Zinc intakes and food sources in vegetarian compared to non-vegetarian adolescent females in New Zealand

Annabel Lucy Cowan

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Abstract

Background: The number of individuals choosing to follow a vegetarian, or reduced meat, diet is thought to be increasing in high income countries such as New Zealand. Consuming a vegetarian diet with adequate macro- and micro-nutrients is achievable, however a vegetarian diet needs to be appropriately planned. The adequacy of zinc intake, in particular, has been reported to be lower in those following a vegetarian diet. This is due to both the exclusion of flesh foods, a rich source of bioavailable zinc, and due to higher intakes of food components that have an inhibitory effect on zinc absorption in vegetarian diets. This is of concern due to the plethora of roles zinc plays in the body, including in the immune system and numerous metabolic pathways. It also plays an important role in growth and development as individuals progress from childhood to young adulthood. However, data comparing the zinc intakes of vegetarian and non-vegetarian adolescent females do not exist in New Zealand.

Objective: To compare the zinc intakes, adequacy of zinc intake, and food sources of zinc in healthy adolescent female vegetarians and non-vegetarians in New Zealand.

Design: The SuNDiAL (Survey of Nutrition, Dietary Assessment, and Lifestyle) project was a cross-sectional, observational study. Participants were recruited throughout New Zealand through high schools, or recruitment targeted at vegetarians. Participants were females 15 to 18 years of age who were asked to self-identify as either vegetarian or non-vegetarian.

Methods: Online questionnaires were completed to gather demographic and lifestyle information. Two 24-hour diet recalls were used to collect dietary data: one face-to-face, with a second non-consecutive one collected via telephone. The second diet recall was to
allow usual intake to be calculated using the Multiple Source Method (MSM). The diet recalls were entered into, and analysed, in FoodWorks, a dietary assessment software programme which uses NZ food composition tables to calculate nutrient intakes. The prevalence of inadequate intake was determined using the estimated average requirement (EAR) cut-point method.

**Results:** The Candidate’s analysis comprised 250 participants: 31 vegetarians and 219 non-vegetarians. Zinc intake on average was 7.5mg and 9.5mg per day for vegetarians and non-vegetarians, respectively. This difference of -2.0mg/day was statistically significant. Using the EAR cut-point method with an EAR at 6mg per day, 16.1% of the vegetarians were observed to be at risk of inadequate intake compared to 6.4% in the non-vegetarian group. However, when the EAR was increased to 9mg per day based on recommendations for strict vegetarians, 80.6% of the vegetarians had an inadequate intake. The ‘grains and pasta’ food group provided the highest proportion of zinc in the diets of both groups. The vegetarians were consuming more zinc (15.2%) from ‘vegetables’ than the non-vegetarians (6.6%).

**Conclusion:** When using an EAR recommended for those following a vegetarian diet, a large proportion of the vegetarian group had inadequate intake which warrants further investigation. This indicates vegetarians may be at a higher risk of having inadequate zinc status. Further research into the biochemical zinc status of vegetarians will give more insight into the risk of deficiency.

**Key words:** Zinc, intake, adolescent females, vegetarian, non-vegetarian, New Zealand.
Preface

The concept, study design and data collection protocols were developed by the Principal Investigators (PIs), Dr Jill Haszard and Dr Meredith Peddie, from the University of Otago. The PIs developed a ‘Protocol Manual’ which outlines further details regarding the background and an overview of the study (Appendix A). The PIs gained ethical approval, applied for funding and oversaw data collection. Liz Fleming (MSc dietary assessment, NZRD University of Otago) provided training and assistance with FoodWorks, the dietary assessment software. Dr Jill Haszard was responsible for the processing and part of the statistical analysis of the raw data. Write up of this thesis was supervised by Associate Professor Anne-Louise Heath from the Department of Human Nutrition, University of Otago.

Data collection for this thesis was part of the SuNDiAL study (Survey of Nutrition, Dietary Assessment and Lifestyle). Data were collected by 29 data collectors (including the Candidate), who were all Master of Dietetics (MDiet) students. The Candidate carried out data collection in the Wellington region in the second semester of the 2019 university year.

The candidate was responsible for the following under supervision:

- Co-ordinating with Kapiti College staff to organise school visits to undertake data collection.
• Alongside another student, delivering a presentation, answering questions about
the study and distributing take home information to potential participants at
Kapiti College.
• Collecting preliminary details from participants.
• Collecting anthropometric data and two 24-hour recalls from 12 participants at
Kapiti College.
• Organising collection of blood and urine samples from 19 participants at Kapiti
College.
• Entering 24 diet recalls into FoodWorks.
• Assisting the data collectors in Whangarei who recruited a large number of study
participants. This involved contacting and telephoning 12 of their participants
and carrying out their second 24-hour diet recalls.
• Entering the 24 hour diet recalls for the 12 Whangarei participants into
FoodWorks.
• Assigning phytate per 100 grams values to 2,124 food items and recipes in
FoodWorks.
• Statistical analysis of the supplement intake for 35 participants.
• Data cleaning, calculating descriptive statistics and forming all tables and
figures.
• Interpretation of the results.
• Writing of this thesis.
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**Dr Meredith Peddie, Dr Jill Haszard** (Primary Investigators) and **Tessa Scott**, who organised and ran the SuNDiAL project. Without your careful planning and organisation this study would not have happened. I believe our research has filled an important gap in the literature for New Zealand. I look forward to the end results when the SuNDiAL study is completed at the end of 2020.

To my Master of Dietetics (MDiet) colleagues **Lauren Tye, Joshua Hodges** and **Anna Summerfield** who were in Wellington completing their thesis at the same time. Your company, support and humor throughout the past five months have made this experience much more enjoyable.

To my **family** back at home in Timaru, always checking in on me and sending care packages to keep me going. Not only during my thesis but throughout my past five years of University studies.

To all the **study participants**, without you this study would not have been possible. Thank you for your time and for sharing personal information with us.
To various cafes around Wellington – in particular Maranui Beach Café, Spruce Goose, and Prefab Eatery for a supply of superb coffee, sweet treats and Wi-Fi connection when I needed a break from the library. Thesis writing was always much better with a change of scenery.
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List of Abbreviations

ANS - Adult Nutrition Survey 2008/09

BMI - Body Mass Index

EAR - Estimated Average Requirement

FFQ - Food Frequency Questionnaire

g - grams

IZiNCG - International Zinc Nutrition Consultative Group

Kg - kilogram

kJ - kilojoules

m – metres

MDiet - Master of Dietetics

mg - milligrams

MoH - Ministry of Health

N/A - Not applicable

NRV - Nutrient Reference Value

NZ - New Zealand
NZRD - New Zealand Registered Dietitian

RDI - Recommended Dietary Intake

SD - Standard Deviation

SuNDiAL - Survey of Nutrition, Dietary Assessment and Lifestyle

WHO - World Health Organization
1. Introduction

Zinc is an essential nutrient for humans (1). During adolescence individuals go through physiological changes including growth and development which require zinc (2, 3), and low intake of zinc can affect such processes (4). In moderate to severe cases this can lead to growth stunting and delay of sexual maturation (3, 5, 6). Zinc also plays an important role in other body functions such as immunity, vision, taste and cognitive development (7). Ensuring adolescent females are achieving adequate zinc nutriture is also of importance as at this age pregnancy is a possibility. Having adequate nutriture for all nutrients, including zinc, is important during pregnancy for both the mother and the developing fetus (8, 9).

The recent EAT-Lancet Commission discussed the benefits for human health and the environment of moving towards more plant-based diets, with low or no flesh foods (10). Additionally, vegetarian diets are thought to be on the rise in high income countries such as New Zealand (11), which warrants research into the adequacy of such diets. When an individual reaches adolescence they are likely to gain more autonomy over the food they eat and may change their eating choices. Some of the eating practices they may follow, including vegetarianism, require careful planning to provide adequate amounts of essential nutrients (12), particularly in cultures where vegetarianism is not the norm. Vegetarians are thought to be at an elevated risk of zinc inadequacy compared to non-vegetarians (3, 4). This is due to: flesh foods, which are generally a rich source of zinc (13), not being included in such diets; the expected increase of food components such as phytate which decrease the bioavailability of zinc in their diets (14-16); and vegetarian
diets often having a lower energy content, all of which can lead to lower nutrient intakes (3). These potential impacts are so significant that recommendations have been made to increase the Estimated Average Requirements (EAR) for zinc by “about 50% for strict vegetarians” (17). This increases the EAR from 6mg to 9mg per day for New Zealand female adolescents who are strict vegetarians. Therefore, it is important to investigate zinc intake and adequacy of zinc intake in New Zealand vegetarian adolescent females, compared to their non-vegetarian counterparts.

The zinc intake of vegetarians and non-vegetarians from a range of age groups has been assessed in the literature internationally (14, 18-21). However, data describing the zinc intake of healthy adolescent females in New Zealand following vegetarian diets do not exist. Overseas data often suggests that adult female vegetarians have a lower zinc intake than non-vegetarian females (18-21). However, the overseas literature assessing the zinc intake of vegetarians compared to non-vegetarians often fails to adequately quantify the effects of the most potent inhibitory food component on zinc bioavailability, phytate (7). The most recent New Zealand Adult Nutrition Survey (NZ ANS 08/09; (22)) estimated zinc intake for female adolescents in New Zealand, however, vegetarians were not distinguished from non-vegetarians (22). Thus, these results cannot be used to indicate the zinc intake and adequacy of female adolescent vegetarians in New Zealand and so there remains a gap in the literature.

The aim of this thesis was to compare the zinc intake and zinc adequacy of healthy vegetarian and non-vegetarian female adolescents in New Zealand.
2. Literature Review

2.1 Literature review methods


Reference lists from original and review articles were also used to find suitable literature. Only publications available in full text and English were used. Studies completed in males only, where males and females were grouped together, or in developing countries, the elderly, or very young children were excluded due to limited relevance to adolescent females in New Zealand (NZ).

2.2 What is vegetarianism?

A vegetarian is defined as an individual who does not consume any flesh foods (red meat, poultry, fish and shellfish) (12). With its increasing popularity in high income countries, such as NZ (11), a range of variations are also becoming common. A Lacto-ovo-vegetarian (LoV) is the formal term for the most common type of vegetarian. Individuals following this dietary pattern consume fruits, vegetables, cereals and grains, nuts and seeds, eggs and dairy products (23). A Lacto-vegetarian (LV) follows the same dietary pattern as an LoV, without consumption of eggs (23). Veganism is another
dietary choice and has no consumption of any foods of animal origin (23). Some individuals also follow ‘low meat’ diets which may limit the frequency of their meat consumption, popularly referred to as “flexitarian”.

Only commercial market research has quantified the prevalence of general vegetarian diets in the NZ population, suggesting an increase in prevalence from 8.6% to 13.3% in males and females aged 14-24 from 2011 to 2016 (11). There is a gap in the scientific literature regarding the proportion of New Zealanders following vegetarian diets.

Reasons for following such diets are wide and vary between individuals. Some common reasons are animal rights and welfare, environmental concerns, health reasons, cultural beliefs and practices, and budget constraints (24-26).

It is important to note that some participants in studies who identify themselves as vegetarian, still consume small amounts of fish or poultry (24). A Finnish study found 80% of the participants who considered themselves ‘vegetarian’ did not actually follow a vegetarian diet when they completed a food frequency questionnaire (FFQ) (27). Incorrect categorisation into the vegetarian group can introduce error and skew results. In regards to zinc intake, flesh foods are generally high sources of zinc (13). Thus, inclusion of participants who consider themselves vegetarians but eat some flesh foods products may cause the zinc intake of the vegetarian group to be higher than its true value.
2.3 Dietary zinc recommendations for vegetarian adolescent females

Zinc is a trace mineral that is widely dispersed within the body (9, 28). It has a range of functions and roles in the body, such as in metabolism, structural aspects of cells, and has a role in the immune system (3, 7, 29). During adolescence puberty increases zinc requirements to aid with physiological changes such as sexual maturation, onset of menstruation and growth (3, 6, 30). This increase in requirements is reflected by increased recommendations. The Ministry of Health (MoH) has developed nutrient recommendations for adolescent females (14-18 years). The Estimated Average Requirement (EAR), the amount estimated to meet nutrient requirements of half the individuals in this life stage and gender group, is 6mg per day (17). The recommended dietary intake (RDI), the intake estimated to meet the requirements of 97-98% of healthy individuals in this life stage and group, is set at 7mg per day (17). Due to multiple aspects of the vegetarian diet leading to increased risk of lower intake and absorption of zinc (3, 31), it is recommended those following a strict vegetarian diet increase their intakes by 50% (32). This shifts the EAR up to 9mg/day, and the RDI up to 10.5mg per day for vegetarian female adolescents.
2.4 Challenges to achieving sufficient intake for vegetarian adolescent females

2.4.1 Physiological

While a vegetarian diet alone has its own challenges to achieve an adequate zinc intake, this can be exaggerated due to increased physiological requirements during female adolescence (3). Physiological changes associated with puberty in early adolescence increase zinc requirements as zinc is required for functions including growth, increased blood volume and sexual maturation (3, 30). Puberty generally occurs in females between the ages of 10 and 15 years (33). Whilst older adolescent (14-18 years) females would have undergone the bulk of their puberty related changes, increased physiological zinc requirements still remain due to the need to replenish depleted tissue zinc pools (2, 3, 34).

There is no specific storage site for zinc, thus cells rely on plasma zinc to supply zinc as required (28). Homeostatic mechanisms in the body maintain adequate serum zinc (28). Regulation of plasma zinc is maintained by adapting absorption, reabsorption and excretion when zinc intake is insufficient (35). However, this homeostatic mechanism can be disturbed during times of higher physiological requirements, such as adolescence (3). This can make it even more challenging for vegetarian adolescents to achieve sufficient intake.

Three to four years following puberty is thought to be a time when approximately one third of skeletal minerals are accumulated (36). Inadequate zinc nutriture is a concern
during this time, as it may contribute to slower skeletal growth and reduced bone mineralization (3).

2.4.2 Dietary (enhancers and inhibitors)

Zinc is known to be heavily impacted by other food components which are commonly consumed in higher quantities in a vegetarian diet, such as phytate (16). Phytate is considered to have the most impact on zinc bioavailability (7). Phytate is a strong chelator. When this food component chelates with zinc it forms an insoluble complex that cannot be digested and absorbed, and thus passes through the gastrointestinal tract (GIT) unabsorbed (7, 37). This is due to the positive charge on the phosphate groups which are attracted to negatively charged trace minerals, such as zinc (38). Phytate occurs in high quantities in foods such as whole grain cereals, nuts and legumes (39). When red meat, poultry and fish are excluded from an individual’s diet, alternative plant sources of protein are substituted to ensure adequate protein intake. Most of these flesh foods excluded are higher in zinc and lower in phytate than their meat alternatives thus posing a potential challenge for vegetarians to absorb sufficient zinc (3, 16).

The phytate:zinc ratio is a tool developed to estimate zinc absorption dependent on the phytate content of the diet. The World Health Organization (WHO) and International Zinc Nutrition Consultative Group (IZiNCG) developed slightly different cut-offs, shown in Table 2.1. Both organisations used similar methods, however, the WHO used data from single meal and total diet studies, whilst IZiNCG used data from total diet studies. Evidence regarding iron absorption suggests the percentage absorbed from a single meal is significantly different to that from a total diet of similar composition (40,
It is thought the same effect may occur with zinc (7), thus studies looking at total diet are likely to be more reliable and reflective of how much zinc we absorb (42).

Overall, both investigations conclude the higher the phytate:zinc ratio, the lower the absorption of zinc (7).

Table 2.1: Differences between WHO and IZiNC phytate:zinc ratio-based absorption estimates

<table>
<thead>
<tr>
<th>Diet Type</th>
<th>WHO(7)</th>
<th>IZiNCG(7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Highly refined²</td>
<td>Mixed³/ refined</td>
</tr>
<tr>
<td>Study Type</td>
<td>Single meal &amp; total diet</td>
<td>Total diet</td>
</tr>
<tr>
<td>Participants</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Phytate:zinc Ratio</td>
<td>&lt;5</td>
<td>5–15</td>
</tr>
<tr>
<td>Zinc Absorption</td>
<td>50%</td>
<td>30%</td>
</tr>
</tbody>
</table>

¹Based on table in IZiNCG technical brief, chapter 1 (7).
Abbreviations: n: number of studies in analysis, WHO: World Health Organization, IZiNCG: International Zinc Nutrition Consultative Group
¹ Highly refined diets are low in cereal fibre, low in phytate and have adequate protein that typically comes from animal sources.
² Mixed diets where protein comes from animal or fish and vegetarian diets that are based on refined sources.
³ Cereal based diets where >50% of the total energy intake comes from unrefined cereal grains or legumes. Little or no intake of animal protein.

Increasing protein quality and quantity in a meal is also associated with increased zinc absorption (43, 44). This is partially due to protein foods being the major dietary source of zinc (13, 37). The protein type also has an effect on zinc absorption (44). Animal sources have been shown to offset some of the inhibitory effect of phytate (37). The
The proposed mechanism is that the amino acids released during protein breakdown keep zinc in a more bioavailable form, rather than this being an action of the animal protein itself (43).

Increasing zinc content of the diet is associated with a decreasing proportion of zinc being absorbed (37, 43, 45). The proposed mechanism behind this is that zinc is absorbed via zinc specific transporters. At higher zinc quantities in the diet, these transporters become saturated and thus additional zinc cannot be absorbed (37, 46).

Mixed evidence regarding the impact of calcium on zinc bioavailability has been published (37, 38). It is proposed that high calcium in the presence of high phytate may have a synergistic effect on the inhibitory effects phytate typically has on zinc absorption (28, 47). However, the inhibitory effect of calcium on zinc absorption has not been observed in humans, indicating that the inhibitory effect observed in animals has little relevance to humans (37).

### 2.4.3 Behavioural

Adolescent females are easily influenced by others regarding body size and this can result in restrictive diets being adopted (48, 49). A vegetarian diet may be adopted for a number of reasons, one of which may be that it is perceived to be a way to restrict dietary intake. This can lead to poor dietary habits (for example not replacing meat with appropriate protein sources) and lead to low energy and micronutrient intake (3, 4, 14). This can pose yet another barrier for adolescents in attaining sufficient zinc nutriture.
Highly active adolescent females may also find that there are effects of intense physical activity on zinc nutriture. Intense physical activity has been shown to cause increased zinc losses due to increased excretion in urine and sweat (48).

However, adolescents, in particular those who are conscious of their health, may also choose to take zinc containing supplements, which may be beneficial to their zinc intake.

2.5 Methods of measuring zinc intake

Dietary zinc intake can be assessed using a range of methods. When the goal of measuring zinc intake is to estimate the distribution of usual zinc intake within a population, more than one day of food intake data is required (42). This is to be able to correct observed dietary intakes of zinc for intra-individual variation. Intra-individual variation refers to the differences in intake that occur in individuals on a daily basis. When multiple measurements of dietary intake cannot be collected for the entire study cohort, a sub-sample can be used to adjust intakes of the entire group (42, 49). Random and systematic errors occur when measuring zinc intake. Random errors can be reduced by increasing the number of days of dietary data collected (49). Systematic errors cannot be reduced by increasing the amount of data. Instead, it is important to ensure the way it is measured is as accurate and uniform as possible. The majority of the literature assessing dietary zinc intake in developed countries has used weighed food records or 24 hour diet recalls for multiple days which is reflective of IZiNCG recommendations (42). It is also recommended that dietary data are interpreted with data from other measurement methods as well, such as biochemical and clinical data, as this gives more insight regarding risk of zinc deficiency in the chosen cohort (42).
There are a range of quantitative dietary assessment methods, the choice of which depends on time, budget and the skill of the researchers. Weighed food records ask the participant to weigh and record all the food they consume over a chosen period of time. They have been used historically to collect reliable dietary zinc intake data in a range of different settings (42). This method is costly and has a larger burden to the participants than other methods, yet it does result in the most accurate record of intake (42). Estimated diet records are similar, in that they require the participant to record the food they eat during the day, without the use of scales to weigh the food.

Retrospective dietary assessment methods can also be used. Twenty-four hour diet recalls are appropriate for a range of settings as these do not require as much input from the individual being assessed. The participant is asked to recall everything they have eaten in the last 24 hours. This can be done verbally by a trained researcher or by use of a validated online assessment tool which is simple enough for the participants to use themselves. Whilst these are not as accurate as weighed diet records (largely because they require participants to remember everything well, and discrepancies in portions consumed are more likely), it is a less costly method and due to the lower burden on the participants, compliance may be enhanced. Food Frequency Questionnaires (FFQs) are another approach to gather information regarding intake of a nutrient, in this case by assessing intake of foods high in a particular nutrient (or moderately high but eaten frequently or in large amounts) over a period of time. This method is less suitable for measuring zinc intake as it may be harder to quantify accurately due to its wide distribution among many foods.
Information gathered from the chosen dietary assessment method must then be entered into dietary assessment software to estimate the quantity of zinc consumed. The food composition database used must be appropriate for the population the data were taken from, e.g. reflective of the nutritional content of the foods in the country (42). Calculating intake of other food components that impact bioavailability, such as phytate is also important to evaluate the impact of these in the diet. The challenge with this is that composition data regarding phytate are not available in the NZ food composition tables (13).

2.6 Zinc intakes in vegetarian compared to non-vegetarian adolescent females

There are no published data comparing zinc intakes of vegetarian and non-vegetarian adolescent females in NZ. The NZ Adult Nutrition Survey 2008/09 (22) is the most recent assessment of zinc intake in a large representative study cohort. Results were weighted to be representative of the residents of NZ. The median (10th, 90th percentile) zinc intake for 15 to 18 year old female adolescents was 8.7 (6.4, 12.1) mg/day, with 6.5% of female adolescents at risk of inadequate intake. It was reported only 1% of the total study cohort had not consumed meat in the four weeks prior to completing their 24-hour diet recall. The proportion of vegetarians from the adolescent cohort was not reported, thus the data cannot be used to infer the zinc intake of vegetarians.

Several studies compare zinc intake in vegetarians and non-vegetarians in older females in other countries. A meta-analysis of 12 studies in females of all ages concluded that the zinc intakes of vegetarians were significantly lower than those of non-vegetarians (mean
difference: -0.90mg/day) (21). However, some of the studies included were very old (a number of them were 33 years ago) and some studies included were in developing countries, thus the data may not be an accurate reflection of NZ eating patterns.

Seven studies that compared zinc intakes between vegetarian and non-vegetarian females, where the cohort’s average age is under 55 years old, were published from 1995 onwards. Studies matching these criteria conducted in countries that are similar to NZ regarding level of economic development, lifestyle and culture have been summarized in Table 2. Three of the seven studies lacked statistical analysis of their results to indicate statistical significance and thus were excluded from Table 2. Of those three studies, two suggested lower zinc intakes in vegetarians (50, 51) and one suggested a higher zinc intake in vegetarians compared to non-vegetarians (52).

Only one study, involving Canadian adolescents, assessed zinc intake in vegetarian and non-vegetarian female adolescents (14). This study found no significant difference in median zinc intake between vegetarian (6.7mg/day) and non-vegetarian (7.9mg/day) female adolescents (14). The data were collected at least 23 years ago so the results are unlikely to be reflective of current NZ eating patterns and the composition of food in NZ. These factors may explain why the zinc intake of NZ adolescents measured in the NZ Adult Nutrition Survey discussed above was higher than both groups of this study. The vegetarians in the Canadian study had a significantly higher intake of phytate than the non-vegetarians (14). Due to this, the Phytate:Zinc molar ratio was also significantly higher in the vegetarian group compared to the non-vegetarians (14 and 9, respectively; (14)). This suggests that whilst the groups had similar total zinc intakes, absorption may differ due to the phytate content of their diets.
Of the studies presented in Table 2.2 in older females, a range of zinc intakes have been observed. Vegetarian zinc intake ranged from 6.7-12.0mg/day, and 7.9-16.0mg/day for non-vegetarians (14, 18-20). Only one of these studies found that vegetarian zinc intakes were not significantly lower than those of non-vegetarians (14). The wide ranges of zinc intakes can be attributed to four main differences between the studies. First, differing definitions of ‘vegetarian’ were used. Some studies classified participants as ‘vegetarian’ if they excluded red meat yet consumed small amounts of poultry or fish (20). This could cause the zinc intake of the vegetarian group to appear higher as fish and poultry contain relatively high levels of zinc (13). Second, the level of guidance given to participants to fill in their diet records differs, as cohorts who were given more guidance (e.g. individually taught how to complete an accurate diet record (18)) were more likely to complete accurate diet recalls. Third, differences in recruitment may have had an impact. One study used a convenience sample recruited from a university (19) where 20% of the participants were studying nutrition. This cohort was, therefore, more likely to be more health conscious and to have a higher education level and income. This type of sampling may also mean they follow a more balanced diet, making them less likely to have low nutrient intakes. Fourth, dietary assessment methods differed. One study in particular used a different method to measure zinc intake and this may have impacted on the results. This study conducted on Australian female young adults reported surprisingly high mean zinc intakes in both vegetarians and non-vegetarians at 12±3.0mg/day and 16±5.0mg/day, respectively (19). This was the only study that used a Food Frequency Questionnaire (FFQ) alone to assess dietary intake of zinc and this is likely to be responsible for the striking results. A paper describing the FFQ validity trial, where energy adjusted nutrient intakes from a 24 hour diet recall were compared to
results from the FFQ, reports that estimated zinc intake from the FFQ (19.3±4.3mg/day) was significantly and substantially higher than the results of the 24 hour diet recall (11.5±2.5 mg/day) (53). Despite the higher values, the significantly lower zinc intake in vegetarians cannot be dismissed entirely as both vegetarian and non-vegetarian zinc intakes were measured using the same FFQ.

These studies suggest that zinc intake in vegetarian adolescents is likely to be significantly lower than non-vegetarians. However, as this has not been investigated in NZ adolescent females, this remains a gap in the literature.
Table 2.2: Studies assessing zinc intake in females from 1995 onwards

<table>
<thead>
<tr>
<th>Reference and country</th>
<th>Participants</th>
<th>Methods</th>
<th>Zinc Intake[^1]</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ball et al. (2000)</td>
<td>Adults</td>
<td>12 day weighed diet record, including three weekend days.</td>
<td>NV: 8.4 ± 2.1mg/day</td>
<td>• Adequacy of zinc intake assessed using Australian RDI. This is not appropriate for determining adequacy so that result cannot be interpreted.</td>
</tr>
<tr>
<td>Australia</td>
<td>NV n = 24</td>
<td></td>
<td>V: 6.8 ± 2.4 mg/day</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V n = 50</td>
<td></td>
<td>Vegetarian zinc intake was significantly lower.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean age: 25.3 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Participants matched for age, sex, smoking status and BMI.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fayet et al. (2014)</td>
<td>Adults</td>
<td>235 item FFQ, measured intake over past three months.</td>
<td>NV: 16 ± 5.0mg/day</td>
<td>• FFQ was the only method used to measure zinc intake.</td>
</tr>
<tr>
<td>Australia</td>
<td>NV n= 200</td>
<td></td>
<td>V: 12 ± 3.0mg/day</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V n= 23</td>
<td></td>
<td>Vegetarian zinc intake was significantly lower.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean age: 22.9 years</td>
<td></td>
<td></td>
<td>• 20% participants were nutrition students.</td>
</tr>
<tr>
<td>Janelle et al. (1995)</td>
<td>Adults</td>
<td>Nine day diet record.</td>
<td>NV: 11.1 ± 3.9mg/day</td>
<td>• Strict inclusion criteria for participants. This may limit generalisability of results.</td>
</tr>
<tr>
<td>Canada</td>
<td>NV n=22, mean age: 27.6 years</td>
<td></td>
<td>V: 8.3 ± 2.1mg/day</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V n= 23, mean age 26.6 years</td>
<td></td>
<td>Vegetarian zinc intake was significantly lower.</td>
<td></td>
</tr>
<tr>
<td>Donovan et al. (1995)</td>
<td>Adolescents</td>
<td>Three day consecutive weighed food records including one weekend day.</td>
<td>NV: 7.9 (6.3, 9.3) mg/day(^2)</td>
<td>V: 6.7 (5.3, 8.3) mg/day(^2)</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Canada</td>
<td>NV n= 29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LoV n= 79</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean age: 18 years</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


\(^1\)Presented as mean (standard deviation) unless stated otherwise

\(^2\)Indicates values presented as median (1\(^{st}\) and 3\(^{rd}\) quartiles).
2.7 Food sources of zinc in vegetarian compared to non-vegetarian adolescent females

The NZ Adult Nutrition Survey 2008/09 (22) collected data regarding food sources of zinc in female adolescents. These data were collected from a large sample size, of participants from all over NZ, so is reflective of a wide range of eating patterns across NZ. However, one limitation as mentioned previously is that vegetarian eating habits were not distinguished from non-vegetarians’. It was reported that only 1% of the participants had not consumed red meat, poultry or fish in the four weeks leading up to the survey, suggesting the results are more reflective of intakes of non-vegetarians.

However, the three highest food groups contributing to zinc intake in female adolescents were plant-based foods that are suitable for a vegetarian diet. These contributors were ‘bread-based dishes’, ‘grains and pasta’, and ‘bread', contributing 12.6%, 12.1% and 8.8% respectively. ‘Beef and veal’ was the next biggest contributor, at 6.9% followed by ‘poultry’ at 6.0% (22).

Not all of the studies presented in Table 2.2 assessed the highest contributors of zinc intakes for vegetarian and non-vegetarian groups. Two of the studies had comments suggesting that zinc intake in non-vegetarians was more likely to come from red meat, fish and poultry, however, this was not supported with data (19, 20). The only study assessing zinc intake of female adolescents found plant-based foods contributed 71% vs 53% of total zinc intakes in vegetarians and non-vegetarians, respectively (14).

Red meat, chicken and poultry, which are commonly consumed foods in a non-vegetarian diet, are rich sources of readily bioavailable zinc (13). These high zinc
density foods did not appear amongst the highest contributors in the studies presented in Table 2.2 that reported the highest zinc contributors. This is likely due to the vast range of commonly consumed zinc-containing foods, that fit into both diets. This may also be due to different flesh foods being classified into numerous different categories (e.g. poultry, pork, beef and veal etc.). If zinc intake from all flesh foods were combined it is likely this food group would become a higher contributor.
3. Objective Statement

Data for this thesis were collected as part of a nationwide survey, SuNDiAL (Survey of Nutrition, Dietary Assessment and Lifestyle). The overall aim of the SuNDiAL survey was to compare the nutritional status, dietary and lifestyle habits, and motivations of vegetarian and non-vegetarian female adolescents in New Zealand.

The aim of this thesis was to compare the usual dietary zinc intake and zinc adequacy of healthy vegetarian and non-vegetarian female adolescents in New Zealand.

The objectives were to, in healthy female adolescents in New Zealand:

1. Determine the usual dietary zinc intake in vegetarians and non-vegetarians.
2. Determine the adequacy of dietary zinc intake in vegetarians and non-vegetarians.
3. Identify the major food sources of zinc in vegetarians and non-vegetarians.
4. Methods

Data for this thesis were collected as part of the nationwide Survey of Nutrition, Dietary Assessment and Lifestyle (SuNDiAL) study. This chapter describes the methods used in the SuNDiAL study that are relevant to this thesis. The study design and protocols were produced by Dr Jill Haszard and Dr Meredith Peddie, the Principal Investigators (PIs). Funding was sourced by the PIs from the Department of Human Nutrition and a Lottery Health Research Grant. The PIs developed a protocol describing the study background and an overview of the SuNDiAL study as a whole (Appendix A). Text in the methods derived from the protocols has been indicated with *italics*.

4.1 Study design

The study had a cross-sectional, observational design with schools recruited across New Zealand. Data were collected in both the first and second semester of the 2019 university year (February 20th – 30th, August 20th – July 30th). The Candidate collected data from the Wellington region in the second semester of 2019.

4.2 Ethics

This study was approved by the Human Ethics Committee, University of Otago (Dunedin, New Zealand), in February 2019 (reference code: H19/004) (Appendix B). The study was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12619000290190).
4.3 Recruitment and participants

Recruitment of participants started in November 2018. Two methods of recruitment were employed: school-based recruitment and targeted recruitment. School-based recruitment aimed to attract healthy adolescent females with a range of dietary habits. Initially recruitment was solely school based, however, only a small number of vegetarians (n=9) were recruited in the first semester so targeted recruitment was introduced. Targeted recruitment was aimed directly at recruiting individuals who followed a vegetarian diet.

The process of school-based recruitment began with an email invitation to the schools to participate in the study. Schools were chosen based on where the data collectors (pairs of Master of Dietetics students) were living for the semester. Schools with a low decile and a large female roll were contacted first. Schools that did not respond within two weeks of the initial email were followed up with a phone call. If this was unsuccessful, a second round of recruitment emails was sent out to other schools, such as those with higher deciles or smaller rolls. The process of recruitment to this point was carried out by the SuNDiAL research team. The research team which will be referred to throughout the methods section consisted of the Principal Investigators, Dr Jill Haszard and Dr Meredith Peddie, and the Study Coordinator, Tessa Scott. If these attempts were unsuccessful, word of mouth was used to recruit schools.

Schools that showed interest were telephoned or emailed to organise dates for the data collectors to visit and give a presentation regarding the study. This presentation gave the potential participants background to the study, what it would require from them, and the
reimbursement details. Participants were reimbursed for their time with a $5 supermarket voucher for each part of the study they completed: questionnaires, first 24-hour recall, second 24-hour recall, blood, urine, accelerometer. The Candidate was part of the team who presented the study on two occasions to students at Kapiti College, the semester two school in Wellington. The potential participants were given the opportunity to ask questions and take home an information sheet. If they were interested in participating, they provided their contact details, either after the presentation or over the following week using a sign-up sheet left at the school. Girls under 16 years of age were required to provide their parents’ contact details when signing up so their parents could provide online consent for their daughter to participate. The participants who signed up were then emailed a link to a REDCap (Vanderbilt University, Nashville) site where they were able to consent to the study and complete the enrolment, dietary habits and attitudes, and motivations questionnaires.

The following schools were recruited for the study:

- Tauraroa Area School (Whangarei)
- Whangarei Girls College (Whangarei)
- Mt Maunganui College (Tauranga)
- Spotswood College (New Plymouth)
- St Catherine's College (Wellington)
- Kapiti College (Kapiti/Wellington)
- Waimea College (Nelson)
• Hornby High School (Christchurch)

• Mt Aspiring College (Wanaka)

• Columba College (Dunedin)

• Kaikorai Valley College (Dunedin)

• Queens’ College (Dunedin)

• Bayfield High (Dunedin)

Targeted recruitment was carried out in Dunedin by the SuNDiAL research team by advertising for females 15-18 years of age who considered themselves to be vegetarian. Advertising was done through Facebook, Instagram and the University of Otago Staff News. Interested females were directed to the study website where a video summarising the study was available to watch in addition to written information. Interested individuals were able to provide their details and were sent the same email with a REDCap link as was used for the school-based recruitment.

At recruitment, the inclusion criteria for the study were: female 15 to 18 years of age, and either enrolled in one of the recruited schools or living in Dunedin and identifying as being vegetarian. Female was defined as self-identifying as female gender. Exclusion criteria were: not being able to speak and understand English, and pregnancy (this was left to the participants to decide).
After recruitment, participants who enrolled and participated in the study were excluded from the Candidate’s analysis if: they did not complete at least one 24-hour diet recall, or if they did not identify as vegetarian or non-vegetarian.

4.4 Data collection

Once participants (or their parent/guardian if they were under 16 years) had consented to take part in the study, they were contacted via text or email by Master of Dietetics (MDiet) students, who will be referred to as the data collectors. The data collectors were students from the University of Otago in their last year of the Master of Dietetics degree. All data collectors took part in training in October 2018 to ensure the protocols regarding data collection were well understood and used. Data collectors were spread across New Zealand in groups of two to four and were responsible for data collection from the participants in their region. The Candidate was located in the Wellington region in a group of four data collectors undertaking data collection in the second semester. Once participants had been contacted by the data collectors, they were booked in for a 30-minute time slot for their first session of data collection. This would take place at either a designated room at the school (school-based recruitment) or the University of Otago Student Dietitian Clinic (targeted recruitment). This included anthropometric measurements, first 24-hour diet recall and booking them in for their second 24-hour diet recall. The General Data Collection Protocol (Appendix C) was followed. For each data measurement taken, the process was explained to the participant and verbal consent was gained.
4.4.1 Questionnaires

Participants were asked to complete three questionnaires: enrolment, dietary habits and attitudes, and motivations. Most of these questionnaires were previously validated (54-57). These were completed by participants online via the REDCap platform. The link to the questionnaire was emailed to participants and they were to complete them in their own time. Participants could pause the questionnaires and come back to them at a later point to complete. Data collectors could see who had not finished completing the questionnaires and were able to prompt the participants to complete them, either in person or via text/email.

For this thesis, relevant data collected from the questionnaires were: ethnicity; date of birth; home address; self-identified vegetarian status (i.e. non-vegetarian, vegetarian or vegan) and, if vegetarian/vegan, length of time that approach had been followed; and supplements used (Appendix D).

Where participants identified with more than one ethnicity, they were placed in the ethnicity category with the highest priority. Ethnicity categories were (high to low priority): Māori, Pacific Islands, Asian, New Zealand European or other.

Home addresses given by participants were used to calculate their New Zealand Index of Deprivation score NZDep13 (58). This score applies to a meshblock of homes in the area thus is not a specific marker of the household socio economic status (SES). A lower score indicates higher SES (least deprived), with a higher number indicating lower SES in the area.
4.4.2 Anthropometry

An inter-reliability study focused on anthropometric measurements was carried out prior to the beginning of data collection to ensure consistency between data collectors. Twenty-seven of the 30 data collectors, including the Candidate, took part. Data collectors were provided with the anthropometry protocol (Appendix E). A convenience sample of 12 adolescent females 15 to 18 years of age consented to having their weight, height and ulna length (not reported in this thesis) measured. Each data collector took these measurements from four participants twice, in a non-successive order. Results were recorded on separate sheets of paper. Inter-rater reliability was assessed using mixed effects intra-class correlation coefficients (ICC). All ICC results indicated agreement between data collectors. The greatest variation occurred when measuring height for the tallest participants. This highlighted to the research team that data collectors needed to be parallel to the participant they were measuring when taking the reading. Following this the research team provided a stepstool to use when measuring height.

The same anthropometry protocol as mentioned above was used for the rest of the SuNDiAL survey to collect height, weight and ulna length. Height was measured using either a Seca 231 or Wedderburn stadiometer issued by the University of Otago. *Participants were asked to remove shoes and any hair buns/braids/ornaments on top of their heads. Participants were asked to stand with their heels together, toes apart pointing outwards. Back of the head, shoulder blades, buttocks and heels were to be touching the stadiometer. Participants were asked to look straight forward, at a 90° angle.* Data collectors, as mentioned above, *used a stepstool to ensure they were parallel*
with the headpiece when taking the measurement. This was to reduce errors. Height was measured to the closest 0.1cm, using the headpiece which was lowered firmly onto the participant’s head.

Weight was measured using a set of electronic scales (see Table 4.1), issued by the University of Otago. The scales were placed on a hard, even surface and the participant was asked to step onto the scales without shoes and bulky clothing, or items in their pockets. The data collector waited for the scales to read and come to a stable number. Weight was recorded to the closest 0.1kg.

**Table 4.1: Scales used for weight measurements**

<table>
<thead>
<tr>
<th>Make</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medisana</td>
<td>PS 420</td>
</tr>
<tr>
<td>Salter</td>
<td>9037 BK3R</td>
</tr>
<tr>
<td>Seca</td>
<td>Alpha 770</td>
</tr>
<tr>
<td>Soehnle</td>
<td>Style Sense Comfort 400</td>
</tr>
</tbody>
</table>

All anthropometric indices were measured in non-successive duplicates unless results varied by more than 0.1cm or 0.1kg. If that was the case the measurement was taken a third time, and an average from the two closest was used.

Body Mass Index (BMI) was calculated as weight (kg) divided by height squared (m²). Due to participants being of an age where they were still growing, it was most appropriate to look at BMI for age z-scores, instead of BMI alone (59). Thus, these were calculated using the World Health Organization (WHO) growth charts (59). These were
then used to classify participants into weight categories. When a participant’s BMI z-score was one to two standard deviations from the mean they were classified ‘overweight’. When their z-score was two or more standard deviations from the mean they were classified as ‘obese’ (59).

4.4.3 Dietary intake

Participants completed two 24-hour diet recalls. Data collectors used the 24-hour diet recall protocol (Appendix F). In summary, the first 24-hour diet recall was taken face to face when the participant met with the data collector. The participant was asked to recall all food and drink items they consumed for the 24-hour period, from midnight to midnight the day before. The data collector recorded as much detail as possible, including brand of foods, quantities, recipes and cooking methods. The use of household measures, food models and a book of images of different portion sizes of commonly consumed foods were used to help estimate the quantity of foods consumed. When possible, if the first 24-hour recall was for a weekday, the second 24-hour recall was booked to report a Saturday or Sunday. The second 24-hour recalls were carried out via phone call or video call, depending on the participant’s preference, using the same protocol but without the props for estimating portion size.
Table 4.2: Days of the week which 24-hour diet recalls were collected

**First 24 h recall**

<table>
<thead>
<tr>
<th>Day of the week</th>
<th>Number</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monday</td>
<td>33</td>
<td>13.2</td>
</tr>
<tr>
<td>Tuesday</td>
<td>56</td>
<td>22.4</td>
</tr>
<tr>
<td>Wednesday</td>
<td>67</td>
<td>26.8</td>
</tr>
<tr>
<td>Thursday</td>
<td>62</td>
<td>24.8</td>
</tr>
<tr>
<td>Friday</td>
<td>27</td>
<td>10.8</td>
</tr>
<tr>
<td>Saturday</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sunday</td>
<td>5</td>
<td>2.0</td>
</tr>
</tbody>
</table>

**Second 24 h recall**

<table>
<thead>
<tr>
<th>Day of the week</th>
<th>Number</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monday</td>
<td>60</td>
<td>27.6</td>
</tr>
<tr>
<td>Tuesday</td>
<td>22</td>
<td>10.1</td>
</tr>
<tr>
<td>Wednesday</td>
<td>17</td>
<td>7.8</td>
</tr>
<tr>
<td>Thursday</td>
<td>16</td>
<td>7.4</td>
</tr>
<tr>
<td>Friday</td>
<td>15</td>
<td>6.9</td>
</tr>
<tr>
<td>Saturday</td>
<td>27</td>
<td>12.4</td>
</tr>
<tr>
<td>Sunday</td>
<td>60</td>
<td>27.7</td>
</tr>
</tbody>
</table>

Twenty-four-hour diet recalls were the chosen method for measuring dietary intake due to their low cost and lower participant burden compared to weighed diet records. This method was chosen over a food frequency questionnaire (FFQ) as the overall SuNDiAL aim was to collect dietary intake data regarding a wide range of nutrients and there is no FFQ currently available to measure the intake of multiple nutrients in New Zealand adolescents.
The second 24-hour recall, on a non-consecutive day, was collected to allow for estimation of ‘usual intake’. This was because dietary patterns often differ between weekends and weekdays. This was collected from as many participants as possible so that the prevalence of inadequate intakes could be estimated (60). The Multiple Source Method (MSM) was used to adjust observed dietary intake data to usual intake by accounting for intra-individual variation (61). Information from participants who provided two 24-hour diet recalls was applied to the whole data set to give adjusted estimates of usual intake for each participant in the study.

4.4.4 Other data

The Candidate organised fitting of accelerometers to measure seven days of 24-hour activity and assisted phlebotomists in collection of urine and blood samples from participants. However, these data were not used for this thesis, instead being collected for the wider SuNDiAL survey.

4.5 Data analysis

4.5.1 Dietary data

After completion of the 24-hour recalls, data collectors, including the Candidate, were responsible for entering the data into FoodWorks version 9 Professional edition (Xyris Software, Melbourne), accessed through the University of Otago. The data collectors were trained by Liz Fleming (Department of Human Nutrition, University of Otago). When recipes were given by participants, appropriate nutrient retention factors (62) and
moisture yield factors (63) were used to ensure the effects of cooking methods were reflected in the data. FoodWorks used FOODfiles 2014 version 2 (The New Zealand Institute for Plant and Food, 2014; (13)) which is the New Zealand food composition database plus selected recipes from the NZ ANS08/09 (22), to generate macro- and micro-nutrient quantities for the diet recalls entered. Newer versions of these data exist; however, they could not be utilised due to time constraints and the time it would take to load the newer food composition databases into FoodWorks. FoodWorks was also able to access recipes from previous studies carried out at the Department of Human Nutrition at the University of Otago. These data include zinc content of food items but not phytate content. When a participant consumed a commercial product that was not available on FoodWorks, the nutritional panel of this product was obtained. A recipe was then created for the product in FoodWorks, where the macronutrients were matched within 10% of the product label.

4.5.2 Zinc supplement data

Data regarding supplement use were gathered as part of the attitudes and motivations questionnaire. Participants gave details regarding their supplement consumption over the last 12 months, specifically: frequency of consumption, nutrients in the supplement, brand, and in some instances a photograph of the packaging. Isabelle Ritchie, a Master of Dietetics student who wrote her thesis on zinc intake in the first semester, also using SuNDiAL data, calculated the zinc intake from supplements reported in the first semester. The Candidate gained permission to access these data and followed the same methods to calculate zinc intake from supplements for the participants who took part in the study in semester two.
The Candidate used online health stores and pharmacies to identify the supplements that contained zinc amongst the supplements reported by the participants and to find the quantity of zinc in each supplement. When a participant reported that they had consumed either a multivitamin or a single mineral supplement that contained zinc, but gave no further details, an average value was assigned: for multivitamins, an average of 6.7mg zinc per supplement was assigned as this was the average of three commonly reported multivitamins in the SuNDiAL survey; for single mineral supplements (zinc), a value of 15mg was assigned as this was the average of three common zinc only supplements available in New Zealand health stores and pharmacies.

The frequency of supplement consumption over the last 12 months was reported as: daily, once per week, more than once per week, monthly, regularly but for a limited time, or not very often. To allow calculation of daily intake of zinc from supplements, these options were assigned numeric equivalents, respectively: 1, 0.14, 0.29, 0.033, 0, and 0. The value for ‘monthly’ was based on the average number of days in a month: $1 / 30.42 = 0.033$. ‘Regularly but for a limited time’ was assigned 0 as the questionnaire asked about intake over the last 12 months which could reflect a short period of time many months before. Once the data described above had been entered for each participant and checked, the frequency of consumption in numeric equivalent was multiplied by the quantity of zinc in the supplement. This gave the daily zinc intake from supplements for each participant.

4.5.3 Phytate data
Due to the significant inhibitory effects of phytate on zinc absorption (37), and the different composition of a vegetarian compared to a non-vegetarian diet, intake of phytate was also assessed.

Data regarding the phytate content of foods, however, are not available in the New Zealand Food Composition database, FOODfiles (13). Thus, these data have to be added manually. A previous Master of Dietetics student, Nicola Hartley, had assigned phytate values to foods in NZ FOODfiles up until 2014 (64) using published literature. Literature with the best food analysis methods and with the closest crop cultivation techniques to New Zealand was prioritised. The phytate values assigned by Nicola Hartley had been checked and approved by Professor Rosalind Gibson (Department of Human Nutrition, University of Otago).

When these phytate values were assigned, they were associated with food IDs relevant to Kai-culator, another dietary assessment software. The current study used a different dietary assessment software programme, FoodWorks. Therefore, the phytate values previously assigned were not linked to the food IDs required for FoodWorks to do the nutrient analysis. The Candidate compared a spreadsheet with Nicola’s previously assigned phytate values (2,717) to a spreadsheet of all the foods selected in FoodWorks for the SuNDiAL participants (2140). When a food between the two spreadsheets matched descriptions, or was very similar, the Candidate copied the phytate value to the new spreadsheet. This then ensured the phytate values were associated with the same foods as before, now with the appropriate food IDs for FoodWorks. Some recipes that had been selected for participants in the SuNDiAL study had not been previously assigned phytate values. Due to time constraints, phytate values could not to be assigned.
to these. Once complete, these data were loaded into FoodWorks to allow the analysis of phytate intake.

4.6 Statistical analysis

4.6.1 Sample size calculation

The sample size calculation was carried out by the SuNDiAL PIs. The participants were recruited in clusters over New Zealand, therefore a design effect of 1.5 was assumed. Only commercial market research regarding the prevalence of vegetarianism in New Zealand existed so the prevalence was assumed to be 20% of the study population (11). A sample size of n=297 was required to detect a difference of 0.5SD, which is considered a “moderate difference”, with 80% power at the 5% significance level. A sample size of 60 vegetarian participants was required to detect this difference in intake of key nutrients of interest (e.g., iron, zinc, and B12). Recruitment was bounded by the 2019 high school year.

4.6.2 Dietary intake and adequacy

The Candidate made scatter plots for the macro- and micro-nutrients of interest to visually identify outliers. No implausible extreme values were observed, thus no outliers were taken out of the data set. For zinc one value was higher than the rest, however, its exclusion did not impact on the results. Due to this, and the value being believable if extreme, it was left in the data set.
The Candidate used Excel 2019 (Microsoft, Washington) to calculate descriptive statistics for the relevant nutrients. The ‘data analysis’ add-on in Excel was used to calculate these. The descriptive statistics and unpaired t tests were used to compare the two groups by calculating the mean difference and associated 95% confidence interval.

An adequacy analysis for zinc was carried out using the Estimated Average Requirement (EAR) cut-point method (65). This was done by comparing the adjusted zinc intake of each participant to the appropriate EAR. The percentage of participants in each group estimated to be consuming less than the EAR was used to indicate the proportion of vegetarians and non-vegetarians at risk of inadequate zinc intake. This was then repeated for the vegetarian group using the proposed adjusted EAR for zinc for ‘strict’ vegetarians, at 9mg per day (17).

To assess the bioavailability of the zinc consumed, to give further insight into adequacy, statistical analysis of zinc intake in relation to both phytate and fibre was also performed. While numerous methods of estimating zinc bioavailability using phytate intake exist, the IZiNCG (International Zinc Nutrition Consultative Group) recommendations were used as they were most suitable, as discussed in section 2.4.2. To look at the effect of phytate on zinc absorption, phytate to zinc molar ratios were calculated in Excel using the following calculation (7):

\[
\text{[phytate (mg)/660)/ (zinc (mg)/65.4]}
\]

The result of the ratio calculation for each participant with phytate data was then used to place each participant into the appropriate category of estimated zinc absorption. A ratio
estimates zinc absorption in the diet to be 34% and a ratio >18 estimates absorption to be 25% (7). Average estimated absorption was then calculated for the vegetarian and non-vegetarian groups.

To see the relationship between fibre and phytate intake, a Pearson's correlation test was carried out in excel. Data adjusted for usual intake for phytate were not available, thus unadjusted fibre intake data was used.

### 4.6.3 Food sources of zinc

The food group classification system used in the NZ Adult Nutrition Survey 2008/09 was used for the current analysis (22). The total amount of zinc intake from each of the 33 food groups was calculated for each participant by Dr Jill Haszard using Stata version 15.1 (Stata Corporation, Texas). The Candidate then used Excel to calculate the mean percentage of zinc intake and standard deviation coming from each food group, for non-vegetarians and vegetarians. Excel was used to calculate the 95% confidence intervals to indicate differences between groups.
5. Results

A total of 282 participants consented and enrolled in the SuNDiAL project. The recruitment and participation of participants has been summarised in Figure 5.1. For the Candidate’s analysis, the sample size was n=250 after the withdrawal of one participant, and 31 exclusions after enrolment. The exclusions were: did not complete at least one 24-hour diet recall (n= 30) and did not identify as vegetarian or non-nonvegetarian (n=1).

The dataset included 31 vegetarians (self-identified) and 219 non-vegetarians. Within the vegetarian group 10 participants identified as vegan, and one as not vegan but not consuming cow’s milk or eggs. Due to the small number of vegans, a separate analysis was not carried out and they were included in the vegetarian group.
Figure 5.1 Participant recruitment and participation
5.1 Participant characteristics

The participant characteristics are presented in Table 5.1. This vegetarian cohort was, on average, four months older than the non-vegetarian cohort, a difference which reached statistical significance. Pacific and Asian ethnic groups were not represented in the vegetarian group, and only represented to a small degree in the non-vegetarian group. Overall, the study sample was predominantly of New Zealand European ethnicity (78%), with 16% being Māori. The distribution of ethnicities was similar to that seen in the New Zealand Census 2013 (66). The proportion of participants with levels of low, medium and high levels of deprivation were similar across the vegetarian and non-vegetarian groups, both having the highest proportion of participants in the low level of deprivation category. Interestingly, of the total study participants 17 %, were in the least deprived category. This is likely to be a lower proportion than the proportion observed in the population of adolescent females in the rest of New Zealand. Body Mass Index (BMI) and BMI z-scores for both groups did not differ significantly. However, the mean difference observed for these participants for BMI was -1.17 kg/m$^2$, which suggests that the vegetarian group may have a smaller body mass overall. This agrees with the proportion of participants in each weight category for both groups as determined by BMI z-score. More of the non-vegetarians in the study were overweight or obese compared to the vegetarians (35.6% vs 22.6%). Of the 31 self-identified vegetarians, 48.4% had been vegetarian for more than two years, and just 6.5% for 1-2 months.
Table 5.1: Demographic characteristics of the participants by vegetarian status¹,²

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Non-vegetarian (n=219)</th>
<th>Vegetarian (n=31)</th>
<th>Mean difference³ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>16.8 (0.9)</td>
<td>17.1 (0.8)</td>
<td>0.32 (0.02, 0.63)</td>
</tr>
<tr>
<td>Ethnicity⁴ (n (%))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NZ European</td>
<td>171 (78.4)</td>
<td>24 (77.4)</td>
<td></td>
</tr>
<tr>
<td>Māori</td>
<td>32 (14.7)</td>
<td>7 (22.6)</td>
<td></td>
</tr>
<tr>
<td>Pacific</td>
<td>6 (2.8)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>9 (4.1)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>NZDep⁶ (n (%))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-3 (low)</td>
<td>80 (36.5)</td>
<td>12 (38.7)</td>
<td></td>
</tr>
<tr>
<td>4-7</td>
<td>100 (45.7)</td>
<td>15 (48.4)</td>
<td></td>
</tr>
<tr>
<td>8-10 (high)</td>
<td>39 (17.8)</td>
<td>4 (12.9)</td>
<td></td>
</tr>
<tr>
<td>Body Mass Index⁷ (kg/m²)</td>
<td>24.1 (4.3)</td>
<td>22.9 (3.8)</td>
<td>-1.17 (-2.64, 0.30)</td>
</tr>
<tr>
<td>Body Mass Index z-score⁸</td>
<td>0.8 (1.0)</td>
<td>0.5 (0.8)</td>
<td>-0.31 (-0.63, 0.02)</td>
</tr>
<tr>
<td>Weight Category⁹ (n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>139 (64.4)</td>
<td>24 (77.4)</td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td>53 (24.5)</td>
<td>5 (16.1)</td>
<td></td>
</tr>
<tr>
<td>Obese</td>
<td>24 (11.1)</td>
<td>2 (6.5)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: n = Number of participants, CI = Confidence interval
¹Mean (standard deviation) unless stated otherwise.
²Participants self-identified into these categories.
³Calculated as vegetarian mean minus non-vegetarian mean.
⁴Where participants identified with more than one ethnicity, they were placed in the ethnicity category with the highest priority. Ethnicity categories were (high to low priority): Māori, Pacific Islands, Asian, New Zealand European or other.
⁵Ethnicity unknown for one participant.
6Household deprivation based on the 10 deciles of the NZDep2013 scale: 1-3 indicates low level deprivation, 4-7 medium and 8-10 high level of deprivation (58).

7Anthropometric measures not collected for two participants.


9Defined as: overweight = BMI z-score 1 to 2 standard deviations from mean; Obese = BMI z-score ≥ 2 standard deviations from mean (59).
5.2 Assigning phytate values to foods

The Candidate assigned phytate values to a total of 1,424 food items and recipes in FOODfiles by aligning the foods consumed in SuNDiAL with the food codes assigned phytate values (64). The constraints faced by the Candidate were: the time of an MDiet thesis, limited data on the phytate content of foods relevant to New Zealand, and the lack of ingredients for some of the recipes in the food composition database. Phytate values could not, therefore, be assigned for 33% of food items and recipes. The Candidate was, however, able to provide full phytate data for 43 participants: 3 vegetarians and 40 non-vegetarians. These data are presented in Table 5.2.

5.3 Zinc intakes and adequacy

Table 5.2 shows the mean intakes of a range of macro- and micro-nutrients, and the phytate:zinc ratios by vegetarian or non-vegetarian classification. The vegetarian group had, on average, an energy intake that was 460kJ/day lower than the non-vegetarians, however, this difference was not statistically significant. It appeared both groups had, on average, relatively low energy intakes, less than the lower end of the range for their estimated energy requirements (9,842 – 19,071kJ/day) (17). The vegetarians in this study had statistically significantly lower intakes of protein, dietary and total zinc than the non-vegetarian group. However, they were consuming significantly more fibre per day.

Vegetarians were consuming on average, significantly less dietary zinc (2mg) and total zinc (1.75mg) than non-vegetarians. When using the EAR for the age and sex of the
participants in this study (6mg/day; (17)) 16.1% of the vegetarians were at risk of inadequate zinc intake, compared to 6.4% of non-vegetarians. However, if the advice is followed that “strict vegetarians’ zinc intake may need to be about 50% higher” (17) this increases the vegetarians’ EAR to 9mg. This adjusted EAR results in 80.6% of the vegetarians having inadequate zinc intake.

The lowest zinc intake was 3.6mg, however this participant also had a low energy intake, of 5185kcal. The highest zinc intake was 21.8mg, which was associated with an energy intake of 10,605kcal, which was higher than the average. Both of these extreme zinc intake values were observed in non-vegetarian participants. They were not excluded from the data set as they represented intakes which are realistic for the wider population to have.

The phytate intake per day of the sub-group that with complete phytate data was 920mg for the vegetarians and 784mg for the non-vegetarians. The phytate:zinc molar ratios were 17.4 and 12.6, respectively. This suggests a zinc absorption of 34% for both groups.

The results of the correlation test between fibre and phytate intake was 0.79. This suggests a strong association between fibre intake and phytate intake.
<table>
<thead>
<tr>
<th></th>
<th>Non-vegetarian</th>
<th>Vegetarian</th>
<th>Mean difference(^3) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy (kJ)</strong></td>
<td>8137 (1812)</td>
<td>7677 (1518)</td>
<td>-460.0 (-1045.78, 125.84)</td>
</tr>
<tr>
<td><strong>Protein (g)</strong></td>
<td>75.3 (19.8)</td>
<td>58.5 (13.7)</td>
<td>-16.5 (-30.92, -2.05)(^4)</td>
</tr>
<tr>
<td><strong>Carbohydrate (g)</strong></td>
<td>228.3 (59.1)</td>
<td>234.6 (41.5)</td>
<td>6.2 (-62.34, 74.82)</td>
</tr>
<tr>
<td><strong>Fibre (g)</strong></td>
<td>24.1 (9.9)</td>
<td>29.3 (9.2)</td>
<td>5.2 (1.77, 8.73)</td>
</tr>
<tr>
<td><strong>Dietary zinc (mg)</strong></td>
<td>9.5 (2.8)</td>
<td>7.5 (1.8)</td>
<td>-2.0 (-2.71, -1.26)</td>
</tr>
<tr>
<td><strong>Zinc from supplements</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg)(^4)</td>
<td>0.5 (2.2)</td>
<td>0.8 (2.4)</td>
<td>0.30 (-0.40, 1.01)</td>
</tr>
<tr>
<td><strong>Total zinc (mg)</strong>(^5)</td>
<td>10.1 (3.5)</td>
<td>8.4 (2.9)</td>
<td>-1.75 (-2.81, -0.68)</td>
</tr>
<tr>
<td><strong>Prevalence of inadequate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>zinc intake using EAR (^6)</td>
<td>14 (6.4)</td>
<td>5 (16.1)</td>
<td></td>
</tr>
<tr>
<td><strong>Prevalence of inadequate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>intake using EAR + 50% (n(%))(^6)</td>
<td>N/A</td>
<td>25 (80.6)</td>
<td></td>
</tr>
<tr>
<td><strong>Phytate</strong>(^7,8)</td>
<td>783.8 (725.2)</td>
<td>921.0 (365.6)</td>
<td>137.2 (-33.7, 608.0)</td>
</tr>
<tr>
<td><strong>Phytate:Zinc ratio</strong>(^7,8)</td>
<td>12.6 (9.8)</td>
<td>17.5 (7.1)</td>
<td>4.9 (-7.8, 17.6)</td>
</tr>
<tr>
<td><strong>Zinc absorption</strong>(^8,10) ((%))</td>
<td>34</td>
<td>34</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Abbreviations: n= number of participants, CI = Confidence interval, EAR = Estimated Average Requirement.
1Calculated from 24-hour recalls, adjusted for usual intake.
2Mean (standard deviation) unless stated otherwise.
3Calculated as vegetarian mean minus non-vegetarian mean.
4Statistically significant values in a row are bold.
5For participants who answered supplement use question: 196 non-vegetarians, 29 non-vegetarians. 25 participants did not answer this question.
6Due to 25 participants not answering question regarding supplement use, total zinc intake data was available for 225 participants.
7EAR for zinc for females 14-18 years = 6mg/day (17).
8Phytate data available for 40 non-vegetarian and 3 vegetarian participants.
9Calculated as (phytate (mg)/660)/ (zinc (mg)/65.4) (7).
10When phytate:zinc ratio 4-18, absorption estimated to be 34%, when ratio >18, absorption estimated to be 25% (7).
5.4 Food group contributions to zinc intake

Figure 5.2 shows the 15 highest food sources of zinc by the average percentage they contributed to the diets of the non-vegetarians and the vegetarians. Table 5.3 shows the percentage contributions of zinc to the diets of the non-vegetarians and the vegetarians for all 33 food groups, the same food groups used in the New Zealand Adult Nutrition Survey (22).

The highest contributor to zinc intake for both groups was ‘grains and pasta’. The second highest contributor of zinc for vegetarians was ‘vegetables’, at 15.7%, although it was a relatively low contributor to the zinc intake of the non-vegetarian group at 6.6%. Non-vegetarians were consuming significantly more zinc from: ‘beef and veal’, ‘poultry’, ‘sausages and processed meats’, ‘pork’, ‘pies and pastries’, ‘egg and egg dishes’, and ‘supplements providing energy’. Some vegetarians were getting small amounts of zinc from some meat containing food groups.
Figure 5.2: Mean proportions of zinc intake by food group by vegetarian status
Table 5.3: Mean proportion of dietary zinc from the 33 major food groups by vegetarian status\textsuperscript{1, 2}

<table>
<thead>
<tr>
<th>Food group</th>
<th>Non-vegetarian n=219</th>
<th>Vegetarian n=31</th>
<th>Mean difference (95% CI)\textsuperscript{3}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grains and pasta</td>
<td>11.1 (12.7)</td>
<td>18.2 (15.4)</td>
<td>7.2 (-9.4, 23.8)</td>
</tr>
<tr>
<td>Vegetables</td>
<td>6.6 (8.7)</td>
<td>15.7 (13.7)</td>
<td>9.1 (-3.9, 22.1)</td>
</tr>
<tr>
<td>Breads including rolls and</td>
<td>9.3 (8.1)</td>
<td>10.8 (7.5)</td>
<td>-1.5 (-3.5, 0.4)</td>
</tr>
<tr>
<td>speciality breads</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bread based dishes</td>
<td>7.8 (15.2)</td>
<td>7.1 (14.2)</td>
<td>-0.7 (-15.5, 14.1)</td>
</tr>
<tr>
<td>Cheese</td>
<td>5.0 (8.0)</td>
<td>5.4 (10.5)</td>
<td>0.3 (-7.7, 8.4)</td>
</tr>
<tr>
<td>Milk</td>
<td>4.2 (6.3)</td>
<td>4.9 (10.1)</td>
<td>0.7 (-6.6, 8.0)</td>
</tr>
<tr>
<td>Fruit</td>
<td>3.0 (4.2)</td>
<td>5.1 (6.5)</td>
<td>2.1 (-1.1, 5.4)</td>
</tr>
<tr>
<td>Potatoes, kumara and taro</td>
<td>3.6 (5.4)</td>
<td>4.4 (6.1)</td>
<td>0.8 (-2.3, 3.8)</td>
</tr>
<tr>
<td>Beef and veal</td>
<td>7.3 (14.2)</td>
<td>0.6 (3.3)</td>
<td>\textsuperscript{4} -6.7 (-9.3, -4.2)</td>
</tr>
<tr>
<td>Nuts and seeds</td>
<td>3.5 (8.9)</td>
<td>4.4 (6.1)</td>
<td>0.9 (-2.7, 4.4)</td>
</tr>
<tr>
<td>Poultry</td>
<td>6.8 (9.2)</td>
<td>0.2 (0.9)</td>
<td>\textsuperscript{4} -6.7 (-8.0, -5.4)</td>
</tr>
<tr>
<td>Snacks sweet</td>
<td>2.0 (3.5)</td>
<td>3.6 (9.1)</td>
<td>1.6 (-4.1, 7.2)</td>
</tr>
<tr>
<td>Food Category</td>
<td>Mean (95% CI)</td>
<td>Lower Bound (95% CI)</td>
<td>Upper Bound (95% CI)</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------------</td>
<td>----------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Breakfast cereals</td>
<td>3.5 (6.9)</td>
<td>1.9 (4.1)</td>
<td>-1.5 (-3.5, 0.4)</td>
</tr>
<tr>
<td>Cakes and muffins</td>
<td>2.0 (3.6)</td>
<td>2.7 (3.4)</td>
<td>0.7 (-0.5, 1.9)</td>
</tr>
<tr>
<td>Savoury sauces and condiments</td>
<td>1.3 (3.0)</td>
<td>2.7 (3.3)</td>
<td>1.4 (0.3, 2.5)</td>
</tr>
<tr>
<td>Sausages and processed meats</td>
<td>3.2 (8.1)</td>
<td>0.6 (2.5)</td>
<td>-2.6 (-4.1, -1.2)</td>
</tr>
<tr>
<td>Pork</td>
<td>3.5 (7.9)</td>
<td>0.2 (1.3)</td>
<td>-3.3 (-4.6, -2.0)</td>
</tr>
<tr>
<td>Biscuits</td>
<td>2.1 (3.3)</td>
<td>1.6 (2.3)</td>
<td>-0.5 (-1.3, 0.2)</td>
</tr>
<tr>
<td>Sugar and sweets</td>
<td>1.4 (3.1)</td>
<td>2.2 (4.7)</td>
<td>0.7 (-1.1, 2.5)</td>
</tr>
<tr>
<td>Snack foods</td>
<td>1.6 (4.2)</td>
<td>1.8 (3.5)</td>
<td>0.2 (-1.1, 1.5)</td>
</tr>
<tr>
<td>Dairy products</td>
<td>1.5 (3.1)</td>
<td>1.4 (3.3)</td>
<td>-0.1 (-1.2, 1.0)</td>
</tr>
<tr>
<td>Non-alcoholic beverages</td>
<td>1.4 (2.9)</td>
<td>1.4 (3.2)</td>
<td>0.0 (-1.0, 1.1)</td>
</tr>
<tr>
<td>Pies and pastries</td>
<td>2.3 (6.5)</td>
<td>0.0 (0.0)</td>
<td>-2.3 (-3.1, -1.4)</td>
</tr>
<tr>
<td>Eggs and egg dishes</td>
<td>1.8 (4.0)</td>
<td>0.4 (1.4)</td>
<td>-1.5 (-2.1, -0.8)</td>
</tr>
<tr>
<td>Fish and seafood</td>
<td>1.1 (3.4)</td>
<td>0.8 (3.1)</td>
<td>-0.3 (-1.3, 0.8)</td>
</tr>
<tr>
<td>Lamb and mutton</td>
<td>1.6 (6.4)</td>
<td>0.0 (0.0)</td>
<td>-1.6 (-2.5, -0.8)</td>
</tr>
<tr>
<td>Food Group Classification</td>
<td>Mean % (SD)</td>
<td>Vegetarian Mean % (SD)</td>
<td>Non-Vegetarian Mean % (SD)</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>-------------</td>
<td>------------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Supplements providing energy</td>
<td>0.1 (0.6)</td>
<td>1.0 (5.3)</td>
<td>0.9 (-1.0, 2.7)</td>
</tr>
<tr>
<td>Soups and stocks</td>
<td>0.4 (2.1)</td>
<td>0.6 (1.6)</td>
<td>0.2 (-0.3, 0.6)</td>
</tr>
<tr>
<td>Puddings and desserts</td>
<td>0.2 (1.1)</td>
<td>0.4 (1.5)</td>
<td>0.2 (-0.1, 0.5)</td>
</tr>
<tr>
<td>Other meat</td>
<td>0.3 (3.6)</td>
<td>0.0 (0.0)</td>
<td>-0.3 (-0.8, 0.1)</td>
</tr>
<tr>
<td>Alcoholic beverages</td>
<td>0.2 (1.6)</td>
<td>0.0 (0.0)</td>
<td>-0.2 (-0.4, 0.0)</td>
</tr>
<tr>
<td>Butter and margarine</td>
<td>0.0 (0.1)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0, 0.0)</td>
</tr>
<tr>
<td>Fats and oils</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0, 0.0)</td>
</tr>
</tbody>
</table>

1. Food group classifications derived from the NZ ANS08/09 (22).
2. Presented as mean % (standard deviation).
3. Calculated as vegetarian mean minus non-vegetarian mean.
4. Values in a row that are statistically significant are bold.
6. Discussion and Conclusion

The vegetarians in this study were consuming statistically significantly less dietary and total zinc than the non-vegetarians. The proportion of zinc provided by different food groups also differed in vegetarians and non-vegetarians, most noticeably with vegetarians consuming more zinc from vegetables, and less from meat. Vegetarian diets have been previously observed to be of higher fibre content which may be associated with a higher phytate intake (18). Higher fibre and phytate (in a subgroup) intakes were observed in the current study. Due to this lower intake of zinc and elevated consumption of phytate, that may inhibit absorption of zinc, the vegetarians were more likely to be at risk of having a zinc intake that is not adequate for meeting their zinc requirements.

The estimated usual dietary zinc intakes in the current study were 7.5mg and 9.5mg per day, for vegetarians and non-vegetarians, respectively. The most recent New Zealand (NZ) Adult Nutrition Survey 2008/09 (ANS 08/09) assessed zinc intake and weighted results to be representative of NZ’s expected resident population. They reported a mean intake of zinc for females 15-18 years of 9.1mg/day (22), a value higher than the vegetarians and slightly lower than the non-vegetarians in the current study. However, the ANS did not distinguish between vegetarians and non-vegetarians. It is likely there were vegetarians in their sample, however it is difficult to estimate the potential impact they may have had on the results due to uncertainties regarding the proportion of vegetarians. If vegetarians in the ANS were, like those in the SuNDiAL study, consuming less zinc than the non-vegetarians, this could have contributed to the slightly lower zinc intake than was seen for non-vegetarians in the current study.

One study, conducted in Canada has compared zinc intake in adolescent vegetarian and non-vegetarian females of a similar mean age to the participants in the
current study. For both groups dietary zinc intakes were lower than those in the current study (6.7mg vegetarians and 7.9mg per day non-vegetarians compared to 7.5mg and 9.5mg per day in SuNDiAL). However, the difference seen in the Canadian study did not reach statistical significance (14). The study had strict classifications for vegetarians and non-vegetarians, in contrast to SuNDiAL where participants self-identified into these categories without definitions. In the current study, four (12.9%) participants who self-identified as vegetarian were consuming some zinc from meat. This is likely to cause the zinc intake of the SuNDiAL vegetarian group to appear higher as meat products generally provide high quantities of zinc (17). The Canadian data are 23 years old and from another country, thus their relevance to NZ adolescents in recent years may be limited. This is due to probable changes in dietary habits and an increase in the availability of vegetarian foods (such as canned beans, and tofu products). The lower zinc intakes seen in the Canadian study are also likely to be due to the lower daily energy intakes (6983kJ for vegetarians and 7089kJ for non-vegetarians; (14)) compared to the SuNDiAL study (7677kJ for vegetarians and 8137kJ for non-vegetarians). A lower quantity of food may be associated with lower nutrient intakes.

Overseas studies in adult females have observed a range of zinc intakes. Mean daily zinc intake ranged from 6.7-12.0mg for vegetarians and 7.9-16.0mg for non-vegetarians (14, 18-20). The intakes in the SuNDiAL study sit towards the lower end of this range. Differences may be due to: the method of dietary assessment, participants being older than SuNDiAL participants, food availability and food composition differing overseas, and differing classifications of vegetarianism. The dietary assessment method can impact the results, for example Food Frequency Questionnaires (FFQ) have been observed to yield a higher mean zinc intake compared to 24 hour diet recalls (53). This may explain the highest zinc intake for vegetarian and non-vegetarians in the range
above, as this was from a study using an FFQ (19). Adults are likely to have more autonomy over what they eat. Thus, their zinc intakes could be higher if this means that they are more likely to replace meat with something else rather than just eat the family meal without the meat as may happen for some adolescent vegetarians. Differences in food availability, cultural practices and food composition overseas may be associated with higher or lower zinc content of the diet. Differing classifications of vegetarianism are likely to have impacted on the results as some participants included in the vegetarian group in the current study consumed small amounts of meat and may, therefore, have a higher zinc intake (13).

The majority of the participants in this study were consuming the estimated average requirement (EAR) for zinc. This agrees with position statements from the American Dietetic Association and Academy of Nutrition and Dietetics that vegetarian diets can provide adequate macro- and micro-nutrients including zinc (12, 23). In the current study, the prevalence of inadequate intake was higher in the vegetarian group at 16.2% compared to 6.4% for their non-vegetarian counterparts. The result for the non-vegetarians was similar to the estimated 6.6% of NZ adolescent females having inadequate zinc intake in the ANS08/09 (22). However, the nutrient reference values for Australia and NZ suggest increasing the EAR by “about 50% for those following a strict vegetarian diet” (17). This is due to the lower bioavailability of zinc in vegetarian diets caused by increased quantities of food components that have an inhibitory effect on zinc (7). This would increase the EAR to 9mg/day for adolescent female strict vegetarians. With the adjusted EAR, 80.6% of the vegetarians in the current study had inadequate intake. However, the vegetarians in this study were not ‘strict’ with 12.9% of them having some of their zinc derived from flesh foods. This may have caused their zinc bioavailability to be higher than it would be for “true” vegetarians.
A population is considered to be at high risk of zinc deficiency when ≥25% of the population have inadequate intake (42, 67). This would apply if the increased EAR is used as 80.6% of the vegetarians in the current study had inadequate zinc intake when using that cutoff. Dietary information gives an estimate of the proportion of the population at risk, however, this needs to be used in conjunction with biochemical markers (49). Zinc status in a population can also be measured by serum zinc, hair zinc and functional abilities (42). These findings warrant further investigation into other zinc markers to confirm the risk of zinc deficiency in adolescent females in NZ.

There were a number of differences in the food groups contributing zinc to these adolescents’ diets. The food group making the highest contribution to zinc intake for both groups was “grains and pasta”. However, the vegetarians were getting a higher proportion of zinc from this food group, at 18.2%, compared to 11.1% in the non-vegetarian group. In the ANS, “grains and pasta” contributed 12.1% of zinc intake for the females of similar age to those in this study, suggesting grains and pasta have a more significant role in vegetarian diets. Grains and pasta can also contribute significant quantities of phytate in the diet thus are likely to be contributing to the vegetarians’ higher phytate intake (68).

Vegetarians in the current study were also getting a large proportion of zinc from “vegetables”, at 15.7%, compared to only 6.6% for the non-vegetarian group. The result for the non-vegetarians was, once again, similar to the expected intake of zinc from vegetables for adolescent females in the ANS, at 5.6% (22).

The non-vegetarians consumed 26.3% of their dietary zinc from meat or meat-based products. Interestingly, the vegetarian group consumed 2.6% of their zinc from these foods too. Strict vegetarians exclude all flesh foods from the diet (12). However, some individuals that consider themselves vegetarian still occasionally consume flesh
foods. Two thirds (1.6%) of the vegetarian group’s zinc intake from flesh foods was from meat and poultry and one third was from fish or seafood. Although this may cause the vegetarian group zinc intake to be higher than it would be if they were strict vegetarians, this may be more reflective of real-life dietary patterns.

Phytate is considered to be the strongest inhibitor of zinc absorption in the diet (7). In the current study the vegetarians consumed more phytate (920mg per day) than non-vegetarians (784mg per day). This is of interest as the inhibitory effect of phytate on zinc absorption is dose-dependent (7). The International Zinc Nutrition Consultative Group (IZiNCG) suggests using the Phytate:Zinc ratio to assess the likely impact of phytate on zinc absorption (7). A higher ratio reflects a lower proportion of zinc being absorbed. In this study, ratios of 17.5 and 12.6 for vegetarians and non-vegetarians, respectively, were calculated based on IZiNCG guidelines (7). This classified both groups into the same level of zinc bioavailability, at 34%, although the phytate intake data were limited to just three vegetarians and 40 non-vegetarians. Fibre intake was positively, and strongly, associated with phytate intake in the current study. Vegetarians in the current study were consuming statistically significantly more fibre than the non-vegetarians. This gives further reason to believe vegetarians may need to consume more zinc than non-vegetarians as they are likely to be consuming more inhibitors, assuming that fibre is a good marker for phytate intake.

The strengths of this study include participants being recruited nationwide so eating patterns across NZ are reflected. A second 24-hour recall was collected for a large subset (n=213; 85%) of participants. Forty percent of the second 24-hour recalls were taken on a weekend day to capture variation that may occur in the diet between weekdays and weekends. Finally, diet recalls entered into FoodWorks were checked by Liz Fleming (MSc in Dietary Assessment) and appropriate corrections were made.
The SuNDiAL study also had several limitations. The most significant was the way in which the 24-hour diet recall data were collected and entered. Twenty-nine data collectors collected these nationwide. Whilst they all received training and had protocols to follow, errors were inevitable. Estimation of portion sizes was difficult, however, this was minimised by the use of household measures and pictures of food portions.

The sample size requirement was not met, reducing the statistical power and therefore the ability to detect a true difference. Only 31 vegetarians were recruited, compared to the 60 that were needed for sufficient power.

In addition, the sample was not representative of individuals of all deprivation levels. There was a higher proportion in the least deprived category than in the wider population. These individuals are likely to consume food of better nutritional quality, such as more meat or more appropriate vegetarian protein alternatives which are more expensive. This may have caused the mean zinc intakes to appear higher. To address this, an attempt was made in the second wave of school recruitment to recruit schools from lower deciles.

Due to time constraints, food composition data from 2014 were used. It is not known what impact evolution of vegetarian foods in recent years, such as the introduction of ‘fake meat’, had on the zinc data. Such products were not included in the 2014 food database therefore had to be entered as estimated recipes. When this was done the recipe was made to match within 10% of the macronutrient content of the product, so did not consider micronutrients.

Self-identification into vegetarian and non-vegetarian categories was a limitation as it was revealed that 12.9% of the vegetarians consumed meat. Due to the higher concentration of zinc in meat, this may have caused the vegetarian zinc intake to appear higher than it would be for strict vegetarians.
The phytate data were limited to a small sub-group for whom complete phytate data could be generated and only included three vegetarians. The accuracy of the available phytate data is unknown as they were based on overseas literature, some of which was 20 years old, and used multiple techniques for determining the phytate content of the foods.

Finally, vegetarians and non-vegetarians in the current study had average energy intakes lower than the reference standards. This raises concerns regarding the accuracy of the estimated nutrient intakes. If real-life food consumption was higher, zinc intake may have been higher. Low reported energy intakes are observed frequently including in the ANS (22, 69). The low energy intakes in the current study do not agree with the body sizes observed. In the SuNDiAL study, 34% of the girls were overweight or obese. If, on average, the girls were not meeting the low end of their energy requirements you would expect fewer overweight or obese girls. This suggests that either low energy reporting was occurring, or the participants may have been dieting.

In conclusion, these data suggest that NZ adolescent women following a vegetarian diet may be at an increased risk of having suboptimal zinc intakes that impact on their zinc status. Using the increased EAR, 80.6% of vegetarians in this study had inadequate intake, warranting further investigation. Further research in this field should recruit a larger vegetarian cohort, with broader demographics and better quantify the intake of phytate. This will allow for more accurate confidence intervals and enable better detection of differences between the groups. Assessing serum zinc in vegetarians would provide more insight into whether they are zinc deficient. Further research into the adequacy of vegetarian diets is particularly important given the expected increases in the numbers of adolescents choosing to follow such diets in the years to come.
7. Application of Research to Dietetic Practice

Dietitians are evidence-based practitioners who provide support for individuals and groups to achieve their best nutrition and health outcomes. This requires support from research so that client education is evidence-based. As nutrition is such a wide and varied field of work, with dietary trends and habits frequently changing, it is important research evolves with these changes.

Vegetarianism is thought to be on the rise (11), as a dietitian the ability to give appropriate dietary advice to those choosing to follow such a diet is now even more important. A vegetarian diet can, when appropriately planned, be sufficient to meet dietary needs (12, 23). However, it is crucial that nutrients that may be consumed in lower amounts in a vegetarian diet are appropriately accounted for. When considering zinc, the bioavailability of zinc in meat products are much higher than vegetarian sources, such as tofu or lentils (15). The quantity of protein in the diet is also known to increase zinc absorption by counteracting the impact of inhibitors such as phytate, however, this is dependent on the protein type (37, 44). That said, ensuring vegetarians have adequate protein in their diet may, therefore, be beneficial for the bioavailability of the zinc they are consuming. In this study, the highest contributors of zinc in the vegetarian diets were “grains and pasta”, “vegetables”, and “breads”. Thus, ensuring adequate intake of these food groups is likely to be pivotal to supporting adequate zinc intake. Foods such as dairy, grains, pasta and legumes may be the most beneficial. Phytate is often consumed in higher quantities in vegetarian diets due to its abundance in the three food groups mentioned above. Phytate is a known inhibitor of zinc intake, thus those consuming more phytate may require more zinc to ensure their absorbable zinc is...
similar to that of someone consuming less phytate. From a dietetic point of view, this can be managed by ensuring individuals are not excessively consuming the foods known to contain high amounts of phytate, such as using food that have gone through processing techniques that reduce phytate, e.g. canning or soaking beans (70).

Throughout this research project, I have been very aware of the limitations of the study design and results. The accuracy of the results, as in all research, depends greatly on the accuracy of data collection. Writing the critique in the limitations section of my Discussion showed me the importance of a well-designed study which considers how errors can be minimised. It also made me think about the importance of clearly listing all limitations and how they may have impacted the results which would allow the reader to make their own judgment and interpretation of the results. This is very important as dietetic interventions and advice are evidence based. Therefore, with any literature it is crucial to consider the limitations and methodology of the study and think about how these factors may have impacted the results. At first, I felt this study had a lot more limitations than strengths. On reflection, this is not a bad thing as it shows transparency as a researcher and acknowledgment of weak areas of the study. This allows readers to interpret the results appropriately. It also gives those planning to carry out further research in the field guidance regarding optimising study design to reduce errors. As a dietitian, reflecting on my work whether it be in a clinical or research setting will always be important. Reflection highlights areas where I can improve and areas where I am doing well. This is required to continuously improve my practice and provide a better level of care for my patients.
8. References

64. Hartley NK. Phytate and zinc intakes of New Zealand toddlers aged 12-24 months: University of Otago; 2014.
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Developed by Dr Jill Haszard and Dr Meredith Peddie
SuNDiAL Project: Survey of Nutrition Dietary Assessment and Lifestyle

2019 & 2020: Adolescent Females

PROTOCOL MANUAL
Updated June 2019

**Funding Agencies:**
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**Study Investigators**

**Principal Investigator:** Dr Jill Haszard¹
Ph. +64 3 479 5683
Email: jill.haszard@otago.ac.nz

**Principal Investigator:** Dr Meredith Peddie¹
Ph. + 64 3 479 8157
Email: meredith.peddie@otago.ac.nz

**Co-Investigator:** Associate Professor Lisa Houghton¹
Ph. +64 3 479 7945
Email: lisa.houghton@otago.ac.nz

**Co-Investigator:** Associate Professor Anne-Louise Heath¹
Ph. + 64 3 479 8379
Email: anne-louise.heath@otago.ac.nz

**Co-Investigator:** Associate Professor Rachel Brown¹
Ph. + 64 3 479 5839
Email: rachel.brown@otago.ac.nz

**Co-Investigator:** Associate Professor Caroline Horwath ¹
Ph. + 64 3 479 7946
Email: caroline.horwath@otago.ac.nz

**Co-Investigator:** Professor Rosalind Gibson¹
Ph. +64 3 479 7955
Email: rosalind.gibson@otago.ac.nz

¹. Department of Human Nutrition, University of Otago, Dunedin, 9054, New Zealand
1. Project Summary

The aim of the SuNDiAL project is to compare the nutritional status, dietary habits, health status, and attitudes and motivations for food choice of vegetarian and non-vegetarian adolescent women. There is very limited data about dietary intake and lifestyle in this age group who exhibit increasing interest in adopting vegetarian eating patterns. Three hundred females aged between 15 and 18 years will be recruited to participate from secondary schools throughout New Zealand between February 2019 and November 2020. Participants will complete two 24-hour diet recalls to provide an estimate of usual dietary intake, provide a blood sample for the analysis of biochemical status of key nutrients, wear an accelerometer for 7 days to provide an estimate of 24-hour activity (sleep, sedentary behaviour and physical activity), answer questions about their attitudes and beliefs about the foods they choose to eat (or not eat) and about their dietary habits. Comparisons of these will be made between vegetarians and non-vegetarians using regression models for nutrient intake and status, and scores from questionnaires; and fractional multinomial logit models for 24-hour activity. Results will be used to inform public health policy about appropriate ways to follow a more plant-based approach to eating without increasing the risk of deficiency of nutrients such as iron and calcium, or inadvertently increasing saturated fat or sodium intakes.

2. Background

The recent publication by the EAT-Lancet Commission on healthy diets from sustainable food systems reports that substantial global dietary shifts to a more plant-based diet is required for health and sustainable food production [1]. Vegetarianism (not consuming meat, poultry or fish) is an increasingly popular eating pattern for many reasons, including the reduced impact on the environment compared to diets high in animal products [2], and the lower risk of non-communicable diseases associated with a plant based diet [3]. Indeed, vegetarians tend to have a body mass index (BMI) that is 1-2 kg/m² lower than their otherwise comparable non-vegetarian peers, and exhibit less weight gain during adulthood [3]. Vegetarians also have a 24% lower risk of ischemic heart disease and a slightly lower risk of some cancers [3]. However, much of the data used to inform us about the health benefits of vegetarianism were collected prior to the 1990s [4, 5]. It is likely that recent increases in convenient, commercially produced vegetarian foods and meals, combined with the increased promotion of the unsubstantiated health benefits of foods such as coconut oil [6], have resulted
in a modern vegetarian diet that is markedly different from that consumed prior to the 1990s. Therefore, it is imperative to examine the modern vegetarian-eating pattern to determine whether it continues to offer the health benefits it once did.

Even when a vegetarian is eating a diet that offers cardio-protective benefits, ensuring the nutritional adequacy of a meatless diet requires care. The dietary intakes of vegetarians, and particularly vegans (those who do not consume meat, poultry, fish, dairy or eggs) may be inadequate for some nutrients including iron, zinc, calcium, vitamin B12, and vitamin D, which could negatively impact their long-term health. Indeed, the bone mineral content of vegetarians tends to be ~4% lower than omnivores [3], potentially putting them at higher risk of fracture and the development of osteoporosis. In addition, the poorer absorption of iron from a vegetarian diet increases the risk of iron deficiency [7]. However, it is unknown how the modern vegetarian diet may exacerbate or protect against the risk of nutrient deficiencies in the current food environment.

Despite the apparent increasing popularity of vegetarian diets we are currently reliant on commercial market research to give an indication of the rates of vegetarianism on New Zealand [8]. Surveys conducted in 2011 and 2015 indicate that rates of vegetarianism in New Zealanders in those aged 14 to 24 years exhibited the largest increase of any age group – from 8.6% to 13.3% [8]. Adolescent females may be at risk of poor iron status even when following a diet that includes iron-rich meat. Furthermore, adolescents represent the future of this country, and may be an appropriate target for instigating the dietary shifts recommended by the EAT-Lancet Commission. These factors together make adolescent females an important population in which to begin an assessment of vegetarianism in New Zealand, examining the health correlates of a modern vegetarian diet, while also assessing the possible risks of nutrient inadequacy and deficiency.

3. Aim of Study

To compare the dietary intakes and habits, nutritional status, health status, motivations, attitudes, and lifestyles of vegetarian and non-vegetarian adolescent females in New Zealand.

4. Objectives

To describe the dietary intake of macronutrients, sugars, fibre, and key micronutrients (iron, zinc, B12, folate, iodine, fluoride, magnesium, riboflavin, and
calcium) in a sample of adolescent females in New Zealand, and to make comparisons between vegetarians and non-vegetarians.

To describe the biochemical micronutrient status of key micronutrients (iron, zinc, vitamins A, B6, B12, folate, selenium, and thiamin) in a sample of adolescent females in New Zealand, and to make comparisons between vegetarians and non-vegetarians.

To describe the 24-hour activity patterns (sleep, sedentary behavior and physical activity) in a sample of adolescent females in New Zealand, and to make comparisons between vegetarians and non-vegetarians.

To determine the attitudes and motivations towards food choice/dietary patterns (e.g., the environment, animal welfare, health) in a sample of adolescent females in New Zealand, and to make comparisons between vegetarians and non-vegetarians.

To describe dietary habits in a sample of adolescent females in New Zealand and to make comparisons between vegetarians and non-vegetarians.

To describe the weight loss intentions and methods of adolescent females in New Zealand and to make comparisons between vegetarians and non-vegetarians.

5. Hypothesis

We hypothesize that vegetarian adolescent females will differ from non-vegetarian females in terms of nutrient intake, micronutrient status, attitudes and motivations, and other lifestyle factors.

6. Study Design

Cross-sectional survey

7. Study Setting/Location

A multi-centered study including at least 14 high schools across New Zealand, along with targeted recruitment of vegetarians. The localities in which data will be collected are determined by where data collectors (MDiet students) will be based in 2019 & 2020. In 2019 these locations are:

- Dunedin
- Wellington
- Christchurch
- New Plymouth
After 6 months of data collection when 145 participants had completed the study, a decision was made to specifically target recruitment of vegetarians as only nine had enrolled in the study. Therefore, in Dunedin, adolescent females who identify as vegetarian, but are not enrolled in a participating school will also be able to participate in the study.

8. Study Population
At least 300 female high school students between the ages of 15 to 18 years from across New Zealand.

9. Eligibility Criteria

9a. Inclusion criteria
Individuals who self-identify as female and are between 15 and 18 years of age, who are enrolled in one of the recruited high schools, or who identify as vegetarian living in Dunedin will take place, and who speak and understand English and are able to complete the required online questionnaires are eligible to participate.

9b. Exclusion criteria
Participants will be asked not to participate in this study if they know that they are pregnant.

10. Study Outcomes

10a. Primary Outcomes
Iron intake and biochemical iron status

10b. Secondary Outcome(s)
Intake and biochemical status of other nutrients
Attitudes and beliefs around food choice, including dietarian identity, food choice motivations.
Dietary habits
BMI z-score
24-hour activity patterns (time spent in sedentary behavior, physical activity and sleep)
Weight loss intentions & methods
11. Study Procedures

11a. Recruitment of participants

There are two methods of recruitment: school-based recruitment and targeted recruitment. The school-based recruitment aims to recruit female adolescents with a broad range of dietary patterns, whereas the targeted recruitment aims to recruit female adolescents who identify as vegetarian.

School-based recruitment

First schools will be recruited and then students at that school will be invited to participate. High schools in the areas in which data collectors are based will be invited to participate. Initially an email invitation will be sent to schools with the largest female roll, from a range of deciles. The PIs (JH and MP) and/or the SuNDiAL coordinator (Tessa Scott) will initiate contact via email from November 2018. Schools that do not respond to the email within two weeks will be sent a second email, and will receive a follow-up phone call. If email and phone contact does not result in the targeted number of schools enrolling in the study (one school per pair of data collectors) schools in the data collection area may be recruited via word of mouth. Interested schools will be phoned to provide information about the dates and times that suit them to have the research teams conducting data collection at the school, as well as information about the facilities and space that they can make available to the research team.

Master of Dietetic students (the data collectors) will visit participating schools early in the term to initiate recruitment of students within the school. This visit may include a presentation to school or year group assemblies, and may also involve providing the school with electronic and/or print information that can be circulated with school newsletters etc.

School students who are interested in participating will be given the opportunity to provide their name, age, and email address after the in-school presentation, where information sheets will be available. Alternatively, school students who are will also be able to visit the study website (www.otago.ac.nz/sundial) where they will be able to read more information about the study, watch a short video about what is involved in participating, and read the information sheet. Individuals interested in participating can then provide their name, age, email address, and high school to the website. An ID number will be assigned to the participant at this point and the SuNDiAL coordinator will email the participant a link to a REDCap questionnaire, where they will complete online consent, and answer a
series of questions about demographics and health. Potential participants are free at any time to contact investigators via phone or email to ask questions about participating in the study. Where a participant is under 16 years of age, they will be asked to provide the email address of a parent/guardian, who will then be contacted via email, and asked to provide online consent for their daughter to participate before the link to the study is sent to the participant.

**Targeted recruitment**

Advertising for females aged 15 – 18 who identify as vegetarian will take place in Dunedin. Advertising will be in local papers and social media. Females who are interested in participating will be directed to the study website (www.otago.ac.nz/sundial), where they can view a video about the study, read the information sheet, and if they wish to participate they will provide their contact details (name and email address) so that a link to the online consent and enrolment forms can be sent to them.

**11b. Study procedure**

Once participants have completed online consent, and answered the initial demographic, vegetarianism, and health questions they can continue on to complete the rest of the online questionnaires or return to complete them later. These additional questionnaires assess dietary habits, and attitudes and motivations to food choice, as well as weight-loss intentions and methods. These are administered on the web application REDCap and are completed in their own time.

Participants will be contacted by data collectors (either by phone or email) to be scheduled to attend a visit with study investigators at school during school time (if they are part of the school-based recruitment group), or at the clinic after school (if they are part of the targeted recruitment group). During this visit participants will carry out a 24-hour dietary recall with one of the Master of Dietetics students. Height, weight and ulna length will be measured in duplicate. It is expected that this part of the data collection visit will take approximately 60 minutes to complete.

Individuals who consent to wear an accelerometer will be fitted with an Actigraph GT3x+ accelerometer to be worn on an elasticated belt over their right hip continuously for 24 hours a day for seven days. Participants wearing an accelerometer will also be asked to complete a sleep and wear time diary over the time which the accelerometer is worn, which will involve recording the times they went to bed and to sleep each night, and to record any time for which the
accelerometer was removed for more than five minutes. Actigraphs and sleep & wear time diaries will be collected eight days after the initial visit by a data collector.

A second 24-hour dietary recall will be undertaken over the phone or by video-call. This will take place the following weekend to capture the variation in dietary intake between days, including weekend days. Completion of this second dietary recall should take participants approximately 30 min.

Participants who consent to providing a blood sample will have one collected by the trained phlebotomist/research nurse at an appointed time. Participants may also provide a urine sample (collection of biochemical samples may occur on a separate day to the 24 h recall depending on the availability of the phlebotomist).
11c. Measurement tools used

**Demographics, vegetarianism, and health status**
After the participant has given consent (and a parent or guardian has given consent if they are 15 years of age) then they will continue on to an online questionnaire assessing demographics, vegetarianism, and health status, such as whether they are diabetic and questions about their menstruation patterns (to estimate iron requirements). This is administered in REDCap.

**Dietary habits, weight-loss intentions & methods, attitudes and motivations for food choice**
Dietary habits, and attitudes and motivations for food choice will be assessed via questionnaires administered in REDCap. These online questionnaires will be available for the participant to complete in their own time after they have consented. The majority of these questionnaires are based on previously validated questionnaires [9-14] where, if necessary, modifications (such as changing of country specific jargon) have been made to make them suitable for New Zealand female adolescents.

**Dietary intake**
Estimates of dietary intake will be calculated for each participant through the completion of two 24 recalls. During each 24 recall participants will be asked to recall everything they eat from midnight to midnight the previous day. Participants will be prompted to recall details such as brands of food items and cooking methods. Participants will be asked to estimate quantities using household measures, food models, and photographs of different portion sizes. The recalled foods and portion sizes will then be entered into FoodWorks 9 (Xyris Software Australia Pty Ltd) by the MDiet students to calculate the energy, macronutrients and micronutrients contained in the recalled diet. FoodWorks uses the most up-to-date and comprehensive food composition tables for New Zealand (FOODfiles 2018 (The New Zealand Institute for Plant & Food Research Limited)) which was enhanced by the inclusion of ANS0809 recipe calculated foods. The administration of a second 24-hour diet recall will allow for an estimation of ‘usual intake’, by using the MSM programme to adjust for the within-person variation in intakes [15].

**Biological specimens**
A total of 10 ml of blood will be collected into two separate tubes (one 4ml and one 6 ml tube) by a trained phlebotomist. The 4 ml tube will be used to by a local laboratory affiliated with Southern Community Laboratories to perform a
complete blood count, and measure Vitamin B12. The 6 ml blood sample will be centrifuged within four hours of collection, by the local laboratory, before being aliquoted into 4 storage tubes and transported on ice to the Department of Human Nutrition at the University of Otago in Dunedin. Samples will be stored at -80°C. Once all blood samples have been collected, one aliquot from each participant will be sent to the VitMin Lab in Germany for analysis of ferritin, soluble transferrin receptor, retinol binding protein, c-reactive protein and alpha-glycoprotein. The remaining blood samples be retained in the Department of Human Nutrition, where they will be analyzed for plasma selenium and plasma zinc, thiamin, plasma folate, B6, Leptin, and IL-6.

The urine sample provided by the participant will be transferred into a storage container and then transported on ice to the Department of Human Nutrition at the University of Otago in Dunedin by the local laboratory who is performing the processing of blood samples. Samples will be stored at -20°C until analysis of iodine concentrations.

The University of Otago, Department of Human Nutrition Micronutrient Laboratory will run the analyses for the biochemistry samples.

24-hour activity
24-hour activity patterns will be assessed using data collected using the Actigraph GT3x+. Data from each accelerometer will be downloaded using Actilife software and deposited electronically onto the password protected SuNDiAL folder on the University’s shared server. Accelerometer data will then be transferred into Stata (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC) where it will be cleaned, wear time and sleep time will be entered, and age appropriate cutoffs used to identify time spent in sleep, sedentary behavior, as well as light, moderate and vigorous physical activity for each 24-hour period.

Anthropometry
All data collectors will be trained to measure height, weight, and ulna length according to study protocols, which are based on published protocols [16, 17]. Height will be measured in duplicate using stadiometers (Seca 213; and Wedderburn), weight will be measured in duplicate using scales (one of Medisana PS420; Salter 9037 BK3R; Seca Alpha 770; or Soehnle Style Sense Comfort 400) that have been calibrated by the research team. Ulna length will be measured in duplicate on the non-dominant side.
11d. Data monitoring and Quality Control

No formal data monitoring will take place.

Data collection will be the responsibility of investigators under the supervision and direction of the PIs. The use of REDCap will minimize the need for extensive data entry and cleaning, although checks of each variable will be undertaken before statistical analysis takes place.

Quality control will be ensured by developing standard operating procedures for all data collection, including:

- Anthropometry (height, weight, ulna length);
- 24-hour dietary recall;
- Collection of blood sample and urine samples;
- Fitting the accelerometers;
- Data entry into FoodWorks

To further ensure the quality of the data collected, all data collectors (MDiet students) will have completed a six-week research methods paper in the previous year, led by the PIs. This focuses on preparing them for this research project. A further two weeks of intensive training in data collection procedures will occur prior to data collection. Any additional data collectors (MSc or PhD students) will undergo the same training.

The intensive training will be led by the PIs (Drs Jill Haszard and Meredith Peddie) with expertise brought in as needed (e.g., to teach about FoodWorks and food composition tables). This will cover obtaining informed consent, conducting research studies, dietary data collection and anthropometric measurements, handling biological samples, and fitting accelerometers.

During data collection, the students will receive ongoing support from the study investigators.

12. Statistical Considerations and Data Analysis

12a. Sample size and statistical power

A sample size of 300 high school students enrolled from 14 high schools gives 80% power to the $\alpha=0.05$ level to detect a 0.5 standard deviation difference (a
“moderate” difference) in continuous outcome variables between vegetarians and non-vegetarians, assuming a prevalence of vegetarianism of 20% and a design effect (for school clusters) of 1.5.

12b. Statistical methods

Statistical analyses will be carried out using Stata (StataCorp. 2017. *Stata Statistical Software: Release 15*. College Station, TX: StataCorp LLC). School clusters will be accounted for in all analyses using appropriate methodology (for example, with the survey command, a sandwich estimator, or as a random effect). Dietary intake estimates will be adjusted for ‘usual intake’ using the Multiple Source Method (MSM) [15]. Estimates of prevalence and means will be reported with 95% confidence intervals. A binary variable for vegetarianism will be created and comparisons between vegetarians and non-vegetarians will be carried out using appropriate regression models. All data management and statistical analysis will be overseen by the study biostatistician, Dr Jill Haszard (PI).

13. Ethical Considerations

13a. Ethical Standards

This study is conducted in full conformity with the current revision of the Declaration of Helsinki, or with the International Conference for Harmonization Good Clinical Practice (ICH-GCP) regulations and guidelines, whichever affords the greater protection to the participant, as well as the laws and regulations of New Zealand. All dietetic students are trained under the Code of Health and Disability Services Consumers’ Rights 1996. They adhere to the New Zealand Dietitians’ Board Code of Ethics.

13b. Quality Assurance

This research will be conducted by researchers who are highly skilled in the technical aspects of this research study, in particular dietary and nutritional assessment.

13c. Risks/safety considerations

**Participant Burden:** To reduce the respondent burden for the participant, questionnaires will be completed online in their own time. For those recruited through schools, completing the second dietary assessment questionnaire outside
of school time will also minimize any further disruption to the participant’s school day.

**Blood sampling:** There is a risk of discomfort, pain and bruising from the blood test. Participants will be informed of these risks, and an experienced nurse or phlebotomist will collect the blood samples and ensure strict aseptic technique is followed during blood collection to minimize any risk of infection.

The data collectors will be briefed on health and safety policies and procedures at their respective schools and will comply with these for the duration of the study. Examples of such policy and procedure will include, but not be limited to:
- Emergency procedures
- Biological sample handling

MDiet students have been vaccinated for Hepatitis B as a requirement for admission into the Dietetics program.

**Informed Consent Process**
Information about the study will be provided to potential participants at either an in-school presentation; and/or through the website, where an informative video about the study can be viewed and a detailed information sheet will be available. The information sheet will also be provided at the school presentation. Participants will be encouraged to discuss this with their family/whanau. Participants can enter their name, age, email address, and school on the website (or on a sign-up sheet at the school presentation) if they are interested in participating. At this point an ID number will be assigned to them and an email sent with a link to the consent form. If at any time potential participants have questions about the study they can contact the investigators via email or phone. Once participants have had all their questions answered they can provide consent by completing the consent form electronically. The participant (or their legally authorized representative) can withdraw consent at any time throughout the course of the study. Consent information will be stored electronically in REDCap.

**Participant Confidentiality**
Participant confidentiality is strictly held in trust by the investigators. This confidentiality is extended to cover testing of biological samples and to the clinical information relating to participants.
Upon enrolment, participants will be assigned a unique identifying code, this is done automatically by REDCap. To preserve confidentiality, during data collection all data will be recorded against this ID number. The information linking the code to the participant’s identity will be stored in a separate password protected file that only the PIs (MP and JH) and the SuNDiAL coordinator will have access to.

Responses to online questionnaires will be electronically linked to study ID numbers, as will accelerometer data. Study ID number will also be written on all biological sample tubes and the recording sheets for anthropometry measurements and 24 h recalls.

Follow-up
Once the data from the study have been analysed, the participants will be provided with an overall summary of the results. Participants are also free to request a copy of their individual data. All participants who provide a blood sample will also be provided with their biochemical haemoglobin, mean cell volume, mean cell haemoglobin, platelets, white blood cells, folate and B12 status, with a note about how to interpret these values. If investigators identify a risk of anaemia in any participant they will be advised to see their general practitioner to speak to them about these results.

Participating schools will also be provided with a summary of both the overall results as well as a summary of selected results obtained from their school (e.g. percentage of girls consuming breakfast, average number of servings of fruits and vegetables etc.)

If student investigators are concerned about an aspect of the nutritional or mental health of a study participant, they may suggest that the participant contact their school counsellor and/or nurse.

Data Management
Data will be collected as per Standard Operating Procedures and cleaned as per standard data entry procedures. Data quality checks will be run on all entered data to check for accuracy, consistency and completeness. The results database will be stored on the investigators’ computers, all of which are password protected. A backup copy may also be stored on the University’s shared server space, but only the PIs (JH and MP) will have the password that will enable access to the data stored on the server.
Information linking participant identity to their ID number will be stored in a separate password protected file that only the PIs (JH and MP) will have access to. The only reason this information will be accessed once the study is completed is if the participant requests their 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 retained for at least 10 years in secure storage.
14. Outcomes and Significance

As a result of the last national nutrition survey, conducted in 2008/09, vegetarianism has been identified as a priority area of nutritional concern by the Ministry of Health. This study will provide a meaningful investigation into the nutritional biochemical status and well-being of New Zealand female adolescents, who, as a population group are likely to have one of the highest rates of vegetarianism. We will be able to assess whether the ‘modern’ vegetarian diet consumed by adolescent females offers substantial benefits or risks over a non-vegetarian eating pattern. The collection of biochemical data, along with dietary intake and beliefs and motives for adopting a vegetarian lifestyle will strengthen the outputs and aid in the description of the health benefits associated with vegetarianism, but also the identification of ‘at risk’ individuals, to support up-to-date government and health agency guidelines for adolescent women.
15. References


10. Steptoe, A., J. Pollard Tm Fau - Wardle, and J. Wardle, Development of a measure of the motives underlying the selection of food: the food choice questionnaire. (0195-6663 [Print]).


Appendix B - Ethical approval
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
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 outlined 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.
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.

Nīhaku noa, nā

Claire Porima
Kaiwhakahuere Pūtere
Senior Project Manager
Office of Māori Development
Te Whare Wānanga o Otago
Ph: +64 3 479 7461
Email: claire.porima@otago.ac.nz
Web: www.otago.ac.nz
Appendix C - General data collection protocol

Developed by Dr Jill Haszard and Dr Meredith Peddie
Daily data collection procedure

Prepared by: Jill
12/02/19

1. Report to the school office. If accelerometers are due to be collected, then set up the collection box at the office.

2. Set up data collection space. Put up SuNDiAL project signs where appropriate. Ensure you have your name badge on.

3. Put anthropometric equipment in a private space.

4. Set up clipboards with a diet recall recording sheet and an anthropometric recording sheet (at the back).

5. Greet your participant and take their name. Take time to develop rapport.

6. Check on the Name & ID spreadsheet that they have enrolled in the study.

7. If they have not then ask them to complete online enrolment by going to the SuNDiAL website, or checking their email for a link to enrol.

8. If they are not on your latest Name & ID spreadsheet but claim to have recently enrolled, give Tessa, Jill, or Meredith a quick call.

9. You cannot collect any data from participants who haven't enrolled.

10. Take the ID number next to their name and write it on the diet recall recording sheet and the anthropometric recording sheet.

11. Carry out the diet recall as per the diet recall protocol.

12. Undertake the anthropometric measures as per the anthropometric protocol.

13. If the participant has opted to wear an accelerometer fit the accelerometer referring to the accelerometer protocol and give the participant guidance on how to complete the wear time log.

14. If they have opted to have blood and/or urine taken then book them in for a time on the Blood booking sheet. Give them an appointment card. If they would rather do the urine sample at home and drop it in, give them a takeaway pack.
15. Thank them very much for their participation and inform them that their voucher will be mailed to them on completion of data collection in their school.

16. Safely store data sheets in a place where they remain confidential (i.e. not just lying around).

17. At the end of the day, collect any accelerometers and logs from the collection box and pack up equipment (if necessary).

18. Store data sheets in a safe place.

19. Send text and/or email reminders about appointments the following day or about returning accelerometers and logs the next day.
Appendix D - Section of questionnaire used to gather supplement information
### Supplement Use

<table>
<thead>
<tr>
<th>Did you take any supplements during the last year?</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

**What type of supplement was it? (Select as many as apply)**

- Multivitamin and/or multimineral
- Single vitamin or mineral
- Oil
- Bran
- Lecithin
- LSA
- Kelp
- Spirulina
- Glucosamine and/or chondroitin
- Echinacea
- Ginkgo
- Hypericum (St John’s Wort)
- Sports supplement
- Other (please specify)

**Multivitamin and/or multimineral: How long did you take the supplement in the last 12 months?**

- Daily
- More than once a week
- Once per week
- Monthly
- Regularly but for a limited time
- Not very often

**Multivitamin and/or multimineral: If you know the brand name and/or the product name please write them here. Please provide as much information about the product as possible.**

---

**Multivitamin and/or multimineral:**

If you are able to take a photo of your supplement packaging, please do so and upload here (you can complete the questionnaire and come back to upload a photo at a later time).

When taking a photo (or two), please make visible the brand and the list of contents.

**Single vitamin or mineral: Please tell us what vitamin or mineral it was:**

---

**Single vitamin or mineral: How long did you take the supplement in the last 12 months?**

- Daily
- More than once a week
- Once per week
- Monthly
- Regularly but for a limited time
- Not very often

**Single vitamin or mineral: If you know the brand name and/or the product name please write them here. Please provide as much information about the product as possible.**

---

02/27/2019 1:01pm www.projectredcap.org
Single vitamin or mineral:

If you are able to take a photo of your supplement packaging, please do so and upload here (you can complete the questionnaire and come back to upload a photo at a later time).

When taking a photo (or two), please make visible the brand and the list of contents.

Please specify the type of oil:

<table>
<thead>
<tr>
<th>Oil: How long did you take the supplement in the last 12 months?</th>
</tr>
</thead>
<tbody>
<tr>
<td>○ Daily</td>
</tr>
<tr>
<td>○ More than once a week</td>
</tr>
<tr>
<td>○ Once per week</td>
</tr>
<tr>
<td>○ Monthly</td>
</tr>
<tr>
<td>○ Regularly but for a limited time</td>
</tr>
<tr>
<td>○ Not very often</td>
</tr>
</tbody>
</table>

If you know the brand name and/or the product name please write them here. Please provide as much information about the product as possible.

Oil:

If you are able to take a photo of your supplement packaging, please do so and upload here (you can complete the questionnaire and come back to upload a photo at a later time).

When taking a photo (or two), please make visible the brand and the list of contents.

Bran: How long did you take the supplement in the last 12 months?

<table>
<thead>
<tr>
<th>Bran:</th>
</tr>
</thead>
<tbody>
<tr>
<td>○ Daily</td>
</tr>
<tr>
<td>○ More than once a week</td>
</tr>
<tr>
<td>○ Once per week</td>
</tr>
<tr>
<td>○ Monthly</td>
</tr>
<tr>
<td>○ Regularly but for a limited time</td>
</tr>
<tr>
<td>○ Not very often</td>
</tr>
</tbody>
</table>

If you know the brand name and/or the product name please write them here. Please provide as much information about the product as possible.

Bran:

If you are able to take a photo of your supplement packaging, please do so and upload here (you can complete the questionnaire and come back to upload a photo at a later time).

When taking a photo (or two), please make visible the brand and the list of contents.
<table>
<thead>
<tr>
<th>Supplement</th>
<th>Question</th>
<th>Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lecithin</td>
<td>How long did you take the supplement in the last 12 months?</td>
<td>Daily, More than once a week, Once per week, Monthly, Regularly but for a limited time, Not very often</td>
</tr>
<tr>
<td>Lecithin</td>
<td>If you know the brand name and/or the product name please write them here. Please provide as much information about the product as possible.</td>
<td></td>
</tr>
<tr>
<td>LSA</td>
<td>How long did you take the supplement in the last 12 months?</td>
<td>Daily, More than once a week, Once per week, Monthly, Regularly but for a limited time, Not very often</td>
</tr>
<tr>
<td>LSA</td>
<td>If you know the brand name and/or the product name please write them here. Please provide as much information about the product as possible.</td>
<td></td>
</tr>
<tr>
<td>Kelp</td>
<td>How long did you take the supplement in the last 12 months?</td>
<td>Daily, More than once a week, Once per week, Monthly, Regularly but for a limited time, Not very often</td>
</tr>
<tr>
<td>Kelp</td>
<td>If you know the brand name and/or the product name please write them here. Please provide as much information about the product as possible.</td>
<td></td>
</tr>
</tbody>
</table>
Kelp:
If you are able to take a photo of your supplement packaging, please do so and upload here (you can complete the questionnaire and come back to upload a photo at a later time).

When taking a photo (or two), please make visible the brand and the list of contents.

<table>
<thead>
<tr>
<th>Spirulina: How long did you take the supplement in the last 12 months?</th>
</tr>
</thead>
<tbody>
<tr>
<td>○ Daily</td>
</tr>
<tr>
<td>○ More than once a week</td>
</tr>
<tr>
<td>○ Once per week</td>
</tr>
<tr>
<td>○ Monthly</td>
</tr>
<tr>
<td>○ Regularly but for a limited time</td>
</tr>
<tr>
<td>○ Not very often</td>
</tr>
</tbody>
</table>

Spirulina:
If you know the brand name and/or the product name please write them here. Please provide as much information about the product as possible.

Spirulina:
If you are able to take a photo of your supplement packaging, please do so and upload here (you can complete the questionnaire and come back to upload a photo at a later time).

When taking a photo (or two), please make visible the brand and the list of contents.

<table>
<thead>
<tr>
<th>Glucosamine and/or chondroitin: How long did you take the supplement in the last 12 months?</th>
</tr>
</thead>
<tbody>
<tr>
<td>○ Daily</td>
</tr>
<tr>
<td>○ More than once a week</td>
</tr>
<tr>
<td>○ Once per week</td>
</tr>
<tr>
<td>○ Monthly</td>
</tr>
<tr>
<td>○ Regularly but for a limited time</td>
</tr>
<tr>
<td>○ Not very often</td>
</tr>
</tbody>
</table>

Glucosamine and/or chondroitin:
If you know the brand name and/or the product name please write them here. Please provide as much information about the product as possible.

Glucosamine and/or chondroitin:
If you are able to take a photo of your supplement packaging, please do so and upload here (you can complete the questionnaire and come back to upload a photo at a later time).

When taking a photo (or two), please make visible the brand and the list of contents.
Echinacea: How long did you take the supplement in the last 12 months?

- Daily
- More than once a week
- Once per week
- Monthly
- Regularly but for a limited time
- Not very often

Echinacea:
If you know the brand name and/or the product name please write them here. Please provide as much information about the product as possible.

Echinacea:
If you are able to take a photo of your supplement packaging, please do so and upload here (you can complete the questionnaire and come back to upload a photo at a later time).
When taking a photo (or two), please make visible the brand and the list of contents.

Ginkgo: How long did you take the supplement in the last 12 months?

- Daily
- More than once a week
- Once per week
- Monthly
- Regularly but for a limited time
- Not very often

Ginkgo:
If you know the brand name and/or the product name please write them here. Please provide as much information about the product as possible.

Ginkgo:
If you are able to take a photo of your supplement packaging, please do so and upload here (you can complete the questionnaire and come back to upload a photo at a later time).
When taking a photo (or two), please make visible the brand and the list of contents.

Hypericum (St John's Wort): How long did you take the supplement in the last 12 months?

- Daily
- More than once a week
- Once per week
- Monthly
- Regularly but for a limited time
- Not very often

Hypericum (St John's Wort):
If you know the brand name and/or the product name please write them here. Please provide as much information about the product as possible.
Hypericum (St John's Wort):

If you are able to take a photo of your supplement packaging, please do so and upload here (you can complete the questionnaire and come back to upload a photo at a later time).

When taking a photo (or two), please make visible the brand and the list of contents.

Sports supplement: How long did you take the supplement in the last 12 months?

- Daily
- More than once a week
- Once per week
- Monthly
- Regularly but for a limited time
- Not very often

Sports supplement:

If you know the brand name and/or the product name please write them here. Please provide as much information about the product as possible.

Sports supplement:

If you are able to take a photo of your supplement packaging, please do so and upload here (you can complete the questionnaire and come back to upload a photo at a later time).

When taking a photo (or two), please make visible the brand and the list of contents.

If Other, please specify:

Other: How long did you take the supplement in the last 12 months?

- Daily
- More than once a week
- Once per week
- Monthly
- Regularly but for a limited time
- Not very often

Other:

If you know the brand name and/or the product name please write them here. Please provide as much information about the product as possible.

Other:

If you are able to take a photo of your supplement packaging, please do so and upload here (you can complete the questionnaire and come back to upload a photo at a later time).

When taking a photo (or two), please make visible the brand and the list of contents.
Appendix E - Anthropometric measures

Developed by Dr Jill Haszard and Dr Meredith Peddie
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 landmarks.

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 watch 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 UNLA LENGTH 1 box on the recording sheet without informing the participants

![Fig 2: Ulna length measurement](image)

REPEAT ALL MEASUREMENTS

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 F - 24-hour diet recall protocol

Developed by Dr Jill Haszard and Dr Meredith Peddie
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

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