Dietary intake and food sources of protein among adolescent females aged 15-18 years living in New Zealand

Brittany Leah Cradock

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Abstract

Background: Adolescence is a life stage characterised by rapid physical growth and development. These rapid changes increase the requirement for protein and energy (1). Protein has a functional and structural role in the body where it makes up a large proportion of skeletal muscle and many other body cells. Protein is, therefore, an important macronutrient during this period of considerable growth. Recent data regarding the dietary protein intake and major protein food sources among this population group is scarce. The aim of this thesis, is to investigate the dietary intake and major food sources of protein for adolescent females aged 15-18 years of age living in New Zealand.

Design: This cross-sectional, clustered study collected information regarding demographics (age, sex, and ethnicity) and nutritional supplement use via online questionnaires. Dietary intakes of all food and beverages were assessed by two non-consecutive 24-hour diet recalls. Data from these diet recalls were entered into FoodWorks and were matched to nutrient lines from the 2016 New Zealand FOODfiles to calculate dietary protein intake. Usual dietary protein intake was calculated using the multiple source method. Height and weight was measured using standard protocols and used to calculate body mass index z-scores.

Results: Data was available for 145 adolescent females aged 15-18 years, who were enrolled in one of the eight secondary schools visited throughout New Zealand. The mean daily intake of protein for adolescent females aged 15-18 years was 73.7g (95% CI 70.1, 77.3). The mean intake of protein expressed per kilogram of body weight per day was 1.2g/kg/d (95% CI 1.1,1.2). Both the mean daily intake and g/kg/d were adequate in meeting the EAR of 45g/d and 0.62g/kg/day respectively. Eleven (8.5%) participants had

an inadequate intake of protein defined as <0.62g/kg/d. Protein intake contributed on average 15 % (95% CI 14.9,16.0) of the total energy intake, which was at the lower end of the acceptable macronutrient distribution range (15-25%). No significant association was observed between protein intake and ethnicity; however, the prevalence of inadequate protein intakes was significantly greater in participants who were classified as overweight or obese. Poultry was the single largest contributor of dietary protein (12.5%), followed by, bread-based dishes (9.7%), bread (8.5%) and grains and pasta (8.4%).

Conclusion: These findings show that the usual dietary protein intakes of healthy adolescent females aged 15-18 years living in New Zealand are adequate. This, along with the confirmation that both animal and plant-based foods contribute to protein intake in this population, will assist the review of dietary guidelines and recommendations for this age group. Further work is required to confirm the preliminary finding that overweight and obese adolescent females are more likely to have inadequate protein intakes.

Preface

The SuNDiAL (Survey of Nutrition, Dietary Assessment and Lifestyle) project is a multi-center study involving secondary schools across New Zealand. The aim of the SuNDiAL project is to investigate the nutritional intakes, dietary habits, health status and attitudes and motivations for food choices in vegetarian and non-vegetarian New Zealand adolescent females. The results of this study will help to inform and update national dietary guidelines for adolescent females. Data was collected by second year Master of Dietetics students from the University of Otago, based in several locations throughout New Zealand.

Multiple phases of data collection will occur within the SuNDiAL project. This thesis will examine the data obtained from phase one of the project (February – April 2019).
This study has been conducted through the Department of Human Nutrition, University of Otago, Dunedin, New Zealand. The primary investigators, Jill Hazsard and Meredith Peddie, were responsible for the SuNDiAL project study design and ethical approval. Jill Hazsard provided her expertise with statistical analysis of the data collected during phase one. The project was funded by the Department of Human Nutrition and Lottery Health Research Grant.

The candidate was responsible for the following:

- Researching and developing the literature review.
- Preparing and delivering a presentation which outlined the study details to the target population.

- Recruitment of 19 female participants aged 15-18 years at one of the eight phase one secondary schools.
- Data collection, including:
    - Providing details to the primary investigators to provide participants with online questionnaires regarding demographic and dietary habits, attitudes and motivations*.
    - Development of a booking system to allow participants to book an appointment with the data collectors.
    - Conducted anthropometric measurements such as standing height, bodyweight and ulna length*.
    - 24-hour dietary recalls.
    - Fitting accelerometers and providing participants with log-books*.
    - Liaising with local laboratory staff to arrange a date for the phlebotomist to collect blood samples*.
    - Booking participants who consented for blood and urine samples to be taken.
    - Setting up a clinic where urine and blood samples were collected.
    - Collection of accelerometers and log-books after seven days.
    - Conducted second 24-hour recalls via a phone call for 19 participants.

      **these measures were conducted by the candidate as part of the larger SuNDiAL study but are not reported in this thesis.*
- Data entry of both 24-hour recalls to FoodWorks. This included developing recipes for and finding substitutions for foods that were not entered in the database.

- Provided anthropometric data and accelerometers to the primary investigators for data analysis.
- Statistical analysis related to the thesis topic.
- Interpretation and discussion of results.
- Write up of all thesis components.

Acknowledgements

I never imagined myself going to University let alone studying for five years and completing my masters. To say I am proud of myself is an understatement. This journey could not have been made possible without the help of many people, whether it be help with an assignment, a shoulder to cry on or even going for a coffee which turned into a two hour “study break”. It’s these little things along the way which has made it possible for me to get to where I am.

I want to thank Sue MacDonell, my supervisor. I could not have written this thesis without your constant support, expertise and words of encouragement. When Wednesday 9am rolled around those weekly check ins were much needed and appreciated.

My fellow classmates of Dietetics. Looking back on the last year and a half we have really been on a rollercoaster of a journey together. No two days were the same and through the good and challenging times we were always there to support one another. I don’t know where I would have been without half of you. I look forward to the future and being able to work alongside you.

Mum and Dad – I am forever grateful for the constant love and support you have given me over the past 5 years. Being in opposite ends of the country hasn’t been easy but the phone calls home with excitement and tears have all been worth it. My precious brother Liam, you are the reason I have carried on with this degree and I am forever grateful knowing you are watching over me every step of the way.

Matt, thank you for being so understanding during the stressful times and celebrating my successes through the good times. Your words of encouragement were appreciated aswell as the tough love you threw in every now and then.

Finally, my friends. I have been so fortunate to meet so many incredible people during my time at Otago. I have never been around so many strangers who many I now call close friends. I have never been through so many highs and lows with so many people but no matter what I have always felt loved and supported from everyone I have been around.

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List of Abbreviations

95% CI	95% Confidence interval
AMDR	Acceptable macronutrient distribution range
BMI	Body Mass Index
EAR	Estimated average requirement
FFQ	Food frequency questionnaire
g/d	Grams per day
g/kg/d	Grams per kilogram per day
IOM	Institute of Medicine
JH	Jill Hazsard
MDiet	Master of Dietetics
MP	Meredith Peddie
MSM	Multiple score method
NRV	Nutrient reference value
n	number from the population
NZEO	New Zealand European and other
PEM	Protein-energy malnutrition
SES	Socio-economic status
SuNDiAL	Survey of Nutrition Dietary Assessment and Lifestyle
UL	Upper level
USA	United States of America
WHO	World Health Organization

1 Introduction

Adolescence is a vulnerable stage of life due to the increased nutrient demands which are required for growth and development. Protein has both a functional and structural role in the body, making up a large proportion of skeletal muscle and other body cells (2). Optimal protein intake in adolescent females is, therefore, essential to help support these accelerated changes.

Protein is the key structural and functional component of many body cells and consists of amino acids which also have specific functional roles. The essential amino acids, are those that the body cannot synthesis, must be obtained in optimal amounts from our diet. However, little is known about the current dietary protein intakes among New Zealand adolescent females aged 15-18 years, nor about the common food sources which contribute to the total protein intake. Further research regarding protein intake among adolescent females is needed to provide health professionals with scientific evidence on which they can base their advice and subsequently ensure that adolescent females are meeting their protein requirements regardless of the dietary patterns they choose to follow.

Therefore, the aim of this thesis is to investigate the dietary protein intake of adolescent females aged 15-18 years of age living in New Zealand, by assessing the actual protein intake and comparing these to the recommendations to assess the adequacy of their diets. In addition, the common food sources which contribute to total protein intake will be investigated.

2 Literature Review

Protein is the major functional and structural component of skeletal muscle and of many body cells. Enzymes, transport molecules, serum albumin, keratin, and collagen are all proteins that are necessary for optimal body function (2). Proteins consist of multiple amino acids which also act as precursors for many coenzymes, hormones and nucleic acids (2). Amino acids, therefore, display metabolic and regulatory versatility based on these physiological functions (3). Adequate dietary protein intake is required during adolescence to support the accelerated growth and development that is a characteristic of this life stage.

This literature review, therefore, provides an overview of the functional roles, dietary requirements and food sources of protein with a focus on those that are important for adolescent females. Methods of assessing dietary protein intake will also be examined.

Literature was obtained from multiple searches of the following databases; PUBMED, and Scopus. The key search words used were; adolescence, protein intake, dietary protein, protein sources, dietary assessment methods, nutrient reference values. Additional literature was sourced from supervisor's archives, as well as from the reference list of the literature reviewed.

2.1 Functional role of Protein

2.1.1 Growth and development:

During the adolescent years, there is an increased demand for nutrients to assist with the rapid growth and development (4, 5). Inadequate dietary protein intake during adolescence has the potential to negatively impact the attainment of optimal height, weight and overall body protein (6, 7). Bone mass increases substantially during the first two decades of life before reaching a plateau in the late teens or young adult years (8, 9). The rate of bone loss in the general adult population has been inversely associated with dietary protein intake, suggesting that higher protein intakes can attenuate bone loss and optimise peak bone mass (10). Furthermore, this study suggests that the skeleton is particularly responsive to adequate dietary protein intakes in the years preceding the onset of pubertal maturation. Emphasising that adequate protein intake is important to ensure the acquisition of peak bone mass (11).

In addition, when energy intake is insufficient to meet demands, dietary protein may be used to meet energy needs. Amino acids are the products of protein digestion and can be used as a substrate for glucose synthesis via gluconeogenesis (12) when carbohydrate sources are depleted. Skeletal muscle is a major source of energy in these conditions due to its large reservoir in the body, however, proteins from all other tissues are also utilized for energy (12). Thus, making protein unavailable for the synthesis of new tissue or tissue repair (4) thereby resulting in a reduction of growth.

2.1.2 Reproduction and Hormones:

With the onset of puberty and menarche, adolescent females are at childbearing age and require adequate energy and protein intakes to optimize reproduction (13). Levels of adrenal hormones, particularly serum estradiol are highest among pubertal females. Inadequate dietary protein will negatively affect hormone synthesis and therefore delay or inhibit sexual maturation (13).

2.1.3 Immune function

Optimal immune function and protection from disease are acquired through adequate dietary protein. Dietary protein provides the substrate for the synthesis of a variety of specific proteins and amino acids that help to regulate key metabolic pathways of the immune response to infectious pathogens (3). For instance, essential amino acids obtained from dietary protein play an important role in the immune response by regulating (1) the activation of T lymphocytes, natural killer cells, and macrophages; (2) cellular redox expression and (3) the production of antibodies and cytokines (14). Adolescence is a vulnerable period of life where choosing to follow certain diets or restricting food intake for weight loss purposes is very common. Displacement of key nutrients such as protein, occurs as dietary intakes are reduced and key food sources are being eliminated or avoided. A lower intake of protein or consumption of low-quality protein can cause adolescents to be more susceptible to infection and inflammation (5, 15). Adequate dietary protein intake which meets an individual's requirements is important to prevent immune dysfunction (15).

2.2 Protein Requirements of adolescent females

2.2.1 Nutrient Reference Values

The Nutrient Reference Values (NRVS) for Australia and New Zealand, describe the required amount of specific nutrients which should be consumed by various population groups to support good health (16). Recommendations are derived to prevent the development of nutrient deficiencies and excessive intakes (16). Current recommendations for protein intake for a normal healthy adolescent female aged 15-18 is 0.77g/kg/d of good quality, highly digestible protein to maintain positive nitrogen balance (16, 17).

Table 2.1 Nutrient Reference Values for protein intake in New Zealand females aged 14-18 years (16).

Nutrient Reference Values	Definition	Protein
Estimated Average Requirement (EAR)	The EAR is the average daily nutrient intake estimated to meet the requirements of 50% of healthy individuals	35g/day (0.62g/kg)
Recommended Daily Intake (RDI)	The RDI represents daily average dietary intake that meets the requirements for nearly 97.5% of healthy individuals in a life stage	45g/day (0.77g/kg)

There is limited data available for setting the EARs of protein for children and adolescents. Consequently, expert working groups have determined the amount of protein required for growth and maintenance in these age groups using the factorial method (16, 18).

The factorial method estimates protein requirements for adolescents by extrapolating data from adult protein requirements (18).

Despite the factorial method being used by most countries to determine protein requirements, the EAR for adolescent females does differ between countries. This variation is due to how the factorial method is used. In New Zealand, the EAR is estimated using the factorial method using estimates of the amount needed for growth and maintenance of fat-free mass (16). Whereas the Institute of Medicine (IOM), in the USA, applied the factorial method to adult estimates of maintenance requirements from nitrogen balance studies. This was done due to the adult nitrogen estimate being similar to the nitrogen estimate of children older than 14 years (18). Regardless of the methodological differences between countries, all protein EARs for adolescent females have been set to ensure a sufficient protein intake to support optimal growth and development.

In New Zealand, the EAR for females aged 14-18 years is 0.62 g/kg/day of protein **Table 2.1** (16). In comparison, the EAR for females aged 14-18 years of age in the USA is higher at 0.71/g/kg/d (18). In conclusion, there is a need for future research to determine an accurate way of estimating human protein nutrition (19) within the same population group taking into consideration the needs required for growth and maintenance.

The tolerable upper intake level is defined as the highest level of daily nutrient intake that is likely to pose no risk of adverse effects (2). No upper limit (UL) for protein has been set in New Zealand due to insufficient data (16, 20). The risk of adverse effects from foods consumed as part of everyday diets is very low compared to the potential adverse effects from protein supplementation. In particular, supplements that consist of individual amino acids which exceed the amounts usually found in the diet (16). Together, the associated risks and limited data that is available makes it impossible to set an upper limit in terms of grams per day (16).

2.3 Sources and intakes of Dietary Protein for Adolescent Females

Twenty amino acids are necessary for optimal growth and metabolism. Nine of these are considered essential and must, therefore, be obtained from dietary sources as their carbon skeletons cannot be synthesized by the body (21). In contrast, non-essential amino acids can be synthesized and utilized by the body without relying on dietary sources (22). Dietary protein is obtained from both animal and plant foods and is considered a complete protein source when it provides all the essential amino acids.

2.3.1 Protein Quality

Protein quality reflects the number of essential amino acids that a protein source provides. High quality or complete proteins are those which provide all essential amino acids (21). Examples include all animal sources of dietary protein such as eggs, red meat, milk, fish and poultry. In contrast, plant-based proteins, such as legumes and nuts, are categorised as incomplete proteins due to the absence of one or more of the essential amino acids (21). Consumption of a variety of plant-based proteins, however, results in a complementary effect where all the essential amino acids are provided by a combination of foods (19).

2.3.2 Dietary sources of protein for adolescent females

During the literature search, national surveys and cross-sectional studies conducted in New Zealand, Canada, and Western Europe were retrieved. These studies highlighted the contribution that specific food sources made to the total protein intake of adolescent females. Data from the last New Zealand, national nutrition survey (2008/09) showed, the highest contributor to protein intake in this demographic group was the bread based dishes (12.6 % of total protein intake) followed by bread (10.8%) and poultry which contributed 9.8% of total protein intake (23). This finding is similar to that observed in Canadian adolescent females where cereal products were also the major source of dietary protein (41%) followed by meat, fish, and poultry (23%) (24). These findings, however, contrast with the literature review of adolescent females living in Western Europe by Rolland-Cachera, who concluded that protein intake was mostly derived from animal sources representing approximately two-thirds of total protein intake (25).

In summary, further research is required to understand the sources of protein that adolescent females are consuming that attribute to their total protein intake. Potentially differing dietary habits amongst this age group means we cannot assume that their protein intake is coming solely coming from animal sources but possibly from a variety of animal and plant-based sources or just plant sources alone. More recent findings would be beneficial in gauging if dietary patterns are changing over time in this population.

2.3.3 Protein intake of adolescent females

The 2008/09 New Zealand Adult National Nutrition Survey (08/09 ANS) found that the mean intake of protein for adolescent females was 67g/day (95% CI 63,71g/day) this exceeded the EAR (35g/day) and, therefore, inadequate protein intakes were virtually

absent (0.7%) (23). Total protein intakes, however, were found to be lower than those of adolescent females in other western countries. For instance, a comparison of daily protein intakes of adolescent females aged 15-19 from Western Europe ranged from 74.0 - 75.6 g/day (25). The same study reports that protein intake was high, both in absolute value and as a percentage of the daily energy intake. It was evident that adolescent females consumed large amounts of protein foods in their diet.

In addition, researchers from Southern Ontario, Canada, assessed the dietary intakes of adolescent females and compared those who consumed vegetarian or semi-vegetarian diets with those who consumed omnivorous diets. The mean intake of protein in females aged 14-19 years who followed an omnivorous diet was 58g/day and this was the only group to meet the recommended protein intake of 46g/day (26). Those who relied on vegetarian protein sources had a lower mean protein intake than those who followed an omnivorous diet. Those following a semi-vegetarian diet and lacto-ovo-vegetarian diet consumed 54g/day and 51g/day, respectively (24). **Table 2.2**, shows comparisons for protein intake among adolescent females internationally.

Table 2.2 International estimated average requirements (EAR) for protein and the percentage of inadequate protein intakes of adolescent females (15 yrs to 19yrs)

Country	EAR g/kg/d	RDI g/d	Mean protein intake g/day (95% CI)	Prevalence of inadequate protein intake (%)
New Zealand	0.62[16]	45[16]	67 (63, 71)[23]	0.7[23]
United States of America (USA)	0.71[18]	46[18]	58 (39, 77)[24]	-
Western Europe	0.84[27]	47[27]	74 - 75.6[25]	-

RDI, recommended daily intake; EAR, estimated average requirement; CI, confidence interval

Direct comparisons between countries and studies are complicated by variations in survey methods, populations, and expression of dietary intake data (25). In future, the application of consistent methodology would be beneficial for comparisons between countries for this population group. Furthermore, there is a lack of data regarding dietary protein intakes in adolescent females (24). Thus, further research in this area is warranted due to this population receiving little attention

2.4 Protein storage, inadequate and excessive intakes

2.4.1 Protein Storage

The largest reservoir of protein storage in the body is skeletal muscle (~43%), while other structural tissues such as skin (collagen) and blood (haemoglobin) contribute lesser amounts (~15% each) to total protein storage (2).

Other organs such as the brain, lungs, heart, and bone contribute to the remainder of the total protein storage. However, the proportion of these varies with developmental age (18).

Dietary protein intake can vary from day to day, and when diets are high or low in protein, the body is able to adapt in the short term to compensate for these changes and keep protein stores consistent. However, severe disease states can cause rapid protein loss due to increased demand for protein. In this instance, there is no protein storage to meet future needs (18).

Amino acids are lost in the body due to the mechanisms of oxidation, excretion, or conversion to other metabolites (2). Therefore, there is no site of storage for amino acids due to the nature of the processing that amino acids undergo in the body.

2.4.2 Inadequate intake

Inadequate intake is defined as the amount of protein consumed that is insufficient to meet the body's needs (28). Low intakes of dietary protein can lead to reduced growth in children or a loss of lean mass in adults (28). The adverse effects of inadequate protein intake can lead to increased susceptibility to diseases, and a higher risk of infection due to a compromised immune system (28, 29).

2.4.3 Protein deficiency

A protein deficient state occurs when the content of protein in the body is reduced, most likely as the result of an increase in requirements (e.g. infection or stress), an increase in losses (hemorrhage, burns) and/or a failure of the conservation systems (7). The physical signs of protein deficiency include poor musculature, dull skin, thin and fragile hair and in some cases signs of oedema (2).

Protein-energy malnutrition (PEM) is classified as a nutrient deficiency and is characterised by insufficient protein and non-protein energy (carbohydrates and fat) (2). Severe protein-energy malnutrition deficiencies are further classified as Marasmus or Kwashiorkor. Marasmus is a general wasting due to the body adapting to a state of starvation following severe deprivation of energy and all nutrients (28, 30). Kwashiorkor, however, results from inadequate dietary protein but relatively normal caloric intake. Kwashiorkor can be exacerbated in a state of infection and clinically presents as generalized oedema and a distended abdomen. (28, 30). Deficiencies of this extent, however, are most prevalent in underdeveloped countries (30) and are rarely seen in adolescent females living in New Zealand.

2.4.4 Excess dietary protein intake

Insufficient protein intake and the resulting health consequences of Kwashiorkor and Marasmus have been widely studied (31). Less is known, however, about the health implications of protein intakes that are above the recommended level (31).

High protein diets are referred to as daily protein consumption of greater than or equal to 1.5g/kg/day (32). This equates to almost three times greater than the EAR for adolescent females. Findings from the Institute of Medicine Dietary Reference Intakes (DRI) report concluded that there was a lack of evidence for making recommendations for upper levels of protein intake (18). Instead, an acceptable macronutrient distribution range (AMDR) of 10-35% of total energy from protein is recommended (32). Similarly, New Zealand has an AMDR of 15 – 25% of total energy from protein (16).

High protein diets can potentially impact kidney health due to the role kidneys have in nitrogen excretion and the potential for high protein diets to over-stress the kidneys (21). Although high protein diets cause changes in renal function that may be harmful to those with pre-existing renal disease, there is insufficient research to relate these findings to healthy individuals who have normal renal function (32, 33). In addition, evidence suggests that protein-induced changes in renal function are likely a normal adaptive mechanism of the body to ensure functional limits are met by healthy kidneys (32).

Limited data on protein and amino acids from food sources make identifying adverse risks difficult. While protein intake from food sources has been found to have a low risk of adverse effects (18) the same does not apply for protein supplements. Evidence suggests that protein supplementation poses a high risk for adverse effects in the general population (18). Therefore, caution is warranted when using supplementation of single amino acids at levels significantly above the amount normally found in food (2).

2.5 Assessment of Dietary protein intake

2.5.1 Dietary assessment methods

Dietary assessment methods are used to determine the nutrient intake of individuals or population groups (34). Assessment methods include 24-hour dietary recalls, diet histories, food frequency questionnaires and weighed food records (35). The method of choice for assessing food or nutrient intake depends primarily on the objective of the study. Choosing the most appropriate dietary assessment method is determined by the nutrient, and the population being studied. The 24-hour dietary recall has been established as the most appropriate method of assessing the mean nutrient intake of a population (35, 36). Appropriate methods for assessing population groups are summarised in **Table 2.3** (34).

Table 2.3 Methodology to measure nutrient intakes in populations, adapted from Gibson (2005) (34).

Level	Desired information	Preferred approach
One	Mean nutrient intake of a group	Single 24-hour recall or 1-day food record, with a large number of subjects and adequate representation of all days of the week.
Two	Proportion of the population at risk of inadequate intake	Repeated observations on each individual or subsample using 24-hour recall or 1-day food record.

2.5.1.1 The 24-hour diet recall

The 24-hour recall is a quantitative record of all food and beverage consumed in the previous 24-hour period (37). When conducted in a population, a single 24-hour recall is appropriate for estimating population means, however, it is not an effective tool to predict individual health outcomes (36). A 24-hour recall can be either self – or interviewer administered, however, like all dietary assessment methods there are limitations that can lead to misrepresentation of actual intake among individuals (20). Inevitable limitations of dietary assessment by a 24-hour recall are that all information is dependent on the respondent's memory, and only short term intake is captured. Furthermore, some respondents may alter their diet intentionally to avoid the burden of recalling, resulting in under or over-reporting their actual intake (20). To overcome these limitations, trained interviewers are required, as are repeat recalls for a subset of the population (20). Regardless, the 24-hour recall has the benefit of being suitable for participants with low literacy levels and is relatively quick to administer, thereby having a low respondent burden (34).

2.5.1.2 Food frequency questionnaire

Food frequency questionnaires (FFQ) are a semi-quantitative questionnaire widely-used in dietary assessment (35). This method enables the assessment of long-term dietary intake as it assesses the frequency of specific foods and food groups consumed over a period of months or years (20). Food frequency questionnaires are termed semi-quantitative if information regarding portions, energy, and specific nutrient intakes can be derived from the results (34). Food Frequency Questionnaires should be developed specifically for each study population as dietary patterns may vary due to culture, individual's preference and age groups (20).

2.5.1.3 Adjusting for usual intakes

A single 24-hour recall only focuses on short term intake, therefore, it is not a good estimate of an individual's usual nutrient intake considering the day to day variation (20, 34). Multiple 24-hour recalls in a subset of the study population are needed to adjust for intra-individual variation and therefore accurately estimate usual nutrient intake (34, 36). Adjustments to determine usual intakes are made using methods such as the Multiple Source Method (MSM) whereby it assumes that the data provided from multiple 24-hour recalls provides unbiased measurements for usual intake taking into consideration the inter and intra-individual variation (38). The MSM comprises of three steps; Firstly, the probability of consumption of food on a day is estimated, this is usually conducted through food frequency questionnaires (FFQ). Secondly, the usual amount of food intake on days of consumption is estimated. Thirdly, the usual intake on all days is calculated by multiplying the probability of consumption with the usual amount of food intake (38).

2.6 Conclusion

Based on the limited literature, there is a need for further research on protein intake in adolescent females. Adolescence is a vulnerable stage of life due to the rapid growth and development that occurs, therefore, evidence that is relevant to this population is of importance. It has been over a decade since the dietary intakes of adolescents have been investigated in New Zealand and no study looking specifically at nutrient intake in adolescent females has been conducted, therefore, this leaves a gap in the research for this population as current recommendations for adolescent females are derived from adult recommendations and studies.

This literature review has highlighted the functional roles of protein and the importance that dietary protein has on adolescent females. However, due to the limited amount of research, it has also highlighted that further research on this population group is necessary to assess protein intakes and to identify common sources of dietary protein. These results will provide a basis for developing future dietary guidelines tailored specifically to this population group.

3 Objective Statement

The overall aim of the SuNDiAL project is to investigate the nutritional intakes, dietary habits, health status, and attitudes and motivations for food choices among New Zealand adolescent females.

The focus of this thesis is to investigate the intake and food sources of protein in adolescent females aged 15-18 years of age living in New Zealand. It has been over a decade since data regarding protein intake has been studied in New Zealand adolescent females, therefore, this thesis will provide the most recent findings of dietary protein intake and food sources in adolescent females. Specific objectives are to:

1. Assess the adequacy of dietary protein intakes among New Zealand adolescent females aged 15-18 years.
2. Identify common food sources which contribute to the protein intake in this population.

4 Methods

This study represents phase one of a larger nationwide cross-sectional, clustered study known as the SuNDiAL project: Survey of Nutrition Dietary Assessment and Lifestyle. Data regarding dietary intake, anthropometry, physical activity and attitudes and beliefs about food choice were collected from eight secondary schools throughout New Zealand. The methods described below are those used to collect the data which is presented in this thesis to investigate the dietary intake and food sources of protein.

Ethical approval was obtained by the University of Otago Human Ethics Committee (Health): H19/004 and SuNDiAL is registered with the Australian New Zealand Clinical Trials Registry: ACTRN12619000290190 (Appendix A).

4.1 School and participant recruitment

4.1.1 School recruitment

Secondary schools were recruited by the study co-ordinators (**Figure 4.1**). Schools were initially chosen by their location, school decile, and having a female role greater than 400 for co-educational schools and 200 for all-girls school. School deciles in New Zealand represent the socio-economic status of the school's student community. Decile ratings are calculated from national census data and range from one to ten with one being the lowest and ten the highest. School deciles were considered during recruitment to ensure that a range of deciles were represented. Initial contact with selected secondary schools was by email in November 2018. Schools that did not respond to this initial email within two weeks were sent a second email and received a follow-up phone call. If recruitment by email and phone did not result in the target number of secondary schools, data collectors directly recruited school's in their allocated location by phone

and personal visits. Schools that showed interest in the SuNDiAL project were asked to provide an indication of suitable dates and times for data collection to commence. They were also asked to provide information regarding the availability of school facilities and space where data collection could occur.

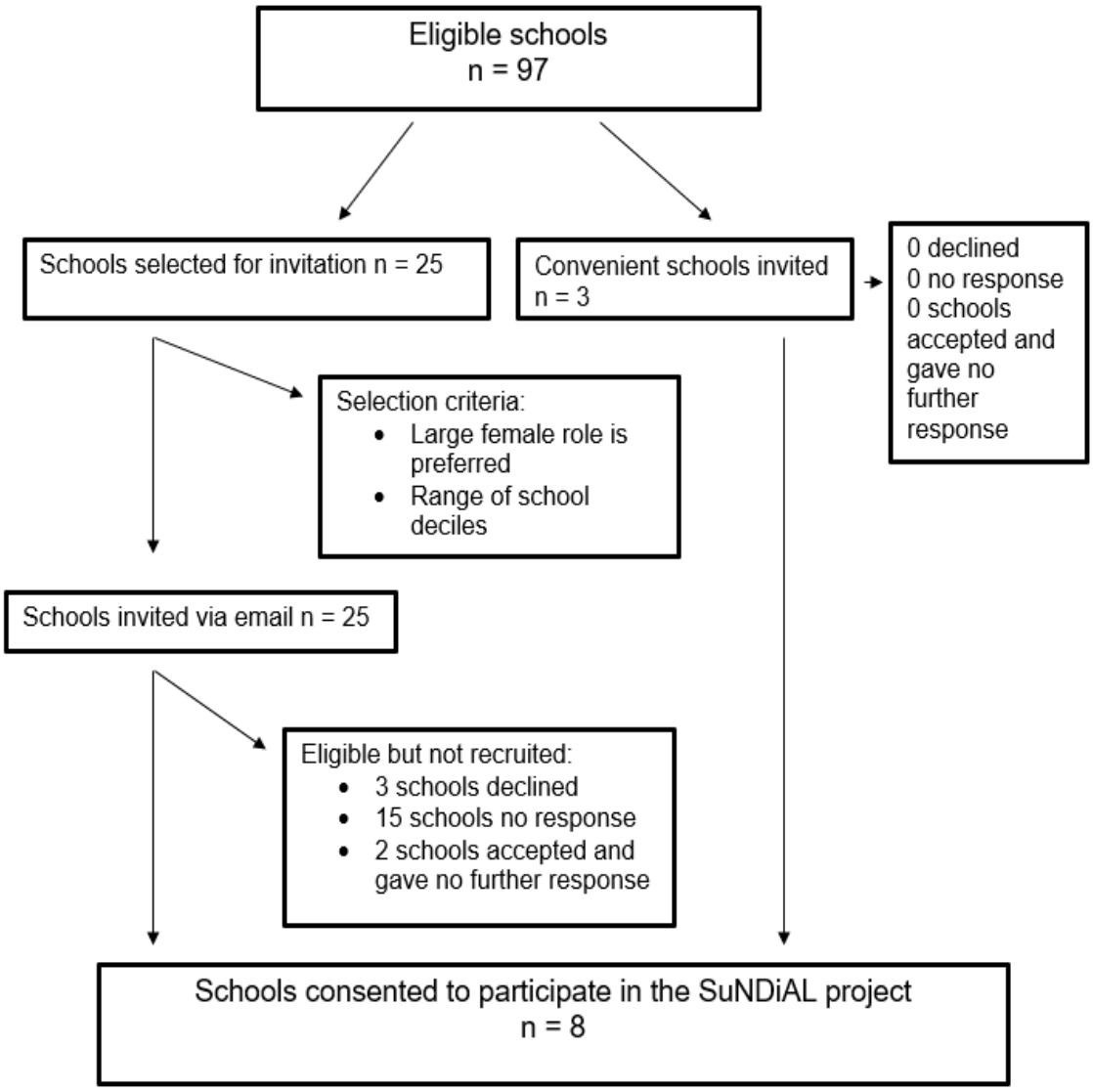

Figure 4.1. Flowchart for school recruitment

4.1.2 Participant recruitment

Participant recruitment began early in the 2019 academic year. Data collectors visited their allocated school to deliver a presentation outlining details of the study purpose and protocol at school or year group assemblies (Appendix B). Presentations were prepared by the data collectors and were reviewed by the study co-ordinators to ensure consistency and accuracy of the content. Secondary school students who were interested in participating provided their name and email address after the presentation. Potential participants aged 15 years at the time of recruitment were also asked to provide the email address of a parent/guardian who were then contacted and asked to provide consent for their daughter to participate in the SuNDiAL project. **Figure 4.2** provides data for the number of people initially recruited, those excluded and the final number of participants whose data was analysed.

SuNDiAL coordinators emailed interested students a link to the study website and online eligibility and consent portal (www.projectredcap.org) (Appendix C). To be eligible participants needed to be enrolled in one of the recruited secondary schools, female, aged between 15 and 18 years of age and speak and understand English. Participants who knew they were pregnant and could not give informed consent were considered to be ineligible.

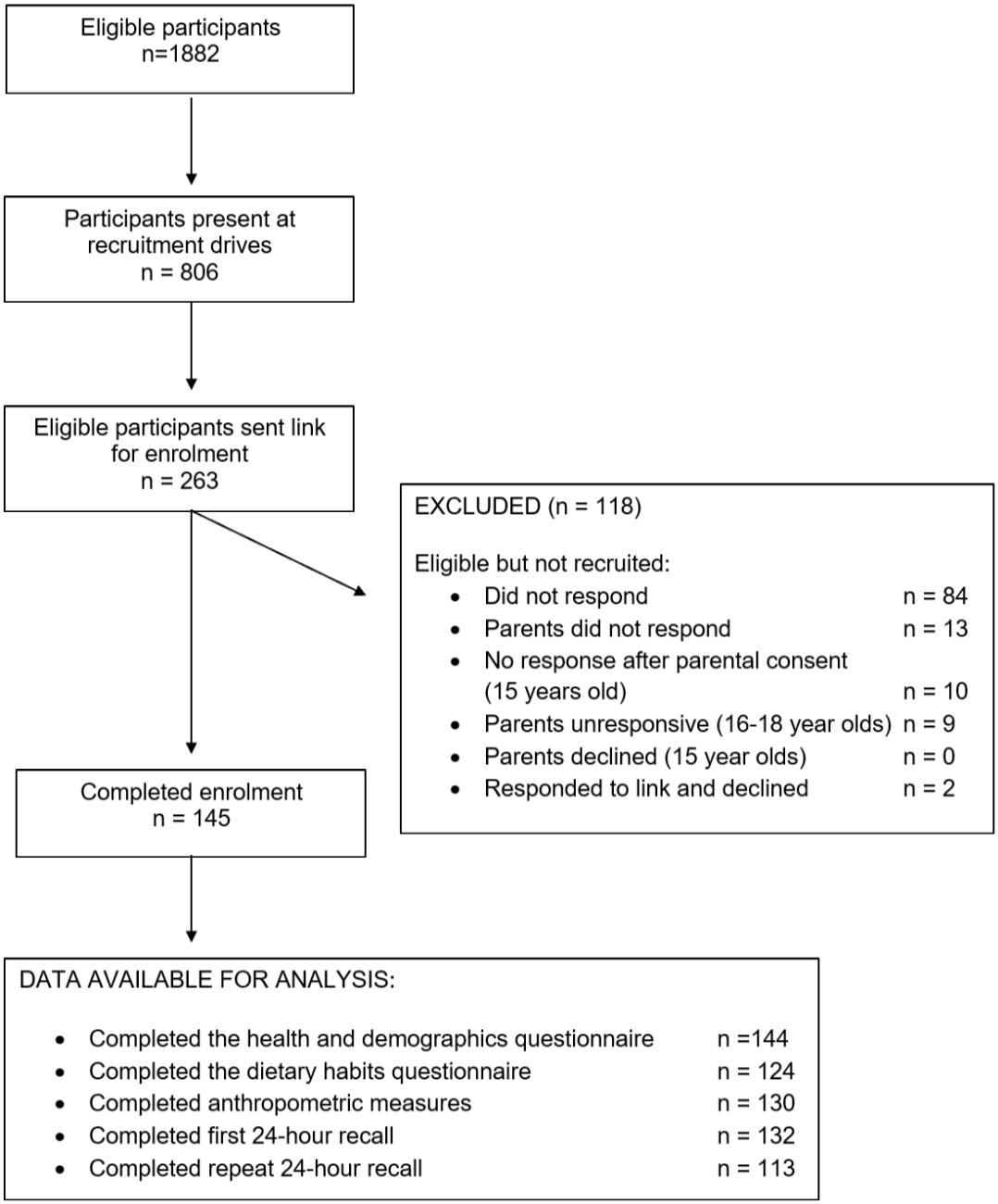

Figure 4.2 Flowchart of participant recruitment

4.2 Data collection

Data collection was conducted by pairs of final year Master of Dietetics students (data collectors) who were trained by the study coordinators to follow standardised study protocols. Data collection occurred in the schools from February 2019 – March 2019.

4.2.1 Demographic and health data

After consent was provided and eligibility was confirmed, participants were assigned a unique study identifier and directed to the online demographic, health and food habits questionnaire (Appendix C). The questionnaire used validated tools to explore dietary identity (39) and attitudes and motivation for food choices (40-42). Where necessary, the questionnaires were modified to suit the New Zealand food environment. This collected self-reported information regarding demographics (age, sex, and ethnicity), and nutritional supplement use. Data regarding dietary habits and attitudes, behaviours, and motivation for food choices were also collected via the online questionnaires. Clarification of questionnaire answers and missing information was obtained by the data collectors during the onsite school visits. Socio-economic status was recorded at the school level by noting the school decile.

4.2.2 Dietary intake assessment

Two non-consecutive 24-hour diet recalls were collected for each participant over a two-week period. The first was carried out in person at the first school visit and the second was undertaken via a phone call or video call which occurred the following weekend. Separating the 24-hour diet recalls allowed for observation of variation in dietary intake between days, including weekends. Both recalls followed the same protocol (Appendix D).

This included recording all food and beverages that were consumed in the previous 24 hours (12am – 12am). A ‘quick list’ was conducted first where the type and name of foods and beverages consumed was obtained. Following the completion of the quick list, the data collector reviewed the list and asked further questions to obtain detail regarding portion sizes, brands of food, time of consumption and methods of cooking

(Appendix E). Leftovers or second helpings were also recorded. Participants were asked to estimate quantities of food and beverages consumed using standardised measuring tools. These included household measures, food models and photographs of different portion sizes to ensure consistency and accurate reporting of consumption.

Data obtained from the two 24-hour recalls were entered into FoodWorks 9 professional (Xyris Software, Australia) by the data collectors. FoodWorks is a nutrient analysis software programme which matches foods to nutrient lines of the 2016 New Zealand FOODfiles (Plant & Food Research, New Zealand). This allows for calculation of total energy, macronutrient and micronutrient content of the 24-hour recalls. When the exact food item was unavailable in the FoodWorks database, substitutions with generic food items of similar nutrient compositions (within a 10% match of energy and macronutrient content) were made. Substitutions were derived from previous University of Otago dietary assessment studies (43) where common New Zealand food sources had previously been assessed. Information obtained from these studies enabled the substitutions and default foods to be generated for the Codebook SuNDiAL 2019 S1. The codebook ensured consistency for data entry between data collectors.

When there was, no suitable substitution available, a representative recipe was developed in FoodWorks. The representative recipes were created by entering all the item's ingredients in order from highest to lowest amounts. When nutrient compositions didn't fall within 10% of the 100-gram values of the nutrient information of the original food, an appropriate alternative was decided by consensus with dietary assessment experts from the Department of Human Nutrition, University of Otago. Additional assumptions were made when information reported was limited. For instance, if the participant did not know the size of a food item, the medium option was chosen as the

default option on the database. Homemade recipes were also entered as a recipe. The participant provided all the ingredients and quantities of each ingredient. When the quantity or weight of a food was not provided, a food weight estimation default in the codebook was used. Once the recipe was created, the portion the participant consumed was entered in their 24-hour recall in FoodWorks.

Dietary supplements such as whey protein powders have been included in the dietary analysis where possible, whereas micronutrient and herbal supplement usage has been assessed using the dietary habits questionnaires.

4.2.3 Anthropometry

Standardised study protocols (Appendix F) were used to obtain anthropometric data. Participants were asked to remove their shoes and all measures were taken in duplicate. If the first and second measurements differed by greater than 0.5 units, a third measurement was taken. The two closest measurements were then averaged to provide the final height and weight (Appendix G).

Standing height was measured using the SECA 213 stadiometer (Seca Corporation, Hamburg). Results were recorded to the nearest 0.1cm. Body weight was measured and recorded to the nearest 0.1kg using SOEHNLE scales (style sense comfort 400, Leifheit Group, Nassau, Germany). Body Mass Index (BMI) was calculated from the average height and weight measurements using the equation, $BMI = weight\ (kg) / height\ (m^2)$ and BMI Z-scores were calculated based on the World Health Organisation (WHO) BMI-for-age growth chart for 5-9 year old females (44). The BMI Z-scores were used to classify participants as follows: thinness < -2 standard deviations (SD); normal healthy weight ≥ -2 SD to ≤ + 1SD, overweight >+1SD or obese >+2SD (44).

4.3 Statistical Analysis

All descriptive statistical analysis was carried out using Microsoft Excel (version 15.33, 2017 Microsoft). Data for body weight is presented as normal (BMI Z-score < 1SD) or overweight and obese (BMIZ-score > 1SD). Descriptive statistics are used to present demographic data including percentage, mean and 95% confidence intervals (95% CI).

Dietary intake data was adjusted for usual intake using the Multiple Source Method (MSM) programme (Version1.0.1, R and Catalyst, a perl application) (38). Calculating usual intake allows for estimates of day-to-day variation based off the two 24-hour recall data. The information obtained from the usual intake was then applied to the whole dataset to give an adjusted estimate of usual intake for each participant.

Protein intake is presented as usual daily intake (g/d) and usual daily protein intake by body weight (g/kg/d). This latter measure was calculated using the daily intake of protein (g/day) and dividing it by body weight (kg) for each participant. Participants with intakes below the EAR of 0.62g/kg/d (16) were calculated to describe the prevalence of inadequacy. Contribution of protein to total energy intake was calculated by using the average daily protein intake (g/d) and applying the Atwater factor of 4kcal/g for protein to then determine the percent that protein contributes to total energy intake (%).

Food groups were determined by categorizing each food into its corresponding food group using the Foodgroups codebook provided by the study co-ordinators. The mean proportion (%) and 95% CI of protein intake for each of the 33 major food groups was

calculated. The top 10 foods contributing to protein intake were then determined based on the 10 highest percentages. The top 10 food groups were also classified as being of primarily animal or plant origin. Seven of these top 10 food groups were classified as animal protein (poultry, beef and veal, pork, milk, sausages processed meat, fish and seafood and bread-based dishes), with the remaining three classified as plant-based protein. Bread-based dishes were classified as a source of animal protein as they include pizza, filled sandwiches, burgers and hotdogs all of which include animal based protein in greater amounts than the protein from plant sources (eg. bread and pizza dough). Differences in mean protein intake for bodyweight, were examined using the difference of the mean statistical test.

5 Results

5.1 Participant characteristics

One-hundred and forty-five adolescent females aged 15-18 years from eight schools participated in phase one of the SuNDiAL project. Demographic and anthropometric characteristics of study participants are shown in **Table 5.1**. Three quarters (78%) of participants were aged 16 – 17 years old at the time of the study and a similar proportion (71%) self-identified as NZ European or Other ethnicity (NZEO). Almost two-thirds (65.3%) of participants were classified as having a normal bodyweight with the remainder being classified as overweight (25.4%) or obese (9.2%).

Table 5.1 Demographic and anthropometric characteristics of 145 New Zealand female adolescents[1]

Participant characteristic	n	n (%)
Age, years (mean (SD))	145	16.7 (± 0.8)
15		28 (19.3)
16		54 (37.2)
17		60 (41.4)
18		3 (2.1)
Ethnicity	144	
NZEO		104 (72.2)
Māori		29 (20.1)
Asian		6 (4.2)
Pacific		5 (3.5)
Bodyweight	130	
Normal (BMI Z-score ≥-2 and ≤+1SD)		85 (65.3)
Overweight (BMI Z-score = +1 SD)		33 (25.4)
Obese (BMI Z-score = +2 SD)		12 (9.2)
School Decile[2]	145	
1-2		0 (0.0)
3-4		14 (9.7)
5-6		64 (44.1)
7- 8		49 (33.8)
9- 10		18 (12.4)

[1] Data presented as n (%) unless otherwise stated.

[2] No Participants attended Decile 1, 2, 4 or 9 schools.

5.2 Dietary Protein intake

The mean usual protein intake of the study population was 73.7g (95% confidence interval (CI) 70.1, 77.3) (**Table 5.2)**, When converted to the usual daily intake of protein per kilogram of body weight, the mean intake was 1.2g/kg/day (95% CI 1.1, 1.2) and the overall prevalence of inadequate protein intake was low (8.5%). Protein intake contributed an average of 15.4% (95% CI 14.9,16.0) of total energy intake with very few (n=2) consuming less than the lower New Zealand AMDR range of 15% (6.7% and 7.8 %). No-one exceeded the upper level of the AMDR as the highest contribution of protein to total energy was 24.3%.

Table 5.2 Usual daily protein intake, and prevalence of protein inadequacy in New Zealand adolescent females

Participant characteristic	Mean protein intake					Prevalence of Inadequate Intake[1]
	n	g/day	(95% CI)	n	g/kg/day	(95% CI)	n (%)
All participants	132	73.7	(70.1, 77.3)	130	1.2	(1.1, 1.2)	11 (8.5)
Age, years							
15		79.2	(69.7, 88.7)		1.3	(1.1, 1.5)	2 (1.5)
16		72.0	(66.4, 77.7)		1.1	(1.0, 1.2)	4 (3.1)
17		73.2	(68.5, 77.8)		1.1	(1.0, 1.2)	5 (3.8)
18		71.3	(60.2, 82.3)		1.2	(1.0, 1.3)	0 (0.0)
Ethnicity							
NZEO		74.7	(70.7, 78.8)		1.2	(1.1,1.3)	5 (3.8)
Māori		70.3	(62.1, 78.5)		1.1	(0.9, 1.2)	4 (3.1)
Asian		64.7	(47.2, 82.1)		1.2	(0.8, 1.6)	1 (0.8)
Pacific		93.5	(68.4, 118.6)		1.3	(0.9, 1.7)	0 (0.0)
Bodyweight [2]	130			130			
Normal		74.3	(70.1, 78.5)		1.3	(1.2, 1.4)	1 (0.8)
Overweight and obese		72.7	(66.1, 79.2)		1.0	(0.9, 1.1) *	10 (7.7)
School Decile							
3-4		64.8	(53.7, 75.9)		1.0	(0.7, 1.2)	4 (3.1)
5-6		71.4	(66.0, 76.7)		1.1	(1.0, 1.2)	6 (4.6)
7- 8		77.7	(72.1, 83.3)		1.2	(1.1, 1.3)	1 (0.8)
9- 10		79.4	(68.0, 90.7)		1.3	(1.1, 1.5)	0
Contribution to total energy (%)		15.4	(14.9, 15.8)				

[1] Inadequate intake defined as < 0.62 g/day [2] Normal = BMI Z-score ≥-2 and ≤+1SD; Overweight and Obese ≥ +1SD.
*significant difference in the mean of normal vs overweight and obese
BMI, body mass index; CI, confidence intervals; NZEO, New Zealand European or Other

5.2.1 Associations between Ethnicity, Decile, BMI and Protein intake.

Pacific participants appeared to have a higher mean usual daily intake of protein to those of NZEO, Māori or Asian ethnicity. When expressed as protein intake per kilogram of bodyweight (g/kg/day), however, this difference was no longer apparent.

Lower usual protein intake was observed for participants attending decile 3 schools when compared to decile 10 schools, with a mean usual daily intake of 64.8g (95% CI 53.7, 75.9) and 79.4g (95% CI 68.0, 90.7) respectively. However, caution is warranted when interpreting these results as the school decile is not representative of individual participant's socioeconomic status within a high or low decile school.

Usual daily protein intake, expressed as both total grams per day and grams per kilogram per day, was higher for participants classified as normal bodyweight compared to those who were overweight or obese. The difference was only significant, however, when expressed as grams of protein per kilogram per day (Table 5.2). The prevalence of inadequate intake appears to be highest among those who are in the overweight and obese category (7.7%). The total daily intake appears to have no significant difference, however, when usual daily intake is converted to grams per kilogram per day there is a significant association between normal and overweight and obese category.

5.2.2 Major food sources of dietary protein

The top 10 food groups which contributed to total protein intake in this cohort of adolescent females are displayed in **Table 5.3**. Poultry (chicken, duck and turkey) was the single largest contributor of protein, followed by bread based dishes (pizza, filled sandwiches, burgers, hotdogs), bread and grains and pasta. The main source of protein was largely from animal based foods which contributed 43.8% compared to 21.4% from plant based foods (bread, grains and pasta, vegetables). The top 10 food groups contributed 65.2% of total protein intake with the remaining 34.8% coming from the remaining 23 food groups, each of which contributed less than 3.1% of total protein **(Table 5.3).**

Consumption of dietary protein supplements was reported by four participants who all consumed whey protein powders. One participant reported daily consumption of whey protein and this was included in her 24-hour dietary recalls and is thus accounted for in her usual protein intake. Three other participants also reported consumption of whey protein powders, however consumption by these participants was irregular (less than once per week) and they did not report the dosage used. Contribution of protein from dietary supplements for these participants has, therefore, not been accounted for in their usual protein intake.

Table 5.3 Top 10 food sources contributing to protein intake in 132 New Zealand adolescent females aged 15-18 years.

Food groups	Contribution to total protein intake	
	Mean %	95% CI
Poultry	12.5	(10.0, 15.1)
Bread based dishes	9.7	(6.9, 12.5)
Bread	8.5	(7.3, 9.6)
Grains and pasta	8.4	(6.9, 9.9)
Beef and veal	5.6	(3.7, 7.5)
Pork	4.6	(3.2, 6.0)
Vegetables	4.5	(3.4, 5.7)
Milk	4.5	(3.3, 5.6)
Sausages and processed meat	3.8	(2.3, 5.3)
Fish and seafood	3.1	(1.7, 4.5)

6 Discussion

To our knowledge this is the first New Zealand study in recent years to examine the nutritional intake and status of adolescent females aged 15-18 years. The overall mean dietary protein intake was greater than the recommended EAR of 35g/d and the prevalence of inadequate protein intake was low. Protein intake contributed an average of 15% of total energy intake; falling at the lower end of the New Zealand AMDR of 15-25%. This suggests that most participants were consuming adequate protein to meet their requirements as only two participants had protein intakes that were below the recommended 15% (6.7% and 7.8 %). Protein intakes appeared to be greater for those who self-identified as Pacific ethnicity, those who attended higher decile schools and for those participants who were classified in the normal bodyweight compared to overweight or obese. Protein was provided by both plant and animal sources with poultry being the single largest contributor to dietary protein intake.

6.1 Protein intake in adolescent females

The daily protein intake of adolescent females in the present study is consistent with the findings from the study conducted in Western Europe (25). Intakes in both New Zealand and Western Europe appear greater than the protein intake of American adolescent females (18). Intakes among all countries, however, remain within the recommended intake based on their countries EAR. The differences in average daily protein intake could be due to the dietary patterns which may influence the overall protein intake.

Both animal and plant based sources contributed to total protein intake, however, animal based sources contributed the majority of total protein intake in the present study. These findings are consistent with those found in Western Europe where protein intake was mostly derived from animal sources (25). A study of Western European females aged 15-19 years reported a mean protein intake that was sourced predominantly from animal sources and that was higher than recommendations (74-75.6g/d vs 47g/d) (25). This was in contrast to studies both internationally and in New Zealand that reported lower protein intakes (58g and 67g respectively) (23, 24). In these latter studies the main contributor to protein intake was cereal and bread based dishes. This suggests that those who consume predominantly animal based sources of protein have a higher total protein intake compared to those who predominantly consumed plant based sources.

6.2 Inadeqaute protein intake

The prevalence of inadequate protein intake was low in the present study, with 8.5% of the population consuming less than the EAR of 0.62g/kg/d of protein. Most of the inadequate intakes were participants who self-identified as NZEO (n = 5) followed by Maori (n=4). Comparatively, in the 08/09 ANS the prevalence of inadequate protein intake was virtually absent (0.7%) and of those, the prevalence was highest in Pacific female adolescents (1.5%) (23). The change in the prevalence of inadequate intake, albeit small, may be due to the changes in food consumption as reflected in the contribution of the top food groups that contributed to protein intake and as discussed in section **6.4** below.

Of the eleven participants who had inadequate protein intake ten (91%) were classified as overweight or obese. The total mean daily protein intake per day was not

significantly different between the normal and overweight and obese categories (74.3g and 72.7g, respectively), however, when protein intake is converted to grams per kilogram of body weight per day, the inadequate intakes become more apparent between these two bodyweight categories. The high prevalence of inadequate intakes in participants that were classified as overweight or obese may potentially be due to under-reporting of actual intakes. Under-reporting has been noted in adolescents who have a higher body weight, due to concerns with body image (45, 46). Nonetheless, it is of concern that female adolescents who are classified in the overweight or obese category. Future work in the SuNDiAL study will examine if this trend is present with a larger and more representative sample size and whether under-reporting is the confounding issue. In the meantime, this finding highlights that higher total protein intakes should be considered for female adolescents with high BMIs to ensure that they are able to meet their requirements.

6.3 Demographic associations with protein intake

Variations in both mean daily protein intake and the prevalence of inadequate protein was observed between demographic groups. Pacific participants appeared to have the highest protein intake of all ethnicities and Asian participants the lowest, yet the prevalence of inadequate protein intakes in both groups is virtually absent. This differs to the results of the 08/09 ANS where the protein intake of adolescent females was lowest for Pacific participants. It is important to note, however, that the allocation to ethnic groups differed between the present study and the 08/09 ANS as the NZEO ethnic group included Asian participants (23). Similarly, it is not possible to say that the differences noted in the present study regarding the protein intake of the various ethnic groups can be applied to the wider female adolescent population of New Zealand as the sample size of both Pacific and Asian ethnic groups was small and,

therefore, not a true representation of the population distribution. Further research with a larger sample size would allow for more variability and therefore, true representation of protein intake among Pacific and Asian females aged 15-18 years living in New Zealand.

6.4 Food sources

The top 10 food groups contributing to protein intake remained consistent between the current study and the 08/09 ANS, where there was a combination of both animal and plant-based sources. In the SuNDiAL study poultry was the single largest contributor to protein intake (12.5%), whereas in the 08/09 ANS, bread-based dishes were found to be the single largest contributor (12.6%) followed by bread (10.8%) and grains and pasta (9.8%). It is important to note, however, that bread based dishes include foods that contain animal proteins such as pizza, hot dogs, filled sandwiches and burgers. It is likely that these animal based proteins contribute greater amount of protein than the plan-based components such as bread and pizza dough. Nevertheless, it is evident that high quality protein is being consumed in substantial amounts based on the proportion of animal products such as poultry, milk, and beef and veal being higher than the proportion of sausages and processed meat which is associated with lower quality protein. As noted above these findings are consistent with international studies. Concluding, both animal and plant based sources are contributing to protein intake among adolescent females, both of which are allowing this population group to meet their recommended amounts of protein per day.

6.5 Strengths and Limitations

This study has several strengths and limitations that need to be considered. Dietary intake data was collected using two non-consecutive 24-hour recalls. This method provided representation of intake on both week and weekend days and allowed usual dietary intakes to be calculated. This accounts for the day to day variation in an individual's intake and more effectively represents the dietary intake of a population compared to a single 24-hour recall (20, 34).

Furthermore, data was collected by trained data collectors (MDiet students) using standardised protocols for anthropometric measures and 24-hour dietary recalls. A standard codebook was also used to ensure that substitutions for foods that were not in the FoodWorks database were correctly and consistently managed by all data collectors. These strengths were used to minimize the potential for data entry errors and inaccuracy which could have occurred due to data being collected and entered by multiple data collectors.

Memory lapses and dietary omissions are recognized as potential limitations of the 24-hour dietary recall method (20, 34). To minimise the impact of memory lapses the three-pass method was used to gather dietary data. The first pass of this method involves a complete list of all food and beverage consumed in the previous 24-hours, followed by the second pass of a detailed description of each food and beverage including cooking methods and brand names. Lastly, the third pass, where estimates of the amount of each food and beverage consumed is obtained using household measures and photos as a guide (34). Future work using the total data set should investigate the potential under-reporting which was not calculated in this phase of the study. Socio-economic status was assigned at the school level rather than for an

individual participant. This limited the interpretation of any association of SES with dietary protein intake. Determining the socio-economic status associated with individual living circumstances would be worthwhile when assessing the overall health and wellbeing of this population group. Despite data collection occurring nationwide, the small sample size cannot be considered representative of New Zealand adolescent females. This is especially relevant to the low recruitment of Pacific and Asian ethnic groups.

6.6 Implications for future research

Given the small sample size, further research of this cross-sectional study would provide a larger nationally representative sample which will ensure that all ethnic groups have a good proportion of participants which will allow for associations between protein intake and ethnicity to be identified. Further research should include consistent standardised scales across all locations, calculation of under-reporting and improving data entry by providing more practice for research assistants to enter food into FoodWorks. All of these would help to ensure confounding issues are minimised. Improving the above issues will allow for further evidence to contribute to sound conclusions regarding protein intake among adolescent females living in New Zealand.

7 Conclusion

In conclusion, examining the diets of adolescent females aged 15-18 years living in New Zealand has provided us with an insight into the protein intakes within this population group. Overall, protein intake is adequate and the top 10 major food groups contributing to protein have been identified. However, there appeared to be no significant differences between protein intake and ethnicity. Conducting further research of a nationally representative sample will be beneficial in identifying trends in protein intake and dietary sources in demographic sub-groups such as different ethnicities.

8 Application to Dietetic Practice

Dietitians are required to work with evidenced based guidelines, while tailoring their interventions and advice to the individual or group they are working with. Therefore, as dietitians, evaluating scientific evidence is a skill that underpins our dietetic practice. Dietitians can use this research to increase their knowledge and awareness of dietary protein intake in adolescent females, taking into consideration any dietary habits that may impact their protein intake and finding appropriate alternatives to ensure they are meeting their requirements. These results will be beneficial for dietitians working in public health and clinical settings to assist education of female adolescents regarding the importance of having a balance diet where all key macronutrients are consumed.

The 08/09 ANS is the most recent study which describes the protein intake of New Zealand adolescents. The present study updates this research regarding the intake and food sources of protein for New Zealand adolescent females. The larger SuNDiAL project will provide information which will allow for recommendations to be tailored to this specific demographic group and will help ensure that appropriate dietary recommendations are made to meet their needs for growth and development.

Dietitians now have research that is clinically relevant to our daily practice. In clinical practice, we assess and prescribe protein based on grams per kilogram of body weight per day which allows recommendations to be tailored specifically to the individual. The results from this study confirm that current recommendations are achievable for individuals. Furthermore, the findings from this study show that overweight and obese participants had the greatest prevalence of inadequate protein intakes. This is

important to consider in dietetic practice to ensure all clients are consuming adequate protein. It illustrates that the grams per kilogram per day (g/kg/d) is a suitable method for clinical practice as we can tailor each individual's protein intake based on their bodyweight to minimise the risk of inadequate protein intake.

8.1 Part B: Reflective practice: What research has taught me

An aspect of my research journey that I have found particularly important for my growth and development as a dietitian was the need for effective communication throughout the research process as well as being resilient to changes. I found the research process challenging due to the nature of the study being so heavily dependent on many people such as the study co-ordinators, supervisors and my fellow classmates (data collectors).

Firstly, the nature of research and the ever-changing data and results was not something that worked well for me. I have been taught that if I have a job or a responsibility to have something done by a certain time I need to stick to it otherwise there will be repercussions for others and I personally do not like knowing that I have let people down. This was probably the key part of my research journey that I struggled with right from the start. Throughout this experience, I managed to see everything as a negative. Our data was given back to us later than anticipated which was hard for us as we have a deadline to meet. I never saw the positive aspect of this which was that our data has been analysed so thoroughly that it is the cleanest data we can have for our target population, a great outcome for research. I struggled knowing that my deadline was now out of my control as data collection and processing did not go as planned and this process took longer than anticipated. This meant I had to reassess my plan in order for me to complete my thesis in a timely but realistic

manner. The constant support from my supervisor and fellow MDiet colleagues is what really got me through. My supervisor knows better than anyone the process of research and that what we were experiencing is a normal part of research. This gave me reassurance to keep going and to be as proactive as I can to ensure that I do meet my deadline. Working so closely with my MDiet colleagues all over the country has taught me to lean on others for support where needed as they are experiencing the same challenges that I am. Knowing this gave me a sense of security as we knew in our minds that we were doing the best we could given the situation and the lack of experience we have as first-time researchers.

Overall, this experience has challenged me and has taught me a lot about myself. I now feel confident for the future whether it be in research or one of the many other aspects of dietetics that I am now prepared to tackle; that everything doesn't go to plan and there are challenges along the way and that's okay. These skills plus the lifelong friendships I have made along the way leave me excited for my future career as a dietitian.

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10 Appendices

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NGĀI TAHU RESEARCH CONSULTATION COMMITTEE
TE KOMITI RAKAHAU KI KAI TAHU

Monday, 17 December 2018

Dr Meredith Peddie
Department of Human Nutrition

Tēnā Koe Dr Meredith Peddie

The SuNDiAL Project 2019: Survey of Nutrition, Dietary Assessment and Lifestyle.

The Ngāi Tahu Research Consultation Committee (the Committee) met on Tuesday, 11 December 2018 to discuss your research proposition.

By way of introduction, this response from The Committee is provided as part of the Memorandum of Understanding between Te Rūnanga o Ngāi Tahu and the University. In the statement of principles of the memorandum it states "Ngāi Tahu acknowledges that the consultation process outline in this policy provides no power of veto by Ngāi Tahu to research undertaken at the University of Otago". As such, this response is not "approval" or "mandate" for the research, rather it is a mandated response from a Ngāi Tahu appointed Committee. This process is part of a number of requirements for researchers to undertake and does not cover other issues relating to ethics, including methodology they are separate requirements with other Committees, for example the Human Ethics Committee, etc.

Within the context of the Policy for Research Consultation with Māori, the Committee base consultation on that defined by Justice McGechan:

"Consultation does not mean negotiation or agreement. It means: setting out a proposal not fully decided upon; adequately informing a party about relevant information upon which the proposal is based; listening to what the others have to say with an open mind (in that there is room to be persuaded against the proposal); undertaking that task in a genuine and not cosmetic manner. Reaching a decision that may or may not alter the original proposal."

The Committee considers the research to be of importance to Māori health.

As this study involves human participants, the Committee strongly encourages that ethnicity data be collected as part of the research project as a right to express their self-identity.

The Committee suggests researchers consider the Southern District Health Board's Tikaka Best Practice document, in particular patient engagement. The document also covers the collection, storage and disposal of blood and tissue samples. This document is available on the Southern District Health Board website. The Committee also refers researchers to Te Mana Raraunga Māori Data Audit Tool, which gives an overview of key Māori Data Sovereignty terms and principles.

The Ngāi Tahu Research Consultation Committee has membership from:

Te Rūnanga o Ōtākou Incorporated
Kāti Huirapa Rūnaka ki Puketeraki
Te Rūnanga o Moeraki

NGĀI TAHU RESEARCH CONSULTATION COMMITTEE
TE KOMITI RAKAHAU KI KAI TAHU

We wish you every success in your research and the Committee also requests a copy of the research findings.

This letter of suggestion, recommendation and advice is current for an 18-month period from Tuesday, 11 December 2018 to 3 June 2020.

The recommendations and suggestions above are provided on your proposal submitted through the consultation website process. These recommendations and suggestions do not necessarily relate to ethical issues with the research, including methodology. Other Committees may also provide feedback in these areas.

Nāhaku noa, nā

Claire Porima
Kaiwhakahaere Pūtere
Senior Project Manager
Office of Māori Development
Te Whare Wānanga o Otāgo
Ph: +64 3 479 7461
Email: claire.porima@otago.ac.nz
Web: www.otago.ac.nz

The Ngāi Tahu Research Consultation Committee has membership from:

Te Rūnanga o Ōtākou Incorporated
Kāti Huirapa Rūnaka ki Puketeraki
Te Rūnanga o Moeraki

Academic Services
Manager, Academic Committees, Mr Gary Witte

H19/004

4 February 2019

Dr J Haszard
Department of Human Nutrition
Division of Sciences

Dear Dr Haszard,

I am writing to let you know that, at its recent meeting, the Ethics Committee considered your proposal entitled "**SuNDiAL Project 2019: Survey of Nutrition Dietary Assessment and Lifestyle Phase 1: Adolescent Females**".

As a result of that consideration, the current status of your proposal is:- **Approved**

For your future reference, the Ethics Committee's reference code for this project is:- **H19/004**.

The comments and views expressed by the Ethics Committee concerning your proposal are as follows:-

While approving the application, the Committee would be grateful if you would respond to the following:

Information Sheet

A typing error was noted on the Information Sheet, under the heading *"Is there any risk of discomfort or harm from participation?"*, line 3, "some" should read "someone".

Consent Form

Please amend the Consent Form to include an option for participants to indicate whether they would prefer for their blood samples to be disposed of using standard methods or with a Karakia.

Please provide the Committee with copies of the updated documents, if changes have been necessary.

The standard conditions of approval for all human research projects reviewed and approved by the Committee are the following:

Conduct the research project strictly in accordance with the research proposal submitted and granted ethics approval, including any amendments required to be made to the proposal by the Human Research Ethics Committee.

Inform the Human Research Ethics Committee immediately of anything which may warrant review of ethics approval of the research project, including: serious or unexpected adverse effects on participants; unforeseen events that might affect continued ethical acceptability of the project; and a written report about these matters must be submitted to the Academic Committees Office by no later than the next working day after recognition of an adverse occurrence/event. Please note that in cases of adverse events an incident report should also be made to the Health and Safety Office:

http://www.otago.ac.nz/healthandsafety/index.html

Advise the Committee in writing as soon as practicable if the research project is discontinued.

Make no change to the project as approved in its entirety by the Committee, including any wording in any document approved as part of the project, without prior written approval of the Committee for any change. If you are applying for an amendment to your approved research, please email your request to the Academic Committees Office:

gary.witte@otago.ac.nz

jo.farrondediaz@otago.ac.nz

Approval is for up to three years from the date of this letter. If this project has not been completed within three years from the date of this letter, re-approval or an extension of approval must be requested. If the nature, consent, location, procedures or personnel of your approved application change, please advise me in writing.

The Human Ethics Committee (Health) asks for a Final Report to be provided upon completion of the study. The Final Report template can be found on the Human Ethics Web Page http://www.otago.ac.nz/council/committees/committees/HumanEthicsCommittees.html

Yours sincerely,

Mr Gary Witte
Manager, Academic Committees
Tel: 479 8256
Email: gary.witte@otago.ac.nz

c.c. Assoc. Prof. L Houghton Department of Human Nutrition

Appendix B: Participant information sheet

Participant Information Sheet

Study title:	The SuNDiAL Project 2019: A survey of nutrition, dietary assessment and lifestyle	
Principal investigators:	**Names:** Dr Jill Haszard & Dr Meredith Peddie **Department:** Human Nutrition **Position:** Research Fellows	Contact phone number: 03 479 5683 03 479 8157

Introduction

Thank you for showing an interest in this project. Please read this information sheet carefully. Take time to think about it and talk with family or friends before you decide whether to take part or not.

If you decide to take part we thank you. If you decide not to take part that won't disadvantage you and we thank you for considering our request.

What is the aim of this research project?

We don't know much about teenage women's food intakes and lifestyles in New Zealand. We suspect that they don't get enough of some nutrients like iron sometimes, and that this can make them feel tired and affect their health. Teenagers often make their own decisions about what foods to eat, but we don't know very much about why they choose the foods they eat. Therefore in 2019 the SuNDiAL project is going to investigate food intakes, nutrition, health, and why female high school students (aged 15-18 years) choose to eat the way they do.

Who is funding this project?

This project is funded by the Department of Human Nutrition, University of Otago, and a Lottery Health Research Grant.

Who are we seeking to participate in the project?

We are looking for at least 300 female high school students who are between 15 and 18 years old. To be eligible to take part, your high school must have agreed to take part in the study, you must speak and understand English, and be able to complete the questionnaires.

If you participate, what will you be asked to do?

If you agree to take part in this study you will be asked to do three things:

1) **Complete an online questionnaire**
   After you have completed the consent process you will be asked to complete a questionnaire that asks questions about your health and some general questions such as what ethnicity you identify with this questionnaire also asks you about your overall eating habits, and why you choose to eat the foods that you do. This questionnaire will take about 30 min to complete.

2) **Attend a session at your school with our research team**
   This visit will take about 60 minutes and you will be asked to:
   - Complete a face to face interview with one of our research team during which you will be asked to recall everything you ate and drank the day before.
   - At this session one of our research team will also measure your height, your weight, and the length of your lower arm – these measurements will be done twice to make sure they are as accurate as possible. This will be done in a private space and you won't be told these measurements unless you ask for them.

3) **Complete a second interview about the food you have eaten on another day**
   Sometime in the 2 weeks after you have finished the session at school you will be contacted by the research team and asked to complete a second interview in which you will be asked to recall everything you ate and drank on a different day of the week than the first interview . This is important because sometimes you can eat quite differently from one day to the next. **This interview will be performed over facetime or zoom, at a time that is convenient for you.**

There are three other parts to the SuNDiAL project that are entirely optional.
Please read the following information carefully before you decide whether to take part in these optional bits of the study. If you agree to do these, but change your mind later, that's OK - there is no disadvantage to not you if you decide not to do these. You will be asked again on the day if you still want to do them.

1) **Provide a blood sample**
   We would like you to provide a blood sample (which would be collected by someone with extensive training in how to collect blood during the session at school), but we understand that not everyone feels comfortable about this so it is entirely up to you if you do this. However, if you do provide a blood sample, we can tell you whether you're iron deficient or not. You can still take part in the rest of the study even if you don't do this bit.

2) **Provide a urine sample**
We would also like you to give a urine ("pee") sample (which is easy for you collect yourself in the bathroom with the equipment we give you, during the session at school). You can still take part in the rest of the study even if you don't do this bit.

3) **Wear an accelerometer for a week**
We would also like you to wear a small red box called an accelerometer on an elastic belt 24 hours a day for the seven days following the session at your school. This will tell us how much time you spend sitting down, moving around, and sleeping. If you choose to wear the accelerometer you will be asked to complete a little diary about the times your took the device off, and what time you went to bed each night on the days that you wear it. One of our research team will return to your school the week after this visit to collect the accelerometer. You can still take part in the rest of the study even if you don't do this bit.

After the completion of the study you will receive a $5 voucher for each component of the study that you complete . That is $5 for completing the online questionnaire, $5 for completing the face to face interview about what you ate in the last 24 hours, $5 got completing the second interview about what you ate; $5 for providing a blood sample; $5 for providing a urine sample or $5 for wearing the accelerometer for a week. Adding to a possible total of $30 in vouchers.

Is there any risk of discomfort or harm from participation?

If you choose to provide a blood sample, you should know that there is a risk of a little pain or discomfort, and possibly a small bruise from the blood test. Any bruising should only last a few days and an experienced nurse or phlebotomist (someone with training to take blood samples) will collect the blood to minimize any discomfort to you.

What specimens, data or information will be collected, and how will they be used?

The answers you provide to the questionnaires and the food questionnaire will be entered into a database with every other participants' answers. All your answers will be kept confidential and stored using an id number, not your name. This information will provide valuable and unique information about the nutrition status of female high school students in New Zealand. Information about why people eat the way they do will also be very helpful if some eating patterns provide health benefits. Ultimately, the results of this study will support the development of up-to-date government and health agency guidelines for young women in New Zealand.

If you provide a blood sample it will be divided into 3 separate parts. One part will be taken to a local laboratory where it will be analysed for Vitamin B12 concentrations and a complete blood count. The

other two parts of your blood sample will be transported to the Department of Human Nutrition at the University of Otago where they will be stored in a freezer until we have finished collecting all the blood samples from around the country. When all the blood samples have been collected, one part of your blood sample will be sent to Germany where it will be analysed for ferritin, soluble transferrin receptor, retinol binding protein, C-reactive protein and alpha-glycoprotein. We are sending this sample to Germany because they have a special machine that can measure these things on a much smaller amount of blood, at a smaller cost, than we can do in New Zealand. The remaining part of your blood sample will remain at the Department of Human Nutrition, where it will be analysed for plasma selenium and plasma zinc, thiamin, plasma folate, Vitamin B6, Leptin, Interlukin-6 and blood lipids.

If you provide a urine sample it will also be transported to the Department of Human Nutrition at the University of Otago where it will be stored in a freezer until it is analysed for iodine concentrations.

Once all of the analysis on your blood and urine samples has been completed they will be disposed of using standard biohazard protocols. On the consent form you can indicate to us if you would like your samples disposed of with a Karakia (Māori Prayer). **We will only test your samples for the things listed here, and won't test them for anything else.**

What about anonymity and confidentiality?

Your information will be identified with an ID number only in the database that contains the results of the study. This database will be stored on the researchers' computers which are password protected. A backup copy may also be stored on the University's shared server space, but only Jill Haszard and Meredith Peddie will have the password so no one else can access the information .

The information linking you to your ID number will be stored in a separate password protected file that only Jill Haszard and Meredith Peddie will have access to. The only reason they would access this information once you have completed the study would be if you requested your individual results. This file will be destroyed once all participants have been given the opportunity to request individual information. The de-identified information collected as part of this research will be kept in secure storage for at least 10 years.

If you agree to participate, can you withdraw later?

You may pull out of the project before the study has been completed (anticipated to be October 2019) without any disadvantage to yourself of any kind. Once data collection is completed and your information is integrated into the study it will no longer be possible to withdraw your information from the study.

Any questions?

If you have any questions now or in the future, please feel free to contact either:

Name: Dr Jill Hazsard **Position: Senior Research Fellow** **Department of Human Nutrition**	Contact phone number: 03 479 5683
Name: Dr Meredith Peddie **Position: Research Fellow** **Department of Human Nutrition**	Contact phone number: 03 479 8157

This study has been approved by the University of Otago Human Ethics Committee (Health). If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (phone +64 3 479 8256 or email gary.witte@otago.ac.nz). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.

Appendix C: SuNDiAL 2019 Participant Information & Enrolment Questionnaire

Thank you for showing an interest in this project. Please read the information about SuNDiAL project carefully. This can be found on our website www.otago.ac.nz/sundial. Take time to think about it and talk with family or friends before you decide whether to take part or not. If you decide to take part we thank you. If you decide not to take part that won't disadvantage you and we thank you for considering it.

Who are we seeking to take part in the project?
We are looking for female high school students who are 15 to 18 years old. To be eligible to take part, your high school must have agreed to take part in the study, you must speak and understand English, and be able to complete the questionnaires.

If you take part, what will you be asked to do?
If you agree to take part in this study you will be asked to do three things:

1) Complete an online questionnaire with three parts to it: (i) health & demographics; (ii) why you choose the food you eat; and (iii) your dietary habits.

2) Attend a session at your school with our research team. This visit will take about 60 minutes and you will be asked to recall the food and drink you've consumed over the last day. You will also have your height, weight, and length of your lower arm measured. These measurements will be done twice to make sure they are as accurate as possible. This will be done in a private space and you may ask for the measurements if you want them.

3) In the next week or two we'll ring or video call you to do a second food and drink recall.

Any questions?

Contact Jill (ph 03 479 5683) or Meredith (ph 03 479 8157) or email us on: sundial@otago.ac.nz

This study has been approved by the University of Otago Human Ethics Committee (Health). If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (phone +64 3 479 8256 or email gary.witte@otago.ac.nz). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.

Electronic consent
Click on the “agree” button below if:

You have read the information about the study
You have had all your questions answered about the study and understand that you can ask for more information at any stage

You know that when the project is completed all personal information that could be linked to you will be removed from the paper records and electronic files for the project, and that these will be placed in secure storage and kept for at least ten years.

You are a young woman who is 15 to 18 years old and isn’t pregnant

You know you can pull out of the study any time before it finishes in October 2019.

If you don’t want to take part in the SuNDiAL project, please click on the “disagree” button.

○ AGREE
○ DISAGREE

Thank you for agreeing to taking part in the SuNDiAL project! If you are female, aged 15-18 years of age and not pregnant, please answer the following two questions:	
What age are you as of today?	○ 15 ○ 16 ○ 17 ○ 18 ○ None of the above
What high school* do you attend?	○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○

*School names have been removed in this appendix to ensure de-identification of all data

Thank you! You are eligible to take part in the SuNDiAL project!

There are three other parts to the SuNDiAL project that are optional. Please read the following information carefully before you decide whether to take part in these optional bits of the study. For each one of these that you do, you will receive a $5 gift voucher from New World or PaknSave.

If you agree to do these, but change your mind later, that's OK - there is no disadvantage to not you if you decide not to do these.

Once all of the analysis has been completed the samples will be disposed of using standard biohazard protocols. On the consent form (below) you can tell us if you would like your blood sample disposed of with a Karakia (Māori Prayer).

Electronic consent

Click on the "AGREE" button below if:
- You have read the information on the website
- You want to take part in these parts of the study

BLOOD SAMPLE:

We would like you to provide a blood sample (which would be collected by someone with extensive training in how to collect blood), but we understand that not everyone feels comfortable about this so it is entirely up to you if you do this. If you do provide a blood sample, we can tell you whether you're iron deficient or not. You can still take part in the rest of the study even if you don't do this bit.

Click on the agree button below if:

You understand the risks of discomfort involved in providing a blood sample
◯ AGREE
◯ DISAGREE

Please click here if you want your samples disposed of with a Karakia (Māori Prayer)

◯ YES
◯ No

URINE SAMPLE:

We would also like you to give a urine sample ("pee or wee") - which is easy for you collect yourself with the equipment we give you. You can still take part in the rest of the study even if you don't do this bit.

Click on the 'AGREE' button below if:

◯ AGREE
◯ DISAGREE

ACCELEROMETER:

We would also like you to wear a small red box called an accelerometer on an elastic belt 24 hours a day for seven days. This will tell us how much time you spend sitting down, moving around, and sleeping. If you choose to wear the accelerometer you will be asked to complete a little diary about the times your took the device off, and what time you went to bed each night on the days that you wear it.

One of our research team will return to your school the week after this visit to collect the accelerometer. You can still take part in the rest of the study even if you don't do this bit.

◯ AGREE
◯ DISAGREE

Contact Information	
What is your name?	_____ (Preferred first name, Last name)
What is your date of birth?	_____
Age	_____
Phone number (mobile would be best - so we can text you reminders)	_____
What is your home address? (This will be the address where we will send your voucher)	_____ (number & street, suburb, city, postcode)

Do you live at this address during school term?	◯ Yes ◯ No
Do you live in a boarding house during school term? (Don't include private boarding)	◯ Yes ◯ No
Please put the name and/or address of the boarding house _____ (number & street, suburb, city, postcode)	

What is the address that you live at during school term?

_____
(number & street, suburb, city, postcode)

Health Information

If you know your height, please write it here:

_____

What unit is this measurement in?	○ centimetres ○ metres ○ feet and inches
If you know your weight (in kg) please write it here:	_____
Have you been diagnosed with diabetes?	○ Yes ○ No
If so, which type?	○ Type 1 diabetes ○ Type 2 diabetes ○ Don't know
Do you avoid eating gluten?	○ Yes ○ No
Have you been diagnosed with either coeliac disease or gluten intolerance?	○ Yes - coeliac disease ○ Yes - gluten intolerant ○ No diagnosis but suspected intolerance or coeliac ○ No
Have you been diagnosed with a food allergy or intolerance? (not gluten)	○ Yes ○ No
Which foods are you allergic or intolerant to? (Select as many as apply)	☐ Eggs ☐ Dairy ☐ Nuts ☐ Shellfish ☐ Other
Other: please specify	_____

Are you vegetarian or vegan?	○ Yes ○ No
Which foods do you eat? (Select as many as apply)	☐ Egg ☐ Milk (not plant milk like soy milk) ☐ Fish or seafood ☐ Chicken or poultry ☐ Meat/red meat occasionally ☐ None of the above
Are you vegan?	○ Yes ○ No
How long have you been following this way of eating?	○ Less than a month ○ Between 1 and 6 months ○ Between 6 months and 1 year ○ Between 1 and 2 years ○ More than 2 years ○ My whole life

The following questions are a bit sensitive, but it is necessary for us to ask them because they can help us understand what nutrients are important for the health of young women your age

How old were you when you had your first period?	○ 11years or younger ○ 12-14 years ○ 15years or older ○ I haven't had a period yet
How long do you usually have from the start of Your period to the start of the next?	○ Less than a week ○ 1-2weeks ○ 3-4weeks ○ 4-5weeks ○ More than 5 weeks ○ I haven't had a period for 3 months ○ The timing of my periods is not regular
How many days does your period usually last? (count your light days as well as your heavy ones)	○ Less than 4 days ○ 4-6days ○ 7-9days ○ 10days or more
Are your periods so heavy that they make it hard for you to go to school?	○ Yes - often ○ Yes - sometimes ○ No
Have you donated blood?	○ Yes ○ No
When did you last donate blood?	○ In the last 4 months ○ Between 4 and 12 months ago ○ More than a year ago
Have you had a nosebleed in the last year?	○ Yes ○ No
Do you have nosebleeds regularly?	○ Yes ○ No
Over the last year, on average how often did you get nose bleeds?	○ More than once a week ○ Once a week ○ Every couple of weeks ○ Once a month ○ Every few months ○ Every 6 months ○ Once a year ○ Less than once a year
Do you use any of the following contraceptives: - Oral contraceptive (eg 'the pill' or 'the mini-pill') - Depo Provera injection - Implant (eg Jadelle) - Hormonal IUD (eg Mirena)	○ No - I don't use those contraceptives ○ Yes - I use one of those contraceptives

Other information

Which ethnic group do you belong to? (Mark those that apply)	☐ New Zealand European ☐ Māori ☐ Samoan ☐ Cook Island Maori ☐ Tongan ☐ Niuean ☐ Chinese ☐ Indian ☐ Other such as Dutch, Japanese, Tokelauan, Please state….
Other: please state	_____
Please let us know which type of gift card you would prefer:	○ New World ○ PaknSave

Thank you for enrolling in the SuNDiAL project!

What happens next?

We are now going to ask you to complete a questionnaire about why you eat the food you do. If you want to complete it at a later time, please click the Save and Return button at the bottom of this page (don't forget to make a note of your code so that you can return to this survey). Or, click the "Submit" button to continue.

You will also get an email and/or text to tell you when you can visit the SuNDiAL clinic at your school to complete the other measurements.

Appendix D: 24-hour recall protocol

24 Hour Recall

Introduce yourself to the participant, thank them for participating in the sundial project and ask them to take a seat.

"I am going to ask you about everything that you ate and drank yesterday. Please try to recall, and tell me about everything that you had to eat at drink, whether it be at home, or away from home, including snacks, drinks and water."

Stage One – Quicklist
"First, we will make a quick list of all the things you ate and drank, and then we will go back over this list and I will ask you more details about the specific foods and drinks, and the amounts."

"It might help you remember what you ate by thinking about where you were, who you were with, or what you were doing yesterday; like going to school, eating out, or watching TV. Feel free to keep these activities in mind and say them aloud if that helps."

"So starting from midnight the day before yesterday, what was the first thing you remember eating?"

Start recording quick list – keep prompting until finished

"That's great. Sometime people forget to tell us about drinks, particularly water when we do this list."

"How much water do you remember drinking yesterday?" (*record*)

"Did you have any other drinks you might have forgotten about?" (*record*)

Stage two – Collect more information
"I am now going to ask you some more specific questions about each food. We also need to work out how much of each food that you ate or drank"

"Lets start at the beginning – the first thing you remember eating was xxxx" *(record)*

What time did you eat/drink that? *(record)*

Go on to collect specific information that is relevant to each food based on the tips provided on the tip sheet. Record as much specific information as you can. Record each food item in a different row.

Use the photos and measurement aids to help the participant estimate the portion size. Remember that brand and package size will always give you the most accurate information.

Before you go onto the next food on the quick list be sure to ask if they added anything to the food they have just described.

Stage 3 – check for any further additions

"Ok, thanks for working with me to provide all of that detail. We are now going to do one more check to make sure there isn't anything else that should be on this list. I am going to read this list back to you. If you remember anything else that you ate while I am reading it back to you please interrupt me and we will record in"

Read through with the participant all the food and drink they have listed

"Is there anything you can think of that we need to add in?" (record as necessary)

"Last Question: Do you know if the salt you use at home contains iodine?" (tick appropriate box)

"Great thank you again. If it is ok with you one day in the next week I would like to ring you and go through this process again on a different day, so that we can get an idea of how the foods you eat change from day to day. What time of the day (outside of school time) would suit you for me to ring you?"

Record preferred times - remember, ideally this second 24 h recall will occur on a randomly selected day, but that might not always be possible (at the very least it should be a different day of the week than today)

Tips Sheet

Remember that the more information you can obtain about each food the more accurate the data is going to be. Please keep in mind that some of your fellow MDiet students are writing their thesis on nutrients (like Folate) that will vary from brand to brand depending on fortification so please be as careful and accurate as possible.

You need to gather more information about each food identified on the Quicklist. Below are some prompts that might help you do this.

Where possible for packaged foods collect the brand name

Potential questions to consider asking (depending on the food reported)

- What is the brand name?
- Was it fresh, canned, frozen or rehydrated?
- Was it home made? Do they know the recipe? If they do record on the recipe sheet) – this is more important for savory foods than baking (as the basic composition of a biscuit or a cake varies much less than the composition of, for example, a stir fry)
- How was it cooked? Was it baked, fried, or boiled
- Was the item coated before cooking, if so what it with flour, batter, eggs, or breadcrumbs etc?
- Was it standard, low fat, low sugar caffeine free?

Do not

- x Collect information about herbs and spices that are used in very small quantities
- x Ask leading questions
- x Ask for recipes for traditional home baking, but do note if it is gluten free.
- x Make assumptions

Do

- ✓ Keep your prompts neutral
- ✓ Ask about cooking method and the type of fat used in cooking e.g. if they say baked, ask what with?
- ✓ Collect brand names for margarine, butter, juices/fruit drinks, breakfast cereals, energy drinks, breads, dairy alternatives (e.g. almond milk) as the micronutrient content of these products can vary considerably from brand to brand.
- ✓ Ask for the recipe for less traditional home baking (e.g. brownies made with black beans, raw caramel slice etc)

Useful Prompts for Specific Food Groups

FRUIT

- Peeled or unpeeled
- Colour? – e.g. red/green apple
- Tinned? – if so was it tinned in syrup or juice, how much of the syrup/juice did they have
- Use photos of tinned peaches, wooden balls, cups or beans to help estimate portion sizes

VEGETABLES

- Fresh, frozen or Tinned (if tinned were they tinned with flavoured sauce/syrup/juice)
- Cooking method – boiled, baked (with fat/oil – what type and how much?), microwaved, steamed etc
- Colour – e.g. red/green capsicums
- Potatoes – with or without skin, if mashed what was added and how much?
- Quantities could be recorded in cups (sliced/whole/mashed/diced) or how much of a whole vegetable (e.g. ½ a medium capsicum)
- Use photos to help estimate portion size for similar vegetables not shown in pictures (e.g. broccoli can be used to estimate cauliflower, peas can be used for corn or bean etc). Use thickness guides and rulers to help estimate sliced vegetables (e.g. cucumber).

DAIRY

- Milk – brand name and fat content (show picture of bottle tops)
- Yoghurt – brand and with fruit or plain/natural or vanilla, reduced fat, low fat
- Ice cream – brand, any additions? If in a bowl use pictures to help estimate amounts.
- Cheese - - type (e.g. Edam, Colby, Feta), brand, grated (in cups or use pictures) or sliced (thickness guides)

NUTS

- Roasted, raw, salted, other favouring, blanched
- Whole, chopped, slivered
- Mixed – with or without peanuts
- How many cups or how many whole nuts? or can use beans to estimate handful size

BREAD

- White, wholemeal, wholegrain, light or dark rye (use photos to help with identification)
- Brand name (important for fortification)
- Toast or sandwich slice (thick or thin)
- For buns – any toppings (don't worry about small amounts of seeds, but do record cheese, bacon etc)

MARGARINE/BUTTER/TABLE SPREAD

- People often use the term butter and margarine interchangeably so collect the brand name (do not comment on the fact they might not have used the correct description)
- Low fat or standard

- Phytosterols (cholesterol reducing)
- Use pictures to help indication of thickness of spread

DRINKS

- Juices/Fruit Drinks
  - Terms used interchangeably so always collect brand information if possible
  - 100% juice or fruit drink
  - No sugar added or sweetened?
  - Added vitamins
  - Commercial or freshly squeezed
  - Did they dilute with water, is so how much
  - Use cups or pictures of cans and bottles to help estimate portion size

- Fizzy drinks
  - Brand
  - Flavour
  - Diet, standard, zero sugar, type of sweetener
  - Caffeinated
  - Use cups or pictures of cans and bottles to help estimate portion size
  -

- Made from liquid (cordial) or powdered concentrate (raro)
  - Brand and flavour details of concentrate
  - Standard or low energy/ low sugar version
  - How much concentrate?
  - Did they make it with water or something else?
  - How much water or other substance was added?

PACKAGED FOODS

- Brand and package size most important
- Did they consume everything in the packet?

MIXED DISHES

- Try and record recipe if possible
- If recipe unavailable try and get as much detail as possible
- Check any protein ingredients, starchy ingredients, vegetables, sauces
- Use photos, cups, plates and bowls to estimate portion size

Appendix E: 24-hour recall data recording sheets

First ☐ or Second ☐ 24 h Recall

Participant ID: _____ Interviewer: _____

Date: _____ Day of the Week: _____

QUICK LIST

Ask about water consumption at the end of quick list

Participant ID: _____ Interviewer: _____

Date: _____ Day of the Week: _____

Time	Description of Food or Drink	Brand	Amount	Leftovers/Second helpings

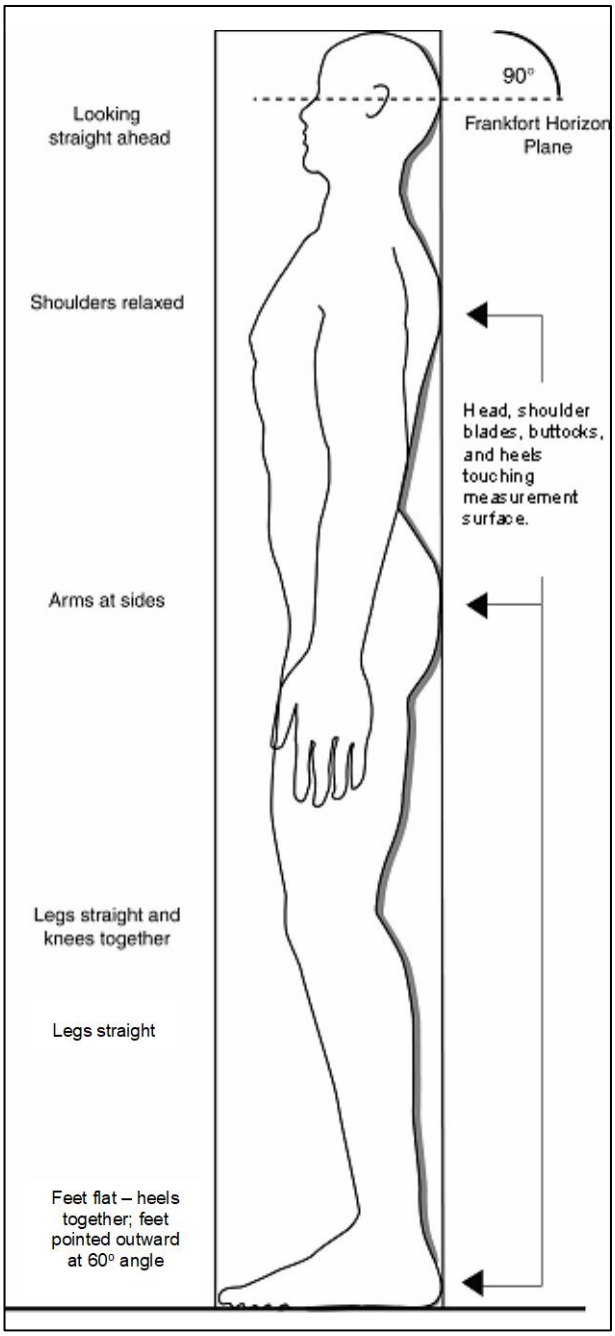
Is the salt used at home iodised? Yes ☐ No ☐ Don't know ☐ Don't use salt

Appendix F: Anthropometry protocol

ANTHROPOMETRIC MEASUREMENTS

Gain verbal consent from the participant for each measurement and explain fully what you will do to obtain them. Before beginning, gain consent from the participant to use non-permanent pen for marking anatomical land marks.

NB: anthropometry tapes have a blank lead before measurement markings start - consider this when reading a measurement.



HEIGHT

1. Ask the participant to remove their shoes, as well as any hair ornaments or buns/braids on the top of the head.

2. If the participant is taller than the investigator, use a step tool to take the measurements. Errors can be minimised by the investigator being parallel to the participant and the headpiece.

3. Tell the participant to stand with their heels together and toes apart pointing outward at approximately a 60-degree angle.

4. Make sure the back of the head, shoulder blades, buttocks, and heels of the participant are touching the backboard/stadiometer.

5. Make sure the participant's head is aligned in the Frankfort horizontal plane, where a horizontal line connects from the ear canal to the lower border of the orbit of the eye.
6. Lower the headpiece to rest firmly on the top of the participant's head and ask the participant to stand as tall as possible and take a deep breath.

7. Record the result to the nearest 0.1 cm in the HEIGHT 1 box on the recording sheet without informing the participants.

WEIGHT

1. Ask the participant to remove any heavy clothing (such as jackets, heavy tops, boots etc). As the participant would have just had their height measurement done, they should not be wearing shoes.

2. Turn on the scales, ensure they are switched on to metric (kg).

3. Ask the participant to step on to the scales so that they are facing away from the display (prevent seeing the weight) cautioning them that they need to step up onto the scales.

4. Wait for the scales to read or come to a stable number.

5. Record the participant's weight to the nearest 0.1 kg in the WEIGHT 1 box on the recording sheet without informing the participant

Ulna length:

Ulna length is measured between the point of the elbow and the midpoint of the prominent bone of the wrist using an anthropometric tape. This value is then compared with a standardized height conversion chart. Participants should be dressed in light clothing with no wrist which or other jewellery on the arm that is to be measured.

1. Measure between the point of the elbow and the midpoint of the prominent bone of the wrist (non-dominant side).

2. Read and accurately record the measurement to the nearest 0.1 cm in the UNLALENGTH 1 box on the recording sheet without informing the participants

REPEAT ALL MEAUREMENTS

Repeat all three measurements again, in the same order, entering the measurements in the HEIGHT 2, WEIGHT 2 and ULNA LENGTH 2 box as appropriate (do no tell participant measurements).

CHECK: are any of the 1st and 2nd measurements are more than 0.5 units apart? If so take a third measurement where required.

Appendix G: Anthropometric recording sheets

Participant ID: _____ Interviewer: _____

Date: _____ Day of the Week: _____