intake (%)		Protein intake (g/kg/d)	
	Low protein (≤0.74g/kg/d)	Adequate protein (>0.75g/kg/d)	Low protein (≤0.74g/kg/d)	Adequate protein (>0.75g/kg/d)		Low protein (≤0.74g/kg/d)	Adequate protein (>0.75g/kg/d)	Low protein (≤0.74g/kg/d)	Adequate protein (>0.75g/kg/d)
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Beef, veal	7.3 (± 12.5)	12.9 (± 15.9)	0.07 (± 0.13)	0.13 (± 0.16)	Milk	12.7 (± 12.4)	12.2 (± 8.3)	0.13 (± 0.12)	0.12 (± 0.08)
Milk	10.1 (± 7.9)	11.0 (± 8.0)	0.10 (± 0.08)	0.11 (± 0.08)	Bread	13.5 (± 9.2)	11.3 (± 6.8)	0.14 (± 0.09)	0.11 (± 0.07)
Bread	17.4 (± 7.6)	10.5 (± 6.7)	0.17 (± 0.08)	0.11 (± 0.07)	Beef, veal	9.4 (± 11.6)	10.0 (± 12.4)	0.09 (± 0.12)	0.10 (± 0.12)
Fish, seafood	6.7 (± 17.0)	9.3 (± 12.5)	0.07 (± 0.17)	0.09 (± 0.13)	Poultry	6.0 (± 10.9)	6.6 (± 11.8)	0.06 (± 0.11)	0.07 (± 0.12)
Pork	4.9 (± 8.2)	9.0 (± 15.5)	0.05 (± 0.08)	0.09 (± 0.16)	Fish, seafood	3.5 (± 10.7)	5.5 (± 11.7)	0.03 (± 0.11)	0.06 (± 0.12)
Poultry	5.8 (± 9.7)	7.5 (± 12.3)	0.06 (± 0.10)	0.08 (± 0.12)	Pork	3.0 (± 6.2)	5.0 (± 8.1)	0.03 (± 0.06)	0.05 (± 0.08)
Cereals	4.9 (± 5.0)	4.6 (± 3.4)	0.05 (± 0.05)	0.05 (± 0.03)	Cheese	4.3 (± 7.8)	5.0 (± 6.0)	0.04 (± 0.08)	0.05 (± 0.06)
Egg, egg dishes	3.7 (± 7.0)	3.8 (± 5.6)	0.04 (± 0.07)	0.04 (± 0.06)	Cereals	4.0 (± 4.0)	5.0 (± 4.6)	0.04 (± 0.04)	0.05 (± 0.05)
Vegetables	4.5 (± 3.2)	3.7 (± 2.2)	0.05 (± 0.03)	0.04 (± 0.02)	Vegetables	4.8 (± 3.1)	4.8 (± 3.3)	0.05 (± 0.03)	0.05 (± 0.03)
Cheese	3.3 (± 7.9)	3.6 (± 5.9)	0.03 (± 0.08)	0.04 (± 0.06)	Egg, egg dishes	3.2 (± 5.9)	3.9 (± 5.8)	0.03 (± 0.06)	0.04 (± 0.06)
Potato, kumara, taro	2.5 (± 2.4)	3.0 (± 1.9)	0.03 (± 0.02)	0.03 (± 0.02)	Mutton (Sheep, lamb)	0.0 (± 0.0)	3.6 (± 9.6)	0.00 (± 0.00)	0.04 (± 0.10)
Mutton (Sheep, lamb)	1.5 (± 4.8)	2.6 (± 9.2)	0.02 (± 0.05)	0.03 (± 0.09)	Fruit	3.3 (± 2.9)	2.8 (± 2.1)	0.03 (± 0.03)	0.03 (± 0.02)
Fruit	3.0 (± 2.7)	2.0 (± 2.0)	0.03 (± 0.03)	0.02 (± 0.02)	Sausage, processed meats	2.7 (± 7.3)	2.3 (± 5.6)	0.03 (± 0.07)	0.02 (± 0.06)
Sausage, processed meats	3.0 (± 7.0)	1.7 (± 4.7)	0.03 (± 0.07)	0.02 (± 0.05)	Cakes	3.1 (± 4.3)	2.3 (± 3.3)	0.03 (± 0.04)	0.02 (± 0.03)
Non-alcoholic beverages	2.3 (± 1.7)	1.7 (± 1.4)	0.02 (± 0.02)	0.02 (± 0.01)	Potato	3.1 (± 2.5)	2.3 (± 1.8)	0.03 (± 0.02)	0.02 (± 0.02)

Table 3b. Top 15 food group intake for Māori and non-Māori men low and adequate protein intake based on the Estimated Average Requirement (EAR)

Food Groups	Māori Men				Food Groups	Non-Māori Men			
	Contribution to protein intake (%)		Protein intake (g/kg/d)			Contribution to protein intake (%)		Protein intake (g/kg/d)	
	Low protein (≤0.85g/kg/d)	Adequate protein (>0.86g/kg/d)	Low protein (≤0.85g/kg/d)	Adequate protein (>0.86g/kg/d)		Low protein (≤0.85g/kg/d)	Adequate protein (>0.86g/kg/d)	Low protein (≤0.85g/kg/d)	Adequate protein (>0.86g/kg/d)
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Fish, seafood	10.9 (± 17.0)	11.2 (± 17.5)	0.11 (± 0.17)	0.11 (± 0.18)	Beef, veal	11.1 (± 13.3)	13.9 (± 16.3)	0.11 (± 0.13)	0.14 (± 0.16)
Poultry	6.6 (± 10.7)	10.4 (± 16.9)	0.07 (± 0.11)	0.10 (± 0.17)	Bread	13.8 (± 8.3)	11.0 (± 6.2)	0.14 (± 0.08)	0.11 (± 0.06)
Beef, veal	12.0 (± 18.2)	9.5 (± 13.7)	0.12 (± 0.18)	0.10 (± 0.14)	Milk	12.6 (± 8.9)	10.0 (± 7.0)	0.13 (± 0.09)	0.10 (± 0.07)
Pork	6.8 (± 11.0)	9.0 (± 15.5)	0.07 (± 0.11)	0.09 (± 0.16)	Poultry	2.7 (± 6.8)	9.8 (± 14.9)	0.03 (± 0.07)	0.10 (± 0.15)
Bread	11.7 (± 8.5)	8.8 (± 5.7)	0.12 (± 0.08)	0.09 (± 0.06)	Fish, seafood	4.6 (± 8.2)	8.0 (± 11.8)	0.05 (± 0.08)	0.08 (± 0.12)
Milk	9.8 (± 7.4)	7.5 (± 5.8)	0.10 (± 0.07)	0.07 (± 0.06)	Cereals	6.4 (± 4.3)	5.4 (± 3.6)	0.06 (± 0.04)	0.05 (± 0.04)
Mutton (Sheep, lamb)	0.2 (± 0.7)	5.9 (± 14.7)	0.00 (± 0.01)	0.06 (± 0.15)	Pork	5.2 (± 8.1)	4.3 (± 8.9)	0.05 (± 0.08)	0.04 (± 0.09)
Cereals	5.5 (± 4.2)	4.5 (± 3.7)	0.06 (± 0.04)	0.04 (± 0.04)	Cheese	3.3 (± 5.3)	4.1 (± 5.3)	0.03 (± 0.05)	0.04 (± 0.05)
Egg, egg dishes	3.0 (± 6.4)	4.1 (± 5.1)	0.03 (± 0.06)	0.04 (± 0.05)	Vegetables	3.9 (± 4.1)	3.8 (± 2.5)	0.04 (± 0.04)	0.04 (± 0.02)
Sausage, processed meats	3.6 (± 9.9)	3.3 (± 8.2)	0.04 (± 0.10)	0.03 (± 0.08)	Egg, egg dishes	4.1 (± 7.0)	3.2 (± 4.7)	0.04 (± 0.07)	0.03 (± 0.05)
Vegetables	3.5 (± 2.4)	3.3 (± 2.6)	0.03 (± 0.02)	0.03 (± 0.03)	Potato	3.6 (± 2.5)	3.0 (± 1.9)	0.04 (± 0.02)	0.03 (± 0.02)
Potato	3.5 (± 3.3)	3.0 (± 1.8)	0.03 (± 0.03)	0.03 (± 0.02)	Mutton (Sheep, lamb)	1.0 (± 4.5)	2.8 (± 8.0)	0.01 (± 0.04)	0.03 (± 0.08)
Cheese	2.5 (± 4.4)	2.1 (± 4.1)	0.02 (± 0.04)	0.02 (± 0.04)	Cakes	1.9 (± 3.6)	2.4 (± 3.2)	0.02 (± 0.04)	0.02 (± 0.03)
Cakes	2.6 (± 4.1)	1.9 (± 2.8)	0.03 (± 0.04)	0.02 (± 0.03)	Pies, pasties	3.4 (± 8.2)	2.2 (± 5.2)	0.03 (± 0.08)	0.02 (± 0.05)
Non-alcoholic beverages	1.8 (± 1.1)	1.8 (± 2.3)	0.02 (± 0.01)	0.02 (± 0.02)	Sausage, processed meats	2.7 (± 6.9)	2.0 (± 5.3)	0.03 (± 0.07)	0.02 (± 0.05)

Table 4. Reduced multivariate regression model predicts protein intake in grams per kilogram per day

Effect	Gender	Estimate	Standard Error	Pr > [t]
Intercept		1.7720	0.8056	0.0283
Age (Years)		-0.01198	0.009609	0.2131
Gender	Men	0.09068	0.03057	0.0032
	Women	0	-	-
Ethnicity	Non-Māori	0.01965	0.03926	0.6169
	Māori	0	-	-
Depression	No	0.09467	0.04314	0.0287
	Yes	0	-	-
SF-12 Physical Health Score		0.001965	0.001450	0.1761

Reduced multivariate regression model was built to predict protein intake in g/kg/d. All variables not significant to P-value of <0.2 were removed except for age and ethnicity. Depression and being a woman were predicted to contribute to having lower protein intake in g/kg/d.

Table 5. Logistic regression model to predict meeting the EAR for protein in grams per kilogram per day

Effect		Odds Ratio Estimates			P-value
		Point Estimate	95% Wald	Confidence Limits	
Age	(one year greater)	1.079	0.943	1.234	0.2697
Gender	Men vs Women	0.759	0.484	1.191	0.2307
Ethnicity	Non-Māori vs Māori	1.360	0.764	2.418	0.2958
Practical support available	No vs Yes	1.245	0.552	2.808	0.5972
Depression	No vs Yes	1.415	0.726	2.756	0.3078
Living alone	No vs Yes	0.958	0.610	1.502	0.8505
NZ Deprivation score	1-4 vs 8-10	0.855	0.485	1.510	0.3806
	5-7 vs 8-10	1.261	0.768	2.071	
Wear dentures	No vs Full	1.335	0.763	2.336	0.0362
	Partial vs Full	1.948	1.170	3.244	
Swallowing problems	Never vs Often or always	0.835	0.245	2.855	0.8244
	Rarely vs Often or always	0.924	0.208	4.101	
	Sometimes vs Often or always	0.615	0.155	2.435	
Chewing problems	Never vs Often or always	1.835	0.684	4.927	0.5193
	Rarely vs Often or always	2.724	0.677	10.965	
	Sometimes vs Often or always	1.599	0.528	4.842	
SF-12 Physical Health Score	(one point greater)	1.009	0.988	1.032	0.4004
SF-12 Mental Health Score	(one point greater)	1.003	0.975	1.031	0.8527
Pension only income	No vs Yes	1.066	0.679	1.673	0.7818

Logistic regression model meeting protein intake based on Estimated Average Requirements (EAR) in g/kg/d. P-value of <0.2 was significant.

Discussion

4.1. Protein Intake and Adequacy

Overall, we found a third of participants (30.9% of women, 33.3% of men), had a low protein intake (median 0.9g/kg/d women, 1.0g/kg/d men) according to the EAR for older adults ≥ 70 years in Australian and New Zealand (≤ 0.75 g/kg/d women, ≤ 0.86 g/kg/d men) (NHMRC, 2006). This prevalence of low protein intake is similar to findings among adults (≥ 85 years) in the Newcastle 85+ study where 28% of participants consumed a protein intake less than the UK RDA of <0.8g/kg/d (Mendonça N. et al., 2018).

In Canada, among participants aged 67 to 84 years in the NuAge study the mean protein intake for men was 1.05g/kg/d and 1.04g/kg/d for women, suggesting most participants had an adequate protein intake based on the RDA (>0.8 g/kg/d) (Farsijani S. et al., 2017). Similarly, among Dutch community dwelling older adults (mean age 77 years) men and women were

observed to consume on average 1.07g/kg/d and 1.05g/kg/d of protein respectively and only 10% of all participants did not meet the RDA ($>0.8\text{g/kg/d}$) (Tieland M. et al., 2012).

The optimal level of protein intake remains to be determined especially for those in advanced age. Australian and New Zealand recommendations for protein are aggregated for adults over 70 years and based on data derived from nitrogen balance studies in young adult men (NHMRC, 2006). The EAR for adults >70 years was increased by 25% over that of young adults (Campbell WW. et al., 2001) without robust data on which to base that estimate. An international study group for optimal dietary protein intake in older people (PROT-AGE) and the European Society for Clinical Nutrition and Metabolism (ESPEN) suggest protein requirements need to be increased to 1.0-1.2g/kg/d for healthy adults over 65 years to overcome anabolic resistance, sarcopenia and reduce the loss of muscle mass (Bauer J. et al., 2013; Deutz NE. et al., 2014).

Using a reduced regression model adjusted for age and ethnicity we found being a woman ($p=0.003$) and the presence of depressive symptoms ($p=0.029$) predicted a lower protein intake adjusted for body weight. Conversely in the Newcastle 85+ study being a woman was associated with adequate protein intake ($\geq 0.8\text{g/kg/d}$) in adjusted models (Mendonça N. et al., 2018).

There are a few studies which suggest that protein intake is higher in men compared to women adjusted for body weight. Among Brazilian older adults aged 60 to 104 years, a higher average protein intake (1.18g/kg/d) was observed in men compared to women (1.01g/kg/d) (Gaspareto N., Previdelli AN., & Aquino RD, 2017). Similarly, among free-living Japanese older adults (≥ 70 years) women consumed significantly less protein (1.5g/kg/d) than men (1.8g/kg/d) (Watanabe R., Hanamori K., Kadoya H., Nishimuta M., & Miyazaki H, 2004). Older women are reported to consume less high quality protein across the day and eat less meat or meat products as a result of being more health conscious than men (Chernoff R, 2004). Among older women who lived alone in the UK, an exploratory study found that the need for cooking high protein foods, their often perishable nature and their high cost were barriers to eating these foods (Best RL. & Appleton KM, 2013) and this may be a contributing factor in the current study as more women lived alone than men.

Our findings observed that the presence of depressive symptoms was associated with consuming less protein and has also been observed among older men (>70 years) in South Korea; those with an inadequate protein intake were almost nine times more likely to be at risk of having depression (Park YH., Choi-Kwon S., Park KA., Suh M., & Jung YS, 2017). It has also been observed that depressive symptoms were significantly increased among those who consumed a low protein diet ($<0.8\text{g/kg/d}$) among older adults with type 2 diabetes and chronic renal disease in Italy (Ciarambino T. et al., 2012). Depression has an impact on appetite and overall food intake (Ávila-Funes JA., Gray-Donald K., & Payette H, 2008) and in the baseline assessment of LiLACS NZ we found depressive symptoms were associated with high nutrition risk for non-Māori (Wham C., Teh R., et al., 2015).

Using a logistic regression model controlled for age and ethnicity we found the oral health of the participants predicted meeting the EAR for protein for both women ($\geq 0.75\text{g/kg/d}$) and men ($\geq 0.86\text{g/kg/d}$). Participants with their own teeth or partial dentures were more likely to have an adequate protein intake than those with full dentures ($p=0.036$). Edentulism was more common in women (54%) than men (41%) which may partially account for the lower protein intake (g/kg/d) observed among women. In New Zealand removing all teeth at a young age was common practice during the early and mid-1900's and at this time New Zealand had the highest rate of edentulous people in a developed country (Sussex PV., Thomson WM., & Fitzgerald

RP, 2010). Inadequate access to dentists and lack of income for on-going dental care led to an 'edentulism epidemic' with individuals being between the ages of 15 to 40 years at the time of extraction (Sussex PV. et al., 2010). In the Newcastle 85+ study, it was observed that a higher protein intake (RDA $>0.8\text{g/kg/d}$) was significantly associated with a higher tooth count (Mendonça N. et al., 2018) which confirms the importance of oral health for eating foods which provide an adequate protein intake. Similarly, among individuals aged 74 years in Japan, those with ≥ 21 versus < 20 teeth had a significantly higher intake of total protein and animal protein (Yoshihara A., Watanabe R., Nishimuta M., Hanada N., & Miyazaki H, 2005) and in Bulgaria individuals aged 47 to 89 years with less teeth also had a significantly lower intake of protein and animal protein (meats) (Pancheva R., Konstantinova D., & Dimova-Gabrovska M, 2018).

Older adults with no teeth or ill-fitting dentures are known to have more difficulties in chewing hard textured foods such as meat (Hutton B. et al., 2002) and denture wearers have a decreased bite force and pain compared to those with natural teeth (Polzer I., Schimmel M., Müller F., & Biffar R, 2010) all of which may compromise protein intake. Our study adds to the body of evidence that the oral health status of adults in advanced age is generally deficient and surveillance and improvement of oral health in older people should be a key priority.

4.2. Protein Distribution

We observed a skewed protein distribution (g/meal), with the highest intake at dinner and the least at breakfast. For women and men respectively protein intake was at breakfast (10.1g, 13.0g), at lunch (14.5g, 17.8g) and dinner (23.3g, 34.2g). In the Newcastle 85+ study a similar skewed distribution pattern was found where participants with an adequate protein intake ($\geq 0.8\text{g/kg/d}$) consumed protein at breakfast (19.5g), lunch (34.3g) and dinner (21.9g) versus those with a low protein intake ($< 0.8\text{g/kg/d}$) had more protein earlier in the day (breakfast 26.1g; lunch 35.0g; and dinner 19.9g) (Mendonça N. et al., 2018). The latter observation may have led to earlier satiety in the day resulting in a lower protein intake at dinner (De Castro JM, 2009). A few other studies have shown an uneven protein distribution especially at the breakfast meal. In Dutch community dwelling older adults (75 to 97 years) protein intake was lowest at breakfast (9.9g), and highest at both lunch (28.0g) and dinner (27.0g) (Tieland M. et al., 2012). The NuAge study also reported that older men and women (67 to 82 years) had a lower protein intake at breakfast (16g, 11g) and similar intakes across lunch (28g, 26g) and dinner (32g, 26.5g), respectively (Farsijani S. et al., 2016). Similarly, among German community dwelling older adults (75 to 85 years) the least amount of protein was consumed at breakfast (16.5g), with slightly more at lunch (24.2g) and dinner (21.9g) (Gingrich A. et al., 2017). As protein intakes of 25 to 30g spread evenly across breakfast, lunch and dinner have been suggested to stimulate muscle protein synthesis (& Paddon-Jones D. & Rasmussen BB, 2009; Volpi E. et al., 2013) and lead to increased muscle mass retention (Farsijani S. et al., 2016) our findings suggest that a food-based approach for increasing protein intake needs to include increased protein at the breakfast meal.

4.3. Protein Sources

For participants with an adequate protein intake ($> \text{EAR}$), beef/veal, bread and milk were the top three sources of protein and sources differed between sex and ethnic groups. In those with

a low protein intake (\leq EAR) bread was the main source followed by milk and beef/veal for Māori women and non-Māori men and women. This differed for Māori men where beef/veal was the main source followed by bread and fish and seafood. For Māori, fish and seafood (kāimoana) are traditional kái (foods) and hold a strong importance in Māori well-being (Cambie RC. & Ferguson LR, 2003) especially where the marae (meeting house) is closer to the sea (Rush EC., Hsi E., Ferguson LR., Williams MH., & Simmons D, 2010). However, due to the shortage of kāimoana as a result of pollution, Māori predominantly consume a Western diet that includes food such as bread and milk that are lower in protein content as reflected in the Māori octogenarians in this study.

Our findings are consistent with the NZANS survey (2008/09) which observed the largest food contributors of protein were bread (14.3%, 14.2%), followed by milk (10.8%, 11.5%) and beef/veal (10.1%, 9.3%) in both men and women aged over 70 years, respectively (University of Otago. & Ministry of Health, 2011).

4.4. Strength and Limitations

Our study is the first to report the determinants of protein intake among Māori and non-Māori octogenarians in New Zealand using a repeat 24-hour multiple pass recall which provides the best available method in this age group (Adamson AJ. et al., 2009). Māori participants were younger with a higher BMI than non-Māori and culturally different food sources of protein were evident. Misreporting and underreporting may have occurred however protein rich foods are usually not understated compared to food items with a negative health image (e.g. cakes, sweets, confectionery) (Macdiarmid J. & Blundell J, 1998). Detailed comparisons between studies may be hindered by different dietary assessment methods and participant characteristics such as geographic location, age, body composition as well as health and nutritional status.

Conclusion

This study shows that a third of the participants did not meet the EAR for adequate protein intake. We observed an uneven mealtime protein distribution pattern and food group contributors to protein intake differed by gender and ethnicity. The odds of consuming an adequate protein intake were higher in participants with their own teeth or partial dentures (adjusted models). Our findings highlight the prevalence and determinants of low protein intake among Māori and non-Māori of advanced age which may help to inform preventative interventions to improve the nutritional health of the oldest old. Our findings may help inform future interventions and food-based strategies such as increasing protein intake at breakfast may improve adequacy and distribution in this population.

4. Conclusions and Recommendations

This study was the first to determine the intakes, distribution, and sources of adequate and inadequate protein in Māori and non-Māori octogenarians. Dietary protein is important to overcome anabolic resistance, sarcopenia and reduce the loss of muscle mass, strength and physical function in the very old (Houston DK. et al., 2008). It is essential to understand the current protein intakes, distribution pattern and their sources to optimise nutritional intake and create recommendations for those in advanced age.

Overall, inadequate protein intakes were observed in 30.9% of women (0.9g/kg/d) and 33.3% of men (1.0g/kg/d) using EAR cut-offs for older adults ≥ 70 years in New Zealand (≤ 0.75 g/kg/d for women; ≤ 0.86 g/kg/d for men) (NHMRC, 2006). The Newcastle 85+ study also found 28% of the oldest old had an inadequate protein intake (\leq RDA 0.8g/kg/d) (Mendonça N. et al., 2018). It is well-known that as adults get older their protein intake may decrease as a result of an overall decrease in food intake. Currently, expert nutrition groups support the idea of increasing the EAR for adults in advanced age as those that meet the current cut-off points for an adequate protein intake may still show signs of sarcopenia, muscle wasting and reduced physical function.

Our study showed that low protein intake was associated with being a woman and showing signs of depressive symptoms. As depression is associated with weight loss in older adults screening for malnutrition in the presence of depressive symptoms may be prudent to identify nutrition risk and refer to a dietitian for assessment and intervention. Participants with their own teeth (no dentures) or who wore partial dentures were more likely to have an adequate protein intake (meet the EAR) compared to those who wore full dentures. It is important for older adults to seek on-going dental care particularly when their dentures may cause them pain or discomfort and may not fit well.

We also found the distribution of dietary protein intake across mealtimes was skewed, with the least amount of protein consumed at breakfast (10.1g women; 13.0g men) and almost a third of the daily protein intake consumed at dinner (23.3g women; 34.2g men). This finding is consistent with the Newcastle 85+ study that observed a skewed distribution in their participants with the breakfast meal (19.5g) containing the least amount of protein compared to lunch (34.3g) and dinner (21.9g) (Mendonça N. et al., 2018). A skewed intake of protein may result in sub-optimal protein synthesis and therefore may compromise the efficiency for older adults to maintain or increase their muscle mass, strength, and physical function.

Recommendations to increase protein intake at the breakfast meal are needed and few studies have investigated the benefits and ways of including eggs into the diet. To increase egg consumption, it is important to reduce negative assumptions of eggs, provide easy to follow recipes to show how eggs can be cooked in a variety of ways and encourage older adults to add eggs to existing meals (van den Heuvel E., Murphy J.L., & Appleton KM, 2018; Van den Heuvel E., Murphy J.L., & Appleton KM, 2018b).

Main sources of protein for participants with an adequate intake (meeting the EAR) were beef and veal for Māori women and non-Māori men, milk for non-Māori women and fish and seafood for Māori men. The second and third key sources of protein were milk, bread and beef/veal which differed between gender and ethnicity whereas, poultry was the second main source for Māori men. Bread was the main source of protein in participants who had an inadequate protein intake whereas beef/veal was the main source of protein for Māori men.

Interestingly, regardless of whether participants had a low or adequate protein intake, they derived most of their protein from milk, bread, and beef/veal.

Encouraging older adults to plan their meals ahead of time and use inexpensive, high protein food sources like eggs may be a good strategy to consume more protein at breakfast. Research regarding protein intake among octogenarians is limited and this is the first time research on protein distribution patterns and food sources have been reported in Māori of advanced age. Findings from our study adds to the body of knowledge of protein needs in advanced age and may help highlight the need for updated food and nutrition guidelines for the very old.

In conclusion, protein intake was inadequate for a third of our participants. Recommendations for older adults to increase their protein intake include eating high quality protein foods at each meal. A good strategy is to ensure a quarter of the plate has a protein food such as eggs, poultry, fish, meat, or meat products at each mealtime. Older adults should be encouraged to take the opportunity to eat meals with others to increase food intake and meal enjoyment. The oral health of adults in advanced age is a concern and strategies are needed to encourage older adults to visit the dentist regularly to prevent further deterioration of oral health and hygiene.

4.1. Strengths

This study has been the first observation of the prevalence of adequate (and inadequate) protein intake, protein distribution and protein food sources in Māori and non-Māori octogenarians. Our findings may have real life implications that can be applied to the oldest old in New Zealand as findings from LiLACS NZ provides a sound representation of Māori and non-Māori octogenarians.

Evaluating protein intake in Māori of advanced age provides knowledge of protein inadequacies, distribution patterns and food sources. Recommendations are needed to incorporate traditional Māori foods as these are culturally important to this population.

The dietary assessment methods used in this study have previously been validated for use among advanced aged older adults in the Newcastle 85+ study and the same protocols were adopted for LiLACS NZ. The Photographic Atlas of Food Portion Sizes, used to identify the quantity of food participants consumed, was adapted for the New Zealand diet (with inclusion of fish, seafood, and traditional Māori foods) therefore sources of protein were accurately identified and recorded.

4.2. Limitations

Misreporting and underreporting are common when recording dietary intake, however protein intake is less likely to be underreported compared to confectionery and high carbohydrate foods due to the negative perception of these foods.

When completing the 24h multiple pass recall, if a participant was unable to report what they ate then a caregiver or carer was able to report intake which may increase the likelihood of inaccuracies. Also, older adults may not have full control over the purchasing of foods and preparing of meals therefore small inaccuracies may occur when reporting the amount of ingredients used and type or brand of the product.

4.3. Recommendations

To increase total protein intake

- To encourage older adults to eat meals with peers and attend social gatherings to eat with others (Clegg ME. & Williams EA, 2018).
- Traditional Māori foods such as fish and seafood should be encouraged when promoting higher protein intake in Māori as consumption of traditional foods may also enhance overall well-being (Rush EC. et al., 2010).

To improve the distribution of protein throughout the day

- Introduce eggs in the breakfast meal to increase protein intake, as eggs are convenient, versatile and is a high-quality source of protein (Mamerow MM. et al., 2014).
- Eggs should be promoted as an everyday food. Negative perceptions of eggs being linked to health problems (high cholesterol) need to be addressed (van den Heuvel E., Murphy JL., & Appleton KM, 2018a) among older adults to reduce barriers to their consumption.

To improve protein intake of animal versus plant sources

- To encourage older adults to consume at least one serving of an animal protein food at each meal as animal proteins have a higher biological value than plant proteins.

Improve factors associated with protein intake

- It is important to identify older adults who show signs of depressive symptoms that may include, losing interest in an activity that previously was an interest, avoiding social settings, have poor appetite and eating less than usual. A malnutrition screening tool may be useful for the very old to determine whether they are at risk of malnutrition and eligible for meal support initiatives.
- The oral health of adults in advanced age is important to ensure that they can eat hard to chew foods such as meat and meat products. Regular dental visits may reduce dental caries, poor oral health and ill-fitting dentures and can identify those most at risk and in need of early treatment (CBG Health Research, 2015).

Future research

Further investigations are needed in large sample sizes of advanced aged adults to determine health outcomes associated with low protein intake to inform recommendations for adequate protein intake.

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6. Appendices

Appendix A: Supplementary results

Appendix B: Journal requirements: *Nutrients*

Appendix A: Supplementary results

Table 2. Energy, protein intake, distribution and requirements for Māori and non-Māori men and women.

² RDI Low Protein							
Women ≤0.94g/kg	70 (60.3)	101 (55.5)	171 (57.4)	-	-	-	0.310
Men ≤1.07g/kg	-	-	-	54 (64.3)	106 (63.1)	160 (63.5)	
² RDI Adequate Protein							
Women >0.94g/kg	46 (39.7)	81 (44.5)	127 (42.6)	-	-	-	0.692
Men >1.07g/kg	-	-	-	30 (35.7)	62 (36.9)	92 (36.5)	

²Low protein and Adequate protein based on Estimated Average Requirements (EAR), for over 70 years old Women 0.75g/kg/day, Men 0.86g/kg/day.

Table 3c. Food group intake for Māori and non-Māori women low and adequate protein intake based on the Recommended Daily Intake (RDI)

Māori Women				
Food Groups	Contribution to protein intake (%)		Protein intake (g/kg/d)	
	Low protein (≤0.93g/kg/d)	Adequate protein (>0.94g/kg/d)	Low Protein (≤0.93g/kg/d)	Adequate Protein (>0.94g/kg/d)
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Milk	10.2 (± 8.0)	11.4 (± 8.0)	0.10 (± 0.08)	0.11 (± 0.08)
Beef, veal	10.7 (± 14.7)	11.2 (± 15.5)	0.11 (± 0.15)	0.11 (± 0.16)
Bread	15.0 (± 7.8)	10.0 (± 6.7)	0.15 (± 0.08)	0.10 (± 0.07)
Poultry	5.0 (± 9.1)	9.8 (± 13.8)	0.05 (± 0.09)	0.10 (± 0.14)
Fish, seafood	7.4 (± 14.9)	9.8 (± 13.4)	0.07 (± 0.15)	0.10 (± 0.13)
Pork	6.6 (± 12.0)	8.8 (± 15.5)	0.07 (± 0.12)	0.09 (± 0.15)
Cereals	4.8 (± 4.5)	4.5 (± 3.3)	0.05 (± 0.04)	0.04 (± 0.03)
Egg, egg dishes	3.7 (± 6.5)	3.9 (± 5.5)	0.04 (± 0.07)	0.04 (± 0.06)
Vegetables	4.3 (± 2.8)	3.6 (± 2.3)	0.04 (± 0.03)	0.04 (± 0.02)
Mutton (Sheep, lamb)	1.5 (± 5.1)	3.3 (± 10.8)	0.01 (± 0.05)	0.03 (± 0.11)
Cheese	3.9 (± 7.8)	2.8 (± 4.4)	0.04 (± 0.08)	0.03 (± 0.04)
Potato, kumara, taro	2.9 (± 2.2)	2.7 (± 2.1)	0.03 (± 0.02)	0.03 (± 0.02)
Sausage, processed meats	2.2 (± 5.8)	2.0 (± 5.5)	0.02 (± 0.06)	0.02 (± 0.05)
Soups	1.7 (± 3.8)	2.0 (± 3.9)	0.02 (± 0.04)	0.02 (± 0.04)
Fruit	2.7 (± 2.6)	1.9 (± 1.6)	0.03 (± 0.03)	0.02 (± 0.02)
Non-Maori Women				
Food Groups	Contribution to protein intake (%)		Protein intake (g/kg/d)	
	Low protein (≤0.93g/kg/d)	Adequate protein (>0.94g/kg/d)	Low protein (≤0.93g/kg/d)	Adequate protein (>0.94g/kg/d)
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Milk	11.9 (± 10.3)	12.8 (± 8.7)	0.12 (± 0.10)	0.13 (± 0.09)
Bread	12.6 (± 8.2)	11.0 (± 6.7)	0.13 (± 0.08)	0.11 (± 0.07)
Beef, veal	10.0 (± 12.3)	9.7 (± 12.0)	0.10 (± 0.12)	0.10 (± 0.12)
Poultry	6.3 (± 11.6)	6.6 (± 11.6)	0.06 (± 0.12)	0.07 (± 0.12)
Fish, seafood	3.7 (± 9.8)	6.5 (± 13.1)	0.04 (± 0.10)	0.07 (± 0.13)
Pork	3.3 (± 5.9)	5.9 (± 9.3)	0.03 (± 0.06)	0.06 (± 0.09)
Cheese	4.5 (± 6.9)	5.2 (± 6.1)	0.05 (± 0.07)	0.05 (± 0.06)
Vegetables	5.1 (± 3.6)	4.5 (± 2.6)	0.05 (± 0.04)	0.05 (± 0.03)
Cereals	5.2 (± 5.0)	4.1 (± 3.6)	0.05 (± 0.05)	0.04 (± 0.04)
Egg, egg dishes	3.7 (± 5.8)	3.7 (± 5.8)	0.04 (± 0.06)	0.04 (± 0.06)
Mutton (Sheep, lamb)	1.9 (± 8.1)	3.5 (± 8.7)	0.02 (± 0.08)	0.04 (± 0.09)
Fruit	3.1 (± 2.5)	2.7 (± 2.2)	0.03 (± 0.02)	0.03 (± 0.02)
Cakes	2.5 (± 3.9)	2.5 (± 3.1)	0.03 (± 0.04)	0.02 (± 0.03)
Potato, kumara, taro	2.6 (± 2.1)	2.4 (± 1.9)	0.03 (± 0.02)	0.02 (± 0.02)
Sausage, processed meats	2.4 (± 6.2)	2.3 (± 6.1)	0.02 (± 0.06)	0.02 (± 0.06)

Table 3d. Food group intake for Māori and non-Māori men low and adequate protein intake based on the Recommended Daily Intake (RDI)

Māori Men				
Food Groups	Contribution to protein intake (%)		Protein intake (g/kg/d)	
	Low protein (≤1.07g/kg/d)	Adequate protein (>1.07g/kg/d)	Low Protein (≤1.07g/kg/d)	Adequate Protein (>1.07g/kg/d)
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Poultry	5.9 (± 10.5)	14.0 (± 19.1)	0.06 (± 0.11)	0.14 (± 0.19)
Fish, seafood	11.1 (± 16.3)	11.0 (± 19.0)	0.11 (± 0.16)	0.11 (± 0.19)
Beef, veal	11.6 (± 17.0)	8.7 (± 13.4)	0.12 (± 0.17)	0.09 (± 0.13)
Bread	10.8 (± 7.8)	8.7 (± 5.6)	0.11 (± 0.08)	0.09 (± 0.06)
Mutton (Sheep, lamb)	0.7 (± 3.0)	8.3 (± 17.9)	0.01 (± 0.03)	0.08 (± 0.18)
Milk	8.9 (± 6.7)	7.7 (± 6.4)	0.09 (± 0.07)	0.08 (± 0.06)
Pork	8.3 (± 13.1)	7.6 (± 15.0)	0.08 (± 0.13)	0.08 (± 0.15)
Egg, egg dishes	3.4 (± 6.0)	4.1 (± 5.0)	0.03 (± 0.06)	0.04 (± 0.05)
Cereals	5.5 (± 4.0)	3.9 (± 3.7)	0.05 (± 0.04)	0.04 (± 0.04)
Vegetables	3.4 (± 2.4)	3.3 (± 2.7)	0.03 (± 0.02)	0.03 (± 0.03)
Potato, kumara, taro	3.4 (± 2.9)	2.8 (± 1.7)	0.03 (± 0.03)	0.03 (± 0.02)
Other Meats	0.9 (± 4.5)	2.0 (± 8.1)	0.01 (± 0.04)	0.02 (± 0.08)
Dairy products	1.7 (± 2.7)	1.8 (± 4.5)	0.02 (± 0.03)	0.02 (± 0.04)
Sausage, processed meats	4.4 (± 10.6)	1.7 (± 4.3)	0.04 (± 0.11)	0.02 (± 0.04)
Cheese	2.6 (± 4.8)	1.7 (± 2.9)	0.03 (± 0.05)	0.02 (± 0.03)
Non-Māori Men				
Food Groups	Contribution to protein intake (%)		Protein intake (g/kg/d)	
	Low protein (≤1.07g/kg/d)	Adequate protein (>1.07g/kg/d)	Low protein (≤1.07g/kg/d)	Adequate protein (>1.07g/kg/d)
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Beef, veal	12.7 (± 15.3)	13.7 (± 16.1)	0.13 (± 0.15)	0.14 (± 0.16)
Poultry	5.1 (± 9.9)	12.3 (± 17.2)	0.05 (± 0.10)	0.12 (± 0.17)
Bread	12.6 (± 7.6)	10.3 (± 5.4)	0.13 (± 0.08)	0.10 (± 0.05)
Milk	11.3 (8.3)	9.8 (± 6.3)	0.11 (± 0.08)	0.10 (± 0.06)
Fish, seafood	6.1 (± 10.5)	8.6 (± 11.7)	0.06 (± 0.11)	0.09 (± 0.12)
Cereals	6.1 (± 4.2)	5.0 (± 3.1)	0.06 (± 0.04)	0.05 (± 0.03)
Pork	4.6 (± 7.7)	4.4 (± 10.2)	0.05 (± 0.08)	0.04 (± 0.10)
Egg, egg dishes	3.5 (± 5.7)	3.5 (± 5.1)	0.03 (± 0.06)	0.03 (± 0.05)
Cheese	4.1 (± 5.7)	3.5 (± 4.6)	0.04 (± 0.06)	0.03 (± 0.05)
Vegetables	4.0 (± 3.5)	3.5 (± 1.8)	0.04 (± 0.04)	0.04 (± 0.02)
Mutton (Sheep, lamb)	1.8 (± 6.4)	3.1 (± 8.5)	0.02 (± 0.06)	0.03 (± 0.08)
Potato, kumara, taro	3.3 (± 2.3)	3.0 (± 1.7)	0.03 (± 0.02)	0.03 (± 0.02)
Pies, pasties	2.7 (± 6.4)	2.3 (± 5.7)	0.03 (± 0.06)	0.02 (± 0.06)
Cakes	2.3 (± 3.5)	2.3 (± 2.9)	0.02 (± 0.04)	0.02 (± 0.03)
Sausage, processed meats	2.3 (± 6.1)	2.0 (± 5.2)	0.02 (± 0.06)	0.02 (± 0.05)

Table 6. Multivariable model predicting protein intake in gram per kilogram per day

Effect		Estimate	Standard Error	Pr > [t]
Intercept		1.3049	0.9314	0.1619
Age (years)		-0.00713	0.01091	0.5135
Gender	Men	0.09278	0.03550	0.0093
	Women	0	-	-
Ethnicity	Non- Māori	0.02016	0.04571	0.6595
	Māori	0	-	-
Practical support available	No	-0.03713	0.06044	0.5393
	Yes	0	-	-
Depression	No	0.07475	0.05470	0.1725
	Yes	0	-	-
Living Alone	No	-0.02979	0.03550	0.4019
	Yes	0	-	-
NZ Deprivation Score	1-4	-0.03952	0.04570	0.3877
	5-7	0.03008	0.03846	0.4346
	8-10	0	-	-
Wears dentures	No	0.01821	0.04428	0.6811
	Partial	0.05053	0.03840	0.1890
	Full	0	-	-
Swallowing problems	Never	0.04523	0.09597	0.6377
	Rarely	0.1078	0.1172	0.3586
	Sometimes	-0.04932	0.1089	0.6510
	Often or always	0	-	-
Chewing problems	Never	0.01013	0.08322	0.9031
	Rarely	0.1468	0.1084	0.1765
	Sometimes	0.04855	0.09260	0.6003
	Often or always	0	-	-
SF-12 Physical Health score		0.002029	0.001723	0.2398
SF-12 Mental Health score		0.000504	0.002255	0.8231
Pension only income	No	-0.01790	0.03572	0.6164
	Yes	0	-	-

Multivariate regression model was built to predict protein intake in g/kg/d. All variables not significant to P-value of <0.2 were removed except for age and ethnicity.

Table 7. Logistic regression model to predict meeting the RDI for protein in grams per kilogram per day

Odds Ratio Estimates					
Effect		Point Estimate	95% Wald Confidence Limits		P-value
Age	(One Year greater)	0.952	0.834	1.087	0.4665
Gender	Men vs Women	2.321	1.522	3.542	<0.0001
Ethnicity	Non- Māori vs Māori	1.344	0.767	2.355	0.3011
Practical support available	No vs Yes	0.556	0.258	1.199	0.1344
Depression	No vs Yes	0.951	0.489	1.851	0.8820
Living alone	No vs Yes	0.947	0.617	1.453	0.8029
NZ Deprivation scores	1-4 vs 8-10	1.314	0.760	2.272	0.4105
	5-7 vs 8-10	1.345	0.847	2.138	
Wear dentures	No vs Full	1.283	0.755	2.179	0.1483
	Partial vs Full	1.570	0.994	2.479	
Swallowing problems	Never vs Often or always	1.084	0.335	3.503	0.6537
	Rarely vs Often or always	1.009	0.242	4.210	
	Sometimes vs Often or always	0.675	0.176	2.583	
Chewing problems	Never vs Often or always	0.632	0.236	1.692	0.0844
	Rarely vs Often or always	2.076	0.559	7.719	
	Sometimes vs Often or always	0.703	0.234	2.108	
SF-12 Physical Health Score	(one point greater)	1.015	0.994	1.037	0.1521
SF-12 Mental Health Score	(one point greater)	1.005	0.977	1.033	0.7431
Pension only income	No vs Yes	0.789	0.514	1.212	0.2790

Logistic regression model meeting protein intake based on Recommended Daily Intake (RDI) in g/kg/d. A P-value of <0.2 was significant.

Appendix B: Journal requirements: *Nutrients*

See <http://www.mdpi.com/journal/nutrients/instructions> for more details.

Manuscript Submission Overview

Types of Publications

Nutrients has no restrictions on the length of manuscripts, provided that the text is concise and comprehensive. Full experimental details must be provided so that the results can be reproduced. *Nutrients* requires that authors publish all experimental controls and make full datasets available where possible (please read the guidelines about Supplementary Materials and references to unpublished data carefully).

Manuscripts submitted to *Nutrients* should neither been published before nor be under consideration for publication in another journal. The main article types are as follows:

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Back Matter

Supplementary Materials: Describe any supplementary material published online alongside the manuscript (figure, tables, video, spreadsheets, etc.). Please indicate the name and title of each element as follows Figure S1: title, Table S1: title, etc.

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