Folate intakes in vegetarian and non-vegetarian adolescent females

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Abstract

Background: Folate is a water soluble B vitamin found naturally among food sources such as dark green vegetables, legumes, beans and offal such as liver. The synthetic form of folic acid is found in fortified food sources and supplements. Nationally and worldwide, folate is a nutrient of concern, as many populations consume intakes below the estimated average requirement. Thus, mandatory fortification of the food supply is common practice for many countries. New Zealand's policy allows the voluntary fortification. It is unknown whether New Zealand adolescent females are achieving their recommended needs, particularly as dietary patterns change, such as increased consumption of plant-based diets.

Objective: The objective of the present study was to evaluate the dietary intake of folate, and major food group contributors among a sample of adolescent vegetarian and non-vegetarian females in New Zealand.

Design: This is a cross-sectional study designed as part of the larger Survey of Nutrition Dietary Assessment and Lifestyle (SuN DiAL) project. Healthy females between 15 to 18 years old were recruited in two phases from 13 high schools, and through targeted recruitment. Participant data were collected via two 24-hour diet recalls, anthropometric measurements, as well as, online sociodemographic and dietary questionnaires. Usual energy and folate intakes (total, natural food folate and folic acid), and the prevalence of folate inadequacy were assessed. Food consumption was categorised into 33 food groups and major contributors to folate intake were calculated.

Results: Two-hundred and eighty-two participants enrolled in the study, with 250 participants completing one 24 hour recall; of those, 213 (85%) completed a second recall. The majority of participants identified as non-vegetarian, with 31 (12%) identifying as vegetarian. The average age of participants was 16.8 years; however, vegetarian participants were slightly older (17.1 years). Over three-quarters of participants were New Zealand European and Other, with approximately 16% of participants classified as Māori and a small proportion of Pacific and Asian participants. Dietary results showed median total intake of folate (IQR) was 306 (232.3, 409.5) µg dietary folate equivalents (DFEs)/day, with an estimated folic acid intake of 33 (0.0, 90.0) µg/day. Over half of all participants consumed folate intakes below the EAR. However, the usual folate intakes among vegetarians were higher, and prevalence of inadequacy was substantially lower than non-vegetarians (32 vs 61%, respectively), despite lower energy intakes among vegetarians. Moreover, folic acid intakes were higher among vegetarians. The major folate contributors for vegetarians were vegetables and bread, as for non-vegetarians the result was reversed. A slightly higher percentage of vegetarian participants were consuming food items from both food groups (87.1 and 83.9% for vegetables and bread, respectively).

Conclusion: Results show that adolescent females, in general, continue to have a high prevalence of inadequate folate intakes although vegetarians had a lower risk of inadequacy compared to non-vegetarians. These findings are a concern as achieving optimal folate intakes are important for reproductive age women. Mandatory fortification has shown to be an effective intervention among this target group globally. More research

is required to better understand the impact of increased voluntary fortification or a mandatory policy on the folate intakes of both New Zealand reproductive women and other population lifecycle groups.

Preface

This thesis was part of the SuN DiAL project. The main aim of the SuN DiAL project was 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.

The candidate, Joshua Hodges was responsible for the following:

Provided SuN DiAL presentations at Kapiti College and performed in participant recruitment within Kapiti College with 3 other Masters of Dietetics students.

Organised and facilitated 24-hour recalls (in person and via phone call) for Kapiti College and Whangarei Girls High School, as well as co-ordinating biological sampling (blood and urine - not included within this thesis). After collections were completed, 24 hour diet recall data were entered into Food works.

Summarised energy and folate nutrient intakes using Microsoft excel, including natural, folic acid and total folate, and evaluated differences in intake among various socio-demographic and BMI subgroups

With supervision and guidance from Associate Professor Lisa Houghton, the writing of this thesis.

The management and statistical analysis of folate data was overseen by the study biostatistician, Dr Jill Haszard (PI).

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List of Abbreviations

5-CH3-H4folate	5-methyltetrahydrofolate
AI	Adequate intake
ANCNPAS	Australian National Children's Nutrition and Physical Activity Survey
ANS08/09	Adult Nutrition Survey 2008 – '09
B12	Cobalamin
B2	Riboflavin
B6	Pyridoxine
B9	Folate
BMI	Body Mass Index
CI	Confidence Interval
CVD	Cardiovascular Disease
D-A-CH	Germany, Austria and Switzerland
DFE	Dietary Folate Equivalent
DHFR	Dihydrogen Folate Reductase
DNA	Deoxyribonucleic Acid
dTMP	Deoxythymidine Monophosphate
dUMP	Deoxyuridine Monophosphate
EAR	Estimated Average Requirement
EPIC	European Prospective Investigation into Cancer and Nutrition Study
FFQ	Food Frequency Questionnaire
FGCP	Folypoly-γ-glutamyl Carboxypeptidase
FOLR1	Folate Receptor 1
Hcy	Homocysteine

LDL-C	Low Density Lipoprotein Cholesterol
LRNI	Lower Recommended Nutrient Intake
MDiet	Masters of Dietetics
MRP	Multidrug-associated Protein
MSM	Multiple Source Method
MTHFR	Methylenetetrahydrofolate Reductase
MTR	Methionine Synthase
MTRR	Methionine Synthase Reductase
NDNS	National Diet and National Survey
NNR	Nordic Nutrition Recommendation
NNS	National Nutrition Survey
NRV	Nutrient Reference Values
NTD	Neural Tube Defect
NZDep	New Zealand Deprivation
NZEO	New Zealand European and Other
PCFT	Proton Coupled Folate Transporter
PRI	Population Reference Intake
RDA	Recommended Daily Allowance
RDI	Recommended Daily Intake
REDCap	Research Electronic Data Capture
RFC1	Reduced Folate Carrier 1
RNI	Reference Nutrient Intake
SAM	S-Adenosyl Methionine
SD	Standard Deviation
SHMT	Serine Hydroxymethyltransferase
SuN DiAL	The Survey of Nutrition, Dietary Assessment and Lifestyles
T2DM	Type 2 Diabetes Mellitus

tHcy	Total Plasma Homocysteine
THF	Tetrahydrofolate
TS	Thymidylate Synthase
UK	United Kingdom
UL	Upper Limit
US/USA	United States of America
USDA	United States Department of Agriculture

1 Introduction

Folate, a water soluble B-vitamin, has many roles within the body, with one of the most important roles as a co-enzyme within single-carbon metabolism. As such, it is required for the synthesis of DNA, and a key nutrient for growth, making it a nutrient of great concern for reproductive aged females (Stanger, 2002). Folate naturally is found most abundantly within dark leafy greens, lentils, beans and liver. Other sources include fortified foods, supplementation and the folate produced by gut microbiome (Combs & McClung, 2017).

Adolescence is a time period where nutrition can become vulnerable. Increasing growth and biological changes cause an increase in both energy and nutrient requirements. The beginning of puberty also brings on the possibility of pregnancy and lactation within the females, which further increases needs (Sawyer et al., 2018). Social changes, like diet choice, disordered eating and body image are features prominent within this age group, which also influence nutritional adequacy. Considering the risk of suboptimal folate intakes, it is concerning to find very few New Zealand studies done on this population group. Of the few studies completed within the last 10 – 20 years, findings suggest that the intakes of both female adolescents and reproductive aged women are much lower than the current estimated average requirement – even with the current voluntary fortification policy (Beck et al., 2015; Evans et al., 2014).

New Zealand established voluntary fortification in 1996 and in 2012, proposed a mandatory fortification policy, which was later rejected. In 2018, The Ministry of

Primary Industries released a report on the current state of folate fortification, showing that cereal (40%), bread (10%) and supplementary foods (8%) were the three main contributors to folic acid within the food supply. It was also highlighted that evidence of the current folate intakes of reproductive aged females, including adolescence, was limited (Ministry for Primary Industries, 2018).

It is of interest to examine whether nutrient intakes have changed due to the growing popularity around plant-based diets, and concurrent evidence supporting better health of those adopting vegetarian or vegan lifestyles (Dinu et al., 2017) As folate is more naturally abundant within plant sources as opposed to meat (except for some offal), it is possible that folate intakes have increased in the populations who adopt plant-based diets. This notion has been supported by previous studies on vegetarian and vegan participants compared to omnivores, which have shown a significant increase in folate intake within those restricting meat (Schüpbach et al., 2017; Segovia-Siapco et al., 2019).

With little data on the exact level of vegetarianism within New Zealand, assumptions are made that adolescent females, often seen as earlier adopters of new dietary trends, are at the forefront of the increasing vegetarian lifestyle. Due to this, research is needed to survey the dietary patterns of this lifecycle group and assess their nutrient intakes, including folate. Therefore, the aim of this study is to describe the dietary total folate and folic acid intakes of a convenient sample of vegetarian and non-vegetarian adolescent female and assess the prevalence of adequacy, with a secondary aim of determining both the major food group contributors of folate and folic acid.

2 Literature Review

2.1 Folate in food, absorption and transport

2.1.1 Chemical structure and properties

Folate is a generic term for a group of water-soluble B9 vitamins comprised of a base structure of a pteridine ring linked to a para-aminobenzoic acid, with a different number of glutamate residues attached. Folic acid is one of several forms of folate. It is a synthetic folate isomer most commonly used for supplementation and food fortification. Folic acid is the most oxidised form and becomes biologically active only after reduction of the pyrazine ring (**Figure 2-1**). Natural food folates are mostly reduced derivative and exist in many chemical forms varying according to the number of glutamates and one carbon moieties (e.g., methyl, formyl, methylene, methenyl) attached (Combs & McClung, 2017).

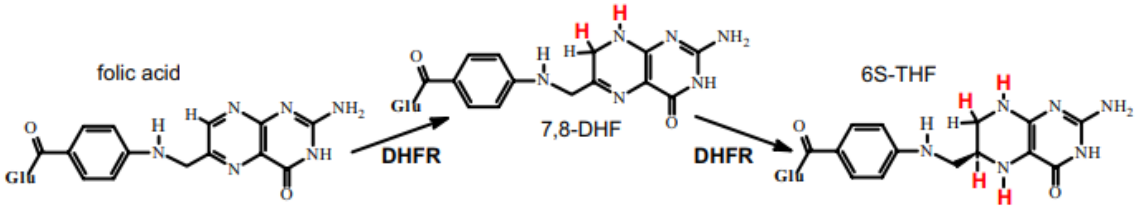

Figure 2-1 Reduction of folic acid by dihydrogen folate reductase (DHFR) to dihydrogen folate and tetrahydrofolate to make biologically active (S. W. Bailey & Ayling, 2009)

2.1.2 Food sources and bioavailability

Folate is naturally found in dark green vegetables, yeast extract, organ meats and legumes such as chickpeas (Saini et al., 2016). Folate is also present in dietary supplements and fortified foods in the form of folic acid. The folate fortification of foods continues to be a voluntary process in New Zealand, with approval to fortify breakfast cereals, bread, and fruit juices. (Ministry for Primary Industries, 2012)

Many colon bacteria are capable of synthesizing a substantial amount of folate (Pompei et al., 2007), with studies suggesting the capability of absorption across the large intestinal tract. A small study conducted with nine participants demonstrated that absorption of colonic folate through caplets to be about 43% (Lakoff et al., 2014). While these findings did not account for the folate that was already synthesised by the colonic microbiome or liver, the results highlight a growing body of research in the area of the potential nutritional impact of the gut microbiome.

In general, the bioavailability of naturally food folate and synthetic folic acid is estimated to be 50 and 85%, respectively. These values consider the many factors that influence the absorption of both forms. For example, natural food folate contains various lengths of glutamyl chains attached to its base structure in contrast to folic acid found in fortified foods, which exists in a mono-glutamyl form. As such, polyglutamate folate has shown to have reduced bioavailability compared to the monoglutamate form. Juicing and other processing methods can result in smaller glutamyl chains, and therefore could increase the bioavailability of folate. (Saini et al., 2016) However, this effect known as

deconjugation has shown to be ineffective on some sources of natural folate, with still very little data the mechanism is not fully understood (Delchier et al., 2016).

Antifolates such as methotrexate can inhibit the absorption of folates through the inhibition of the enzyme dihydrogen folate reductase (Barford et al., 1980; Leblanc et al., 2000).

Lastly, polymorphism of genes related to absorption, transport and metabolism of folate can also influence bioavailability in populations. Specifically, polymorphism of proton-coupled folate transporter has shown to affect the absorption of folate within the small intestine (Van der Meer et al., 2016).

2.1.3 Absorption, transport and storage

Absorption of natural folate in the small intestine occurs mostly on the intestinal brush border via the proton-coupled folate transporter (PCFT) once hydrolysed to the monoglutamate form by the enzyme folypoly-γ-glutamyl carboxypeptidase (FGCP). Folic acid is absorbed directly by PCFT without undergoing any change. Once in the enterocytes folic acid is reduced by dihydrofolate reductase to dihydrofolate and further to tetrahydrofolate (THF). THF is converted to 5-methyltetrahydrofolate (5-CH_3-H_{4f}folate) by methylenetetrahydrofolate reductase (MTHFR). It is then transported to the mesenteric vein by multidrug-associated protein (MRP) and taken up by cells via folate receptors protein 1 (FOLR1) or reduced folate carrier 1 (RFC1) (Saini et al., 2016).

The transporters involved within the absorption of folate have different affinities for the derivatives of folate. RFC makes up most of folate transport and has a higher affinity for

binding the reduced forms of folate compared with non-reduced. Its transport is driven and optimal at a pH of 7.4, there is evidence of an increase in RFC when folate deficiency is present (Visentin et al., 2014). PCFT is a sodium dependent transporter that is required for the absorption of monoglutamate folate across the lining of the small intestine. It prefers a more acidic pH which is well suited to the jejunum of the small intestine. MRP has a role in moving oxidised folates across the enteric membrane (Combs & McClung, 2017).

Storage of the total body folate is estimated to be 50% within the liver as polyglutamates, but there are no definitive estimations to the quantity of folate stored in the body. The range within the literature is from as little as 10 mg up to 100mg (Combs & McClung, 2017; Finglas et al., 2003; Stanger, 2002).

2.1.4 Metabolism and function

Folate derivatives act as important coenzymes within the one carbon metabolism pathway. Tetrahydrofolate (THF), a major derivative of folate, is produced from folic acid via reduction by dihydrofolate reductase (DHFR). Folate derivatives are involved in: (1) biosynthesis of purines and pyrimidines, (2) reversible conversion of serine to glycine, (3) methylation of homocysteine to methionine, (4) which in turn creates the methyl donor S-Adenosyl methionine (SAM). These metabolic functions are vital in DNA synthesis and methylation. As such, common anti-cancer drugs target certain enzymes within the folate metabolism pathway to reduce these reactions from occurring.

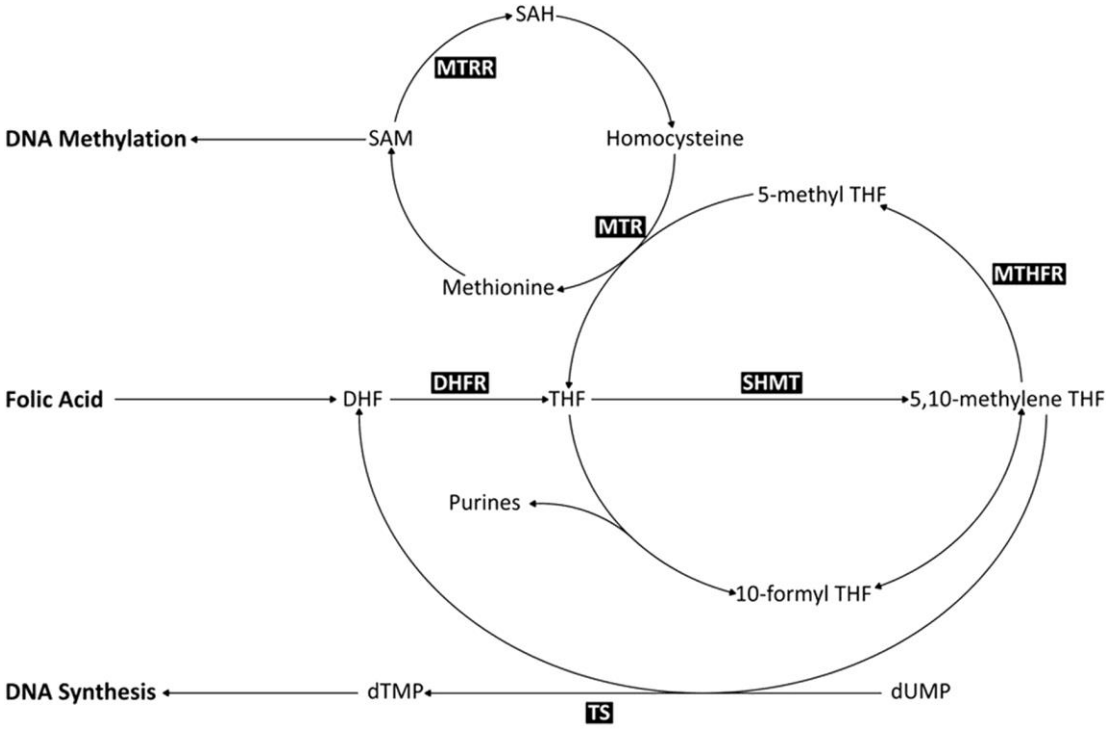

Figure 2-2 Folate metabolism (Lightfoot et al., 2010)

2.1.5 Functions and consequences of folate deficiency

Folate turnover results from losses due to catabolism, excretion and skin (Hild, 1969; Suh et al., 2001). A negative folate balance results in a reduction of pyrimidines and purines and an accumulation in homocysteine levels, leading to adverse effects on health.

Anaemia

Megaloblastic anaemia is a common feature of folate deficiency (Combs & McClung, 2017). The reduced cell division causes large, nucleated erythrocyte precursors called macrocytes and hypersegmented neutrophils, which result due to decreased DNA synthesis and delayed maturation of bone marrow. Common characteristics of anaemia are weakness, fatigue, irritability, breathlessness and lack of concentration. Folate

deficiency will also affect other areas that have higher rates of cell turnover like the intestinal epithelium. This is shown as megaloblastosis of the enterocytes which can cause increased problems such as malabsorption and diarrhoea.

Hyperhomocysteinaemia

High levels of homocysteine (Hcy) due to a reduced concentration of 5-methyl-THF metabolite has been linked with an increased risk of cardiovascular disease and cerebrovascular disease (Ebara, 2017; Finglas et al., 2003). It is unclear, however, whether an increased intake of supplemental folate, B6 or B12 in these populations decreases cardiovascular risk.

Cancer

A low folate status has been associated with several cancers such as colorectal, breast and prostate (Combs & McClung, 2017; Pieroth et al., 2018). The proposed mechanism for this phenomenon is thought to be linked to the reduced conversion of dTMP to dUMP resulting in increased DNA breakage due to the misincorporation of uracil (Combs & McClung, 2017). Studies suggest that folate has multiple affects within cancer as it can both reduce or increase the risk of development and progression of different types of cancers (Kim, 2018).

Neural Tube Defects

In pregnancy, folate is required in the growth and development of the embryo and plays a pivotal role in the closure of the neural tube. Inadequate intakes of folate are associated with neural tube defects (NTDs) such as spina bifida, exencephaly and anencephaly

(Pitkin, 2007). However, low folate status is not the sole cause of NTDs, rather it is hypothesised that maternal folate status interacts with foetal genes as well as other dietary factors affecting the one carbon metabolism pathway (Greene & Copp, 2014). As such, recommendations for the uptake of folic acid during the periconceptional period are in place in most countries because a large number of pregnancies are not identified until after critical time periods (Tamura & Picciano, 2006).

2.2 Dietary requirements for folate

2.2.1 Nutrient reference values

Nutrient reference values (NRV) are a set of recommendations for nutrient intakes that are required for a healthy population. The NRVs are comprised of various intake values, including the Estimated Average Requirements (EAR) set at the level that meets 50% of the population needs, and the Recommended Daily Intakes (RDI) set to meet 97-98% of populations nutrient requirements. The Tolerable Upper Intake Level (UL) is the highest daily intake level considered safe (i.e., unlikely to cause adverse effects) for the general population (National Health Medical Research Council, 2006).

The RDI for dietary folate among New Zealand males and females aged 14 to >70 years is set at 400 µg/day as dietary folate equivalents (DFE). DFEs are used to account for the differences in bioavailability of folate from various food and supplement source. One µg of DFE is the equivalent to 1 µg of food folate, 0.5 µg of folic acid without food or 0.6 µg of folic acid in food, or with foods (Suitor & Bailey, 2000). Dietary folate requirements increase for pregnant and lactating women with a recommendation of 600 and 500 µg DFE/day respectively (National Health Medical Research Council, 2006).

The recommendation for young adults aged 14 – 18 years has been extrapolated from longer term dose-response adult studies comparing 200, 300 and 400 µg/day dosage. A substantial increase in folate biomarkers and an optimal decrease in homocysteine levels has been demonstrated with 400 µg/day compared to the lower doses (National Health Medical Research Council, 2006).

New Zealand's folate ULs are set for the intake of folic acid only, as high intakes of natural food folate have no known adverse effects. Synthetic folic acid supplementation at a high dosage has associations with adverse neurological effects on individuals deficient of vitamin B12, and furthermore increases their deficiency, such as the older adult population (National Health Medical Research Council, 2006).

Dietary folate intake recommendations from the US are similar to Australia and New Zealand. In contrast, German speaking (D-A-CH) and Nordic (NNR) countries have set a lower intake level of 300 µg/day as their population reference intake (PRI) and reference intake (RI), respectively. The intake levels set by D-A-CH in 2013 were based on an intake of 200 µg DFE which was sufficient to support adequate serum and erythrocyte folate concentrations, with a further 30% added along with rounding for underestimation errors (Krawinkel et al., 2014). The NNR in 2004 used dietary studies examining similar biomarkers in addition to circulating homocysteine levels (European Food Safety Authority, 2014).

2.2.2 Dietary folate intakes and the prevalence of inadequacy

Globally dietary folate intakes among female adolescents are varied. Countries that tend to have higher intakes and lower prevalence of inadequacy among female adolescents

were those from North America (R. L. Bailey et al., 2010) and the UK (Dhonukshe-Rutten et al., 2007), In the European countries such as Norway, Sweden, Denmark and the Netherlands, a systematic review found that intakes were the lowest among all females, averaging closer to, and below 250 µg/day (Dhonukshe-Rutten et al., 2007).

The 1997 New Zealand National Nutrition Survey reported a usual intake of folate of 202 – 203 µg DFEs per day among females aged 15 – 24 years. In particular, female adolescents had the highest prevalence of inadequate intakes (between 21.2 – 22.2% using EAR of 150 µg at the time of sampling) compared to other population subgroups (Ministry of Health, 2003). Over 40% of folate intakes in the 1997 survey came from vegetables (18%), breads (13%) and breakfast cereals (11%). The 2008 – 09 Adult Nutrition Survey did not report folate intakes as the food composition data were considered unreliable.

A cross-sectional survey in 2009 of 125 reproductive age New Zealand women reported an average daily folate intake of 362 µg DFEs per day, with 37% of participants not achieving adequate dietary folate intakes as per an EAR of 320 µg DFEs per day (Evans et al., 2014). In addition, a smaller cross-sectional study on 47 adolescent New Zealand female ballet dancers found 61.9% of participants fell below the EAR of 330 µg/day with a mean intake of 301.7 µg/day (Beck et al., 2015).

The 2007 Australian National Children's Nutrition and Physical Activity Survey (ANCNPAS) reported mean intakes of folate of 440.5 µg/day DFE among young females (14 – 16 years), with 28% not achieving intakes meeting the EAR. In 2009, Australia undertook mandatory folic acid fortification of bread flour. An investigation of pre- and

post- fortification intake levels Australia found a 159 µg/day increase in average folic acid intakes for females aged 16 – 44 years and a 233 µg/day increase for 14 – 16-year-old females (Dugbaza & Cunningham, 2012).

The UK's National Diet and Nutrition Survey (NDNS) reported mean total folate intake from food to be 199 µg and 178 µg/day DFE among females aged 11-18 years in 2012-14 and 2014-16, respectively. The results showed that 15% of this population subgroup fell below the lower recommended nutrient intake LRNI of 100 µg/day. Mean folate intakes with and without supplements were higher among child-bearing aged women (16 – 49 years) at 205 µg/day and 239 µg/day, respectively. When comparing to the recommended 400 µg, the gap is substantial even with the use of voluntary fortification. The major folate sources in the 11 – 18 year old group were: cereal and cereal products (39% of total intake), vegetables and potatoes (21%) and meat and meat products (10%) (Roberts et al., 2018).

A smaller UK study published in 2019 found very similar folate intakes for female university students aged 18 – 25 years. The study showed their mean intake of folate was 171.29 µg/day (Farhat et al., 2019). Data from other European countries report slightly higher intakes of folate among adult women. Results from the EPIC study focused on adults in ten European countries including the Netherlands and Scandinavian countries (Norway, Sweden and Denmark) reported folate intakes ranging from 201.1 – 259 µg/day among all adult women. Major folate food sources included: vegetables (19.7 – 21.6%), cereal and cereal products (13.1 – 20.8%) and dairy products (12.5 – 16.5%) (Park, 2012). These findings are supported by another European survey including nine countries

demonstrating mean folate intakes was 180.7 µg/day among females 12.5 – 17.5 years, with over 90% of both male and females not meeting the recommended intake of 400 µg/day DFEs. The major food contributors for the female adolescents in the latter study were vegetables (273.9 g/day, excluding potatoes) followed by fruits (143.76 g/day) and bread and rolls (131.61 g/day) (Iglesia et al., 2017).

2.2.3 Mandatory folate fortification

There are conflicting arguments for, and against the implementation of mandatory folic acid fortification of foods, and as such, the policy of mandatory and voluntary fortification programmes vary worldwide (Wald et al., 2018).

Currently, New Zealand has a voluntary fortification programme which has been in place since 1996 (Ministry for Primary Industries, 2012). As such, the 1997 national survey was based solely on the use of supplementation and did not identify the inclusion of fortification folic acid within the population's intakes. In 2009, Australia-New Zealand proposed a joint mandatory folate fortification policy, however, New Zealand opted out continuing with a voluntary fortification programme including cereal and cereal products, breakfast cereals, cereal flours, pasta, yeast extracts, and fruit juices. In Australia, the policy was passed leading to the fortification of all bread flour with folic acid. Similar to New Zealand, the majority of European countries including the UK do not have a mandatory fortification programme; yet many of these countries have implemented a voluntary folic acid fortification policy.

To date, 81 countries including Canada and USA have a mandatory folic acid fortification policies. (Crider et al., 2011; Wald et al., 2018) The USA introduced

fortification to bread and cereal products at 140 µg/100g in 1998. Recent survey reports indicate a mean folic acid intake of 198 and 171 µg/day DFEs for 12 – 19 year-old and 20 – 29 year-old females, respectively. Moreover, females were consuming substantially higher daily intakes of 489 and 486 µg/day DFEs (Bowman et al., 2017).

A study from the US in 2010 found females age 14 – 18 years were consuming 496 µg of DFE (includes 201µg/day of dietary folic acid), with 19% not meeting the EAR of 330 µg/day (R. L. Bailey et al., 2010). A study using the same dataset found females 19 years and older consumed folate largely from ready to eat cereal (18.7%), yeast breads and rolls (16.6%), and cake, cookies, quick breads, pastry and pies (6.1%) (O'Neil et al., 2012).

A study on the proposed mandatory fortification of folate in New Zealand reported that reproductive age females would consume a median of 535 µg/day DFEs if a mandatory policy was introduced which required breads to be fortified with 80 – 180 µg/100g of folic acid. This fortification model would result in 5% of the participants consuming less than the EAR of 320 µg/day DFEs on average (Evans et al., 2014).

2.3 Vegetarianism

The prevalence of vegetarianism is suggested to be on the rise globally. A 2014 study "guess-timated" using national census data (excluding India) that the prevalence of vegetarianism to be ~40%; countries including; USA, Italy, Great Britain and Germany, are estimated to have a prevalence closer to 9% (Leitzmann, 2014). Similarly, online market research in New Zealand from Roy Morgan Research Ltd report a current prevalence of 10.3% of New Zealanders 14 years or older were either always or mostly

consuming a vegetarian diet. The 14 – 25 year age bracket had the largest increase from 2011 – 2015 with a rise from 8.6 – 13.3% (Roy Morgan Research, 2016). The rise of vegetarianism among various population groups is likely to lead to both health benefits and risks, including those associated with nutrient intakes.

2.3.1 Definition

Vegetarianism is a broad term for a group of dietary restrictions that abstain from some or all animal products. The New Zealand Vegetarian society recognises 6 different types of vegetarians (NZ Vegetarian Society, 2019).

Table 2-1 Vegetarian diet types

Vegetarian Type	Restrictions
Vegan	No animal products consumed or used
Lacto-ovo-vegetarian	Consumes dairy and eggs
Lacto-vegetarian	Consumes dairy
Ovo-vegetarian	Consumes eggs
Pescatarian	Consumes fish
Flexitarian	Plant-based diet (meat consumed in moderation)

2.3.2 Health consequences of vegetarianism

Vegetarianism has shown to reduce the incidences of both ischaemic heart disease and cancer when compared to those eating an omnivorous diet (Palacios & Maki, 2019), although scepticism has arisen for some of these results as those with a vegetarian lifestyle have shown to exhibit many other healthier lifestyle factors. In the US population, vegetarian dietary patterns of the Seventh Day Adventists have often been studied, and may skew health outcomes as this population subgroup also does not smoke,

consume alcohol, exercises regularly and undertakes adequate rest as part of their faith (Palacios & Maki, 2019).

Mechanisms for the reduced incidences of adverse outcomes are suggested to be due to the removal of meats that contain specific nutrients and chemicals that are associated with chronic disease. For example, processed and red meats have higher levels of sodium, nitrates and nitrites, all of which have shown associations with negative health outcomes (Domingo & Nadal, 2017; Palacios & Maki, 2019). Red meat is high in saturated fatty acids that have been linked with an increase in low density lipoprotein-cholesterol (LDL-C) and consequently an increase in cardiovascular disease (Hooper et al., 2015). Cooking of meats has shown to increase the consumption of carcinogens and oxidative factors in the diet. The addition of nutrients like magnesium, vitamin C, E and K, non-haem iron and fibre through substituting meat for a vegetarian diet can also be suggested to create positive health outcomes in these populations (Palacios & Maki, 2019).

The removal of animal products reduces some essential nutrients in the diet that could have detrimental effects on the health of the population. Animal products are rich sources of haem iron, zinc, vitamin B12, calcium, vitamin D and long chained omega 3 fatty acids (Pereira & Vicente, 2013). However, a well-planned vegetarian diet will reduce the risk of suboptimal intake of these nutrients.

2.3.3 Folate intakes among vegetarian female adolescents

Folate is found in a lot of green leafy vegetables, legumes, fruits, and less plentiful in meat products except for offal (such as liver). This suggests that those with a vegetarian or vegan diet would be at a lower risk of folate inadequate diets (Allen, 2008). It is clear

within the literature that female populations and adolescent females consuming vegetarian diets around the world when compared to those with meat in their diets have a higher intake of folate in their diets. These findings include countries like; Canada, India and Sweden, reflecting a diverse intake of foods (Houghton et al., 1997; Larsson & Johansson, 2002; Sivaprasad et al., 2016).

A recent US study on vegetarianism among a sample of 534 adolescent males and females aged 12 – 18-years-old in mostly Adventist communities showed a significant increase in the consumption of folate for vegetarians when compared to non-vegetarians (675.19 vs. 540.77 µg/day ($p < 0.0001$), respectively). Vegetarians in the study had significantly higher intakes of several food groups including breads/grains/pastas/cereals, vegetables, fruits, nuts/nut butters/meat alternatives, and dairy alternatives (Segovia-Siapco et al., 2019).

A study in Canada prior to mandatory folate fortification showed lacto-ovo-vegetarians consumed significantly more folate compared to omnivores (median intakes: 228.3 vs. 163.7 µg/day ($p < 0.05$), respectively) among 224 females aged 14 – 19 years. It also showed a higher prevalence of inadequate intakes in omnivores compared to lacto-ovo-vegetarians with 16% of omnivores consuming less than 2/3 of the RNI (400 µg/day) compared to 7% in the vegetarian group (Houghton et al., 1997).

To date, there have been no studies in New Zealand investigating the dietary folate intakes of vegetarian versus non-vegetarian females. However, worldwide studies have shown that vegetarian females consume a higher level of folate. It is unknown whether

this difference can be assumed to overcome the previous level of inadequate folate intakes seen in the few national studies on the adolescent female population.

Objective Statement

Folate's important role in pregnancy and neural tube defect prevention has been well known for many years yet data on the amount of folate consumed by young reproductive age females are limited. There has been a noted rise in plant-based diets within this population lifecycle group as vegetarian and vegan diets become increasingly popular. As such, a better understanding of dietary intakes and major food sources of folate amongst vegetarian and non-vegetarian adolescent females would be invaluable for future policy, particularly in the area of food fortification.

Research Aims: The aim of the present study was to evaluate the dietary intake of folate among a sample of adolescent vegetarian and non-vegetarian females in New Zealand.

The specific research objectives are:

- To describe the dietary total folate and folic acid intakes of vegetarian and non-vegetarian adolescent females
- To assess dietary folate adequacy of vegetarian and non-vegetarian adolescent females.
- To determine the major food folate and folic acid sources among vegetarian and non-vegetarian adolescent females.

3 Methods

This cross-sectional study was part of the larger Survey of Nutrition Dietary Assessment and Lifestyle (SuN DiAL) project. The aim of the SuNDiAL project was to compare the nutritional status, dietary habits, health status, and attitudes and motivations for food choice of vegetarian and non-vegetarian adolescent women. This thesis focus will be on data relevant to the intake, prevalence of inadequacy and major food sources of folate among vegetarian and non-vegetarian participants.

3.1 Study Participants

Participants were eligible for inclusion in the study if they self-identified as female, were between 15 and 18 years of age, and able to speak and understand English to complete the required online questionnaires. Participants were excluded from the study if they were aware, they were pregnant.

The study was conducted in two recruitment phases: firstly, from February 2019 - April 2019, and the second phase from July 2019 - September 2019. Two methods were used to recruit participants via i) a selection of secondary schools and ii) targeted recruitment of adolescent females that identified as vegetarian.

High schools were selected in locations across New Zealand based on where data collectors were located and then prioritised if they had a roll number of at least 400 students for co-educational schools and 200 students for female only schools. Decile was considered to ensure that the selected schools represented a range from one to ten. Once

selected, schools were sent invitations to participate via email with follow-up phone calls. In the first phase, eight high schools agreed to participate from Dunedin, Christchurch, Nelson, Wellington, New Plymouth, Tauranga and Whangarei; and the second phase, five high schools participated from Dunedin, Wanaka, Wellington and Whangarei. Data collectors visited participating schools to provide information on the study to eligible participants through presentations. Interested students were then invited to sign up via email, where they could access further information and enrol after providing online consent. For participants aged 15 years, parental consent was obtained.

Targeted recruitment of vegetarian participants took place in Dunedin only in the second recruitment phase via social media (Facebook and Instagram) and local newspapers. A link to the study website was promoted and similarly, interested participants were invited to enrol and provide online consent. Ethical approval of the overall SuNDiAL protocol was obtained by the University of Otago Human Ethics Committee (Health): H19/004. The survey was also registered with the Australian New Zealand Clinical Trials Registry: ACTRN12619000290190.

3.2 Data Collection

3.2.1 Sociodemographic, Health and Dietary Habit questionnaires

Socio-demographic and health data, as well as questions on dietary habits, and attitudes and motivations to food choices were collected via online questionnaire (Appendix 1, Appendix 2, Appendix 3). Classification by ethnicity was based on self-reports by the participant, with the option of selecting all of the applicable categories: New Zealand

European, Māori, Samoan, Cook Island Maori, Tongan, Niuean, Chinese, Indian, Other. Rules were then established as per the New Zealand census to assign participants to a single ethnic category when they reported as belonging to more than one ethnic group. Health data were collected including information on vegetarianism and any food restrictions. Dietary habits, and food choice attitude and motivations were collected using an online version of both the Dietary Habits and the Attitudes and Motivations for Food Choice questionnaires. These questionnaires used a combination of scales to assess participants' level of agreement with certain statements regarding their food choices (such as ethical reasoning for following their dietary pattern), how often certain foods/food groups (such as fruit and vegetables, meat and dairy) were consumed and the frequency of dietary habits (such as breakfast consumption). Supplementation use was assessed within the participants dietary habits, with questions pertaining to type, brand, dosage and frequency.

Participants deprivation level was estimated using the New Zealand Deprivation Index 2013 (NZDep). NZDep provides a measure of relative socioeconomic deprivation between geographical areas. The NZDep scale (1 – 10) is a simple way of determining the relative level of deprivation, where 1 encompasses the 10% of New Zealand with the lowest deprivation and 10 is the 10% with the highest level of deprivation. The deprivation scores are determined by multiple factors including unemployment, education, income level and source, access to transportation, home ownership, bedroom occupancy (number of people per room) and access to internet (Atkinson et al., 2014).

Categories used in the present study range from low (1 to 3 on NZDep scale), moderate (between 4 – 7), and high deprivation (from 8 – 10).

3.2.2 Anthropometry

Each participant was contacted for an in-person interview, which was used to gather anthropometric measurements and perform the first of two interviewer-led 24-hour diet recalls. Height and weight data were collected using standardised techniques (Appendix 4) by trained data collectors. Weight was measured using four types of scales: Medisana PS 420, Salter 9037 BK3R, Seca Alpha 770 and Soehnle Style Sense Comfort 400. Height was measured using two types of portable stadiometers: the Seca 213 and Wedderburn stadiometers. Measurements were taken in duplicate to the nearest 0.1 kg for weight and 0.1 cm for height. If the difference of the two measurements were greater than 0.5 kg for weight and 0.5 cm for height, then a third measurement was taken. Body mass index z scores (BMI z-score) were calculated using the WHO growth reference for school-aged children and adolescents (de Onis et al., 2007). A z-score of less than -2 is considered “underweight, -2 to 1 is a “healthy” range, 1-2 is “overweight” and above 3 is termed “obese”.

3.2.3 Dietary intake of folate

Dietary intake was collected via two multi-stage 24-hour recall taken from midnight to midnight of the previous day. Initial interviews were in person by a trained interviewer, with the second recall conducted by either phone call or video call. The recall involved firstly gathering a quick list containing all the foods and beverages consumed during the

24 hours, then a more detailed list was produced, including brands, amounts, cooking methods and any leftovers or comments. A list of prompts was used by each interviewer for commonly consumed foods that were listed by the participants to make the process more accurate. Food quantities were assessed by the use of measuring implements (measuring cups, crockery, and photographed food models) (Appendix 5).

The dietary recall data were entered into FoodWorks 9 (Xyris Software Australia Pty Ltd) and analysed for daily intakes energy (kJ), natural food folate (μg), folic acid (μg) and total folate (DFEs). FoodWorks is comprised of the most up-to-date and comprehensive food composition tables for New Zealand (FOODfiles 2018 (The New Zealand Institute for Plant & Food Research Limited)) along with recipe calculated foods from the latest 2008-09 national representative Adult Nutrition Survey (ANS0809).

To ensure consistency among those entering data, a FoodWorks protocol and training session was held to check for differences and errors before commencing with data entry. For each food and beverage item, data collectors aimed to select the exact item within the FoodWorks database. Where participants consumed items not found in FoodWorks, an appropriate substitution with similar macronutrient content within 10% was made. To aid in making appropriate substitutions, a codebook containing previous substitutions used for certain food items in other studies was made available. The codebook also contained default portion size or food weight estimations to use when such information was not provided from the participant. For food items based on a recipe, each food ingredient was entered into a recipe file and given retention factor and a nutrient yield to allow for the most accurate quantification of nutrients after cooking. As folic acid fortification of bread

is voluntary in New Zealand, a list of all brands of breads fortified with folic acid was provided to ensure accurate measurement of total folate and folic acid intakes among participants (Appendix 6).

3.3 Statistical analysis

A sample size of 300 high school students enrolled from 14 high schools was estimated to provide 80% power to the α=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.

The dietary intake estimates were adjusted for 'usual intake' using the Multiple Source Method (MSM) (Harttig et al., 2011). A second 24-hour diet recall allowed for the estimation of 'usual intake' for both natural folate and total folate DFE, by using the MSM programme to adjust for the within-person variation in intakes (Harttig et al., 2011). In contrast, folic acid was presented as a mean daily intake as adjustment for usual intake was not possible given participants often consumed no folic acid within a day (i.e., a value of zero). The EAR cut-point method was used to estimate the proportion of participants not meeting the requirement for folate (i.e., inadequate intakes). Estimates of prevalence and mean intakes were reported along with 95% confidence intervals. A binary variable for vegetarianism was created to allow for comparison between vegetarians and non-vegetarians.

Data were analysed using both Microsoft Excel, version 1909 (Build 12026.20264) (Microsoft Excel, Redmond, Washington, USA) for the majority of descriptive data and Stata (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC) to calculate the major food sources contributing to folate intakes across the sample population. For the latter, intake of folate provided by the food source was summed across all the individuals and divided by the total amount of folate to determine the mean proportion of contribution to folate intake in each food group.

4 Results

Of the 282 participants who enrolled in the study, a total of 250 participants completed at least one 24 hour recall, with 213 of these participants completing a second recall **(Figure 4-1)**. Three participants declined the collection of anthropometric data. In addition, two participants did not complete the dietary habit questionnaire, which included dietary supplement data.

Table 4-1 presents the sociodemographic and health characteristics of study participants. The majority of the population identified as non-vegetarian, with only 31 of the 250 participants (12%) reporting to be vegetarian. The average age of participants was 16.8 years; however, the subgroup of vegetarian participants was slightly older (17.1 years). The majority of participants were categorised from low to moderate deprivation areas. Over three-quarters of participants identified as New Zealand European and Other, with approximately 16% of participants classified as Māori (22% of vegetarians versus 15% of non-vegetarian) and a small proportion of Pacific and Asian participants. Two-thirds of all participants were classified as normal weight, nearly one-quarter were overweight and approximately 10% classified as obese. A higher proportion of vegetarians (24 of 31; 77%) were normal weight compared to non-vegetarians (139 of 219; 64%) and likewise, a lower proportion of vegetarians were overweight and obese compared to non-vegetarians.

Of the 248 participants that completed the dietary habits questionnaire, 92 (37%) participants were using a supplement; of those, 16 (17.4%) identify as vegetarian, with 38

(15%) of participants taking a multivitamin or B-vitamin containing complex. The folic acid content of these products ranged from 50 to 400 μg per dose. Folate-containing supplements such as spirulina were also reported, although use of this product in the form of tablets, powder and premade smoothies was considerably lower, with 12 (13% of supplement users) participants consuming it less than once per month. The folate content of the spirulina products was unknown.

Table 4-2 shows the estimated usual daily intake of energy and folate and prevalence of folate inadequacy for all participants, and by vegetarian and non-vegetarian status. The usual daily total folate intake of the sample population was 306 μg DFE/day, with an estimated folic acid intake of 33 μg/d. The overall prevalence of inadequate folate intake was high, with over half of all participants consuming folate intakes below the EAR. For those who reported being vegetarian, usual folate intakes were higher, and prevalence of inadequacy was substantially lower compared to non-vegetarian (32% vs 61%, respectively) despite slightly lower energy intakes among vegetarian participants. Moreover, folic acid intakes were higher among vegetarians compared to non-vegetarian participants. Of note, only 26% (8 of 31) of vegetarian participants did not consume folic acid in their diet, whereas 37% (82 of 219) non-vegetarians consumed no folic acid. When looking at the maximum level of folic acid consumed, we found one participant consumed 503 μg/d, derived from fortified food alone not supplements. For both vegetarian and non-vegetarian participants, the prevalence of folate inadequacy was higher for those participants in the higher deprivation category and among NZEO

participants. Less than 10% of Maori, Asian and Pacific participants had overall intakes that fell below the EAR of 330 µg/day for both subgroups.

Mean differences in folate intakes between socio-demographic subgroups are presented by vegetarian and non-vegetarian status in **Table 4-3**. When comparing the vegetarians to non-vegetarians, natural folate and total folate intakes were significantly higher for the vegetarian participants compared to non-vegetarians, with a mean difference (95% CI) of 75.7 µg/day (57.7, 93.7 ug/day) and 82.6 µg DFEs/day (36.7, 128.5 µg), respectively.

Of the vegetarian participants, those with healthy BMIs tended to have higher intakes of folic acid, natural and total folate. Those with low to moderate deprivation levels had significantly higher natural and total folate, whereas, participants of Maori ethnicity or moderate deprivation tended to have higher folic acid intakes. For the non-vegetarian group, Pacific participants had substantially higher intakes of total folate and folic acid intakes compared to NZEO. Similarly, those with low to moderate deprivation tended also to have higher natural folate intakes.

Table 4-4 outlines the 10 top food groups contributing to overall folate intakes. The top two contributors for vegetarians were vegetables and bread, and for non-vegetarians the result was reversed. Vegetarians had a slightly higher percentage of participants consuming both food groups with 87.1 and 83.9% for vegetables and bread, respectively. Breakfast cereals were third and sixth for non-vegetarian and vegetarian participants, respectively. However, only one vegetarian and 80 non-vegetarians consumed them.

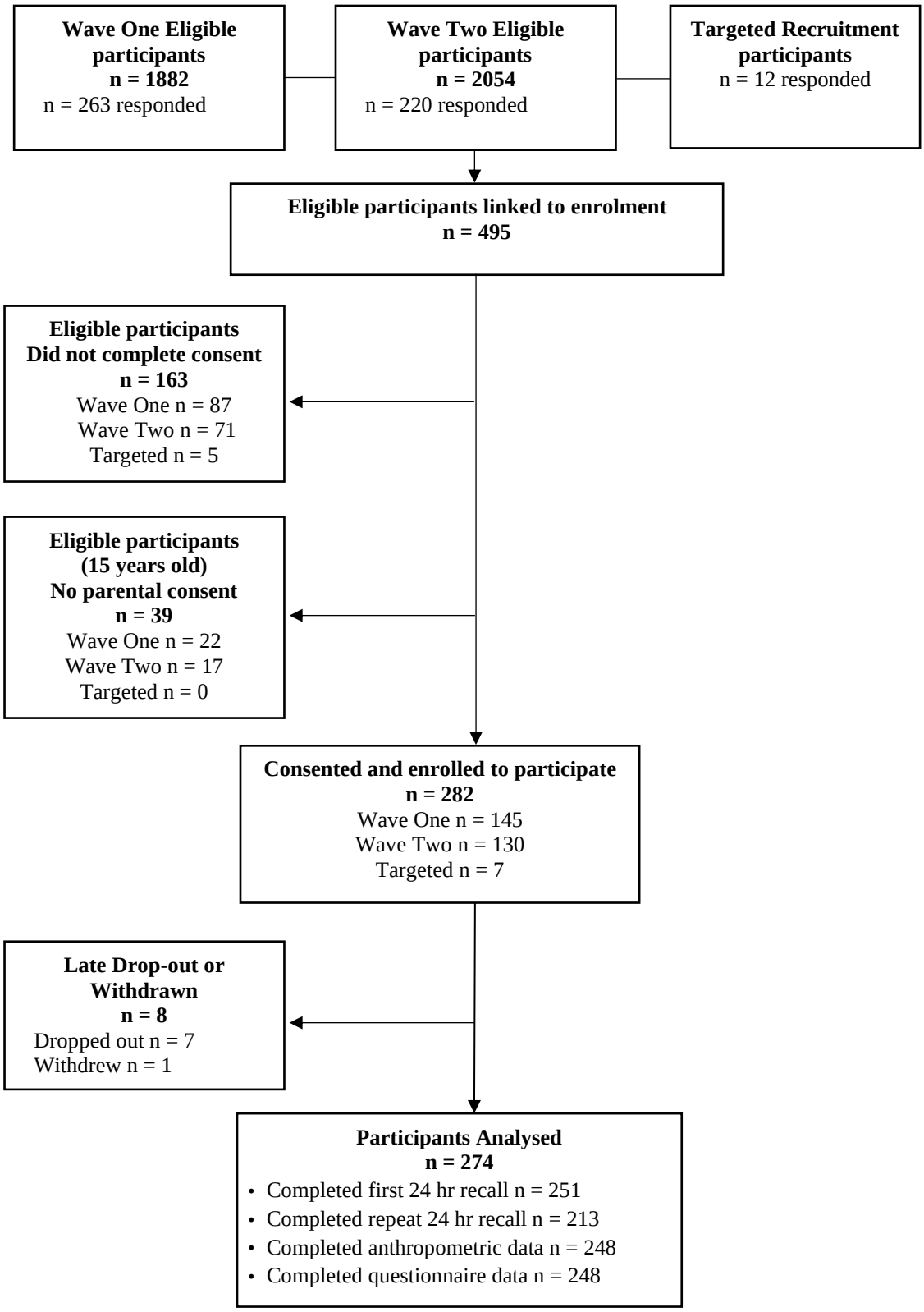

Figure 4-1 Sundial participant flow chart

Table 4-1: Sociodemographic and health characteristics of participants by vegetarian and non-vegetarian status

Characteristics	All participants (n = 250) n (%)[2]	Vegetarian (n = 31) n (%)[2]	Non-vegetarian (n = 219) n (%)[2]
Age, years [mean (SD)]	16.8 (0.8)	17.1 (0.8)	16.8 (0.9)
15 – 16 years	98 (39.2)	12 (38.7)	120 (54.8)
17 – 18 years	152 (60.8)	19 (61.3)	99 (45.2)
Ethnicity[1]			
NZEO	195 (78.0)	24 (77.4)	171 (78.1)
Maori	39 (15.6)	7 (22.6)	32 (14.6)
Pacific	6 (2.4)	0 (0.0)	6 (2.7)
Asian	9 (3.6)	0 (0.0)	9 (4.1)
NZDep Score [mean (SD)]	4.6 (2.5)	4.5 (2.4)	4.6 (2.5)
1 (low)	92 (36.8)	12 (38.7)	80 (36.5)
2 (moderate)	115 (46.0)	15 (48.4)	100 (45.7)
3 (high)	43 (17.2)	4 (12.9)	39 (17.8)
BMI category[1]			
Underweight (<-2)	0 (0.0)	0 (0.0)	0 (0.0)
Healthy (-2 - ≤1)	163 (66.0)	24 (77.4)	139 (64.4)
Overweight (>1 – 2)	58 (23.5)	5 (16.1)	53 (24.5)
Obese (>2)	26 (10.5)	2 (6.6)	24 (11.1)

Abbreviations: NZEO, New Zealand European and Others; NZDep, New Zealand Deprivation; BMI, Body Mass Index.

[1]Missing data: BMI category, n=3; Ethnicity, n=1

[2]Unless specified otherwise

BMI categories defined: Underweight <18.5kg/m^2, Healthy 18.5 – 24.9kg/m^2, Overweight 25 – 29.9kg/m^2, Obese >30kg/m^2.

Table 4-2 Folate intakes by population group and prevalence of inadequate intakes

Population Group	n	Energy (kJ/day) Mean ± S.D.	Natural folate intake (µg/day) Median (25^{th}-75th)	Folic acid intake (µg/day) Median (25^{th}-75th)	Total Folate (µg DFEs/day) Median (25^{th}-75^{th})	Prevalence of intake <EAR[1] % (n)[3]
All participants	250	8079.0 ± 1781.6	219.6 (172.5, 281.7)	33.0 (0.0, 90.0)	306.8 (232.3, 409.5)	57.2 (143)
Vegetarians	31	7676.6 ± 1518.1	272.3 (242.2, 371.0)	53.0 (0.7, 102.0)	431.1 (318.4, 471.4)	32.3 (10)
BMI category						
Healthy	24	7584.8 ± 1705.9	274.9 (246.5, 388.8)	49.3 (1.0, 107.2)	457.3 (321.3, 476.0)	22.6 (7)
Overweight	5	7811.6 ± 300.4	243.8 (238.4, 245.7)	2.2 (0.0, 96.0)	289.0 (283.7, 370.3)	60.0 (3)
Obese	2	8441.4 ± 618.1	309.0 (306.0, 312.1)	84.9 (75.4, 94.4)	435.4 (433.3, 437.6)	0.0 (0)
Ethnicity						
NZEO	24	7811.0 ± 1522.5	273.4 (240.4, 362.7)	59.4 (0.0, 102.0)	435.4 (320.2, 471.4)	25.8 (8)
Maori	7	7215.9 ± 1522.4	272.3 (255.6, 377.8)	45.5 (1.8, 98.6)	362.0 (314.0, 529.4)	6.5 (2)
NZDep category						
Low	12	7297.1 ± 817.3	291.6 (259.1, 411.7)	5.1 (0.0, 58.3)	361.1 (321.3, 463.9)	33.3 (4)
Moderate	15	8069.4 ± 1821.4	269.3 (239.2, 371.0)	96.1 (43.5, 118.3)	462.6 (356.7, 479.6)	26.7 (4)
High	4	7342.4 ± 1902.6	244.8 (236.5, 259.3)	49.1 (1.6, 97.5)	329.7 (282.0, 388.7)	50.0 (2)
Non-vegetarians	219	8136.6 ± 1811.6	207.0 (166.1, 273.3)	28.0 (0.0, 83.2)	291.6 (225.0, 388.1)	60.7 (133)
BMI category[2]						
Healthy	142	8309.1 ± 1849.6	215.2 (175.4, 273.0)	28.0 (0.0, 87.7)	298.3 (238.9, 382.4)	39.7 (87)
Overweight	53	7742.2 ± 1767.8	190.4 (155.1, 273.0)	41.6 (0.0, 78.0)	288.4 (193.4, 403.1)	13.7 (30)
Obese	24	7987.2 ± 1582.7	205.5 (162.1, 276.1)	1.1 (0.0, 45.8)	260.8 (224.1, 353.9)	7.3 (16)

Table 4-2 Continued…

Population Group	n	Energy (kJ/day) Mean ± S.D.	Natural folate intake (µg/day) Median (25^{th}-75th)	Folic acid intake (µg/day) Median (25^{th}-75th)	Total Folate (µg DFEs/day) Median (25^{th}-75^{th})	Prevalence of intake <EAR[1] % (n)[3]
Ethnicity						
NZEO	171	8209.7 ± 1811.9	206.7 (162.9, 271.3)	24.0 (0.0, 73.2)	281.9 (214.2, 374.5)	47.5 (104)
Maori	32	7740.2 ±1455.8	231.0 (193.9, 287.4)	39.0 (11.2, 90.7)	322.1 (281.9, 396.7)	9.6 (21)
Asian	9	7488.2 ± 2250.0	186.3 (174.0, 228.1)	10.0 (0.0, 164.0)	331.0 (207.1, 406.2)	2.7 (6)
Pacific	6	9484.0 ± 2293.0	182.2 (120.3, 302.2)	58.0 (42.3, 253.5	254.8 (199.8, 511.2)	1.0 (2)
NZDep category						
Low	80	8396.0 ± 1942.9	228.1 (180.1, 278.8)	39.0 (0.0, 96.1)	317.4 (255.9, 407.3)	60.0 (48)
Moderate	100	8074.6 ± 1728.2	198.6 (162.5, 271.1)	16.3 (0.0, 65.8)	269.1 (212.9, 360.6)	56.0 (56)
High	39	7762.6 ± 1704.0	190.4 (162.7, 255.6)	46.5 (0.0, 99.8)	290.8 (195.7, 390.2)	74.4 (29)

Abbreviations: NZDep, New Zealand Deprivation; BMI, Body Mass Index; EAR, Estimated Average Requirement; NZEO, New Zealand European and Other; DFE, Dietary Folate Equivalent. [1]EAR = 330 µg DFEs/day [2]Missing data: BMI category, n=3. [3]Number of participants below the EAR-cut off were individually calculated and divided by the total number of participants from the same subgroup and multiplied by 100

Table 4-3 Mean natural folate, folic acid and total folate intake differences among vegetarian and non-vegetarians and the respective socio-demographic and BMI subgroups

Group Comparison	Natural Folate µg/day Mean difference (95% C.I.)	Folic Acid µg/day Mean difference (95% C.I.)	Total Folate µg DFEs/day Mean difference (95% C.I.)
Vegetarian vs non-vegetarian	75.7 (57.7, 93.7)	9.5 (-4.3, 23.3)	82.6 (36.7, 128.5)
Vegetarians:			
BMI category:			
Healthy vs O & O	35.4 (11.7, 59.2)	38.9 (13.1, 64.7)	61.1 (26.2, 96.1)
NZDep category:			
Low vs Moderate	33.5 (-4.1, 71.0)	-39.8 (-67.9, -11.7)	-4.1 (-51.4, 43.3)
Low vs High	78.8 (46.1, 111.5)	-2.6 (-38.8, 33.5)	74.9 (19.8, 130.0)
Moderate vs High	45.4 (14.2, 76.5)	37.1 (4.2, 70.1)	79.0 (27.9, 130.1)
Ethnicity:			
NZEO vs Maori	-13.2 (-59.3, 32.9)	-41.7 (-80.6, -2.9)	-53.6 (-115.3, 8.2)
Non-vegetarians:			
BMI category:			
Healthy vs O & O	9.5 (-3.5, 22.4)	1.8 (-9.8, 13.5)	10.2 (-11.0, 31.4)
NZDep category:			
Low vs Moderate	22.6 (7.3, 37.8)	10.2 (-2.4, 22.8)	26.3 (3.7, 48.9)
Low vs High	58.1 (40.1, 76.1)	1.1 (-16.2, 18.5)	54.3 (22.6, 86.0)
Moderate vs High	35.5 (20.7, 50.3)	-9.1 (-24.8, 6.7)	28.0 (-0.8, 55.9)
Ethnicity:			
NZEO vs Maori	35.2 (19.7, 50.7)	-6.9 (-25.5, 11.7)	25.2 (-5.8, 56.2)
NZEO vs Pacific	-84.2 (-144.8, -23.7)	-99.6 (-164.9, -34.4)	-200.6 (-320.2, -81.0)
NZEO vs Asian	33.4 (-4.0, 70.8)	-0.7 (-19.3, 18.0)	30.6 (-20.6, 81.8)

Abbreviations: BMI O & O, Body Mass Index Overweight + Obese; C.I, Confidence Interval; DFE, Dietary Folate Equivalent; NZDep, New Zealand Deprivation NZEO, New Zealand European and Other

Table 4-4 Major food group contributors of folate intakes by vegetarian and non-vegetarian participants

	Vegetarian n = 31			Non-Vegetarian n = 219		
	Total folate (Dietary Folate Equivalents)					
Ranking	Food Group	% intake (CI)[1]	n (%)	Food Group	% intake (CI)[1]	n (%)
1	Vegetables	16.7 (11.4, 22.1)	27 (87.1)	Bread[2]	16.6 (14.0, 19.2)	179 (81.7)
2	Bread[2]	13.1 (7.2, 19.0)	26 (83.9)	Vegetables	13.4 (11.4, 15.3)	179 (81.7)
3	Savoury sauce, condiments[4]	12.1 (5.3, 18.9)	13 (41.9)	Breakfast cereals	9.3 (6.9, 11.6)	80 (36.5)
4	Fruit	9.1 (4.1, 14.2)	11 (35.5)	Fruit	7.6 (6.2, 8.9)	170 (77.6)
5	Grains, pasta	8.8 (3.6, 14.0)	16 (51.6)	Grains, pasta	5.2 (4.2, 6.2)	154 (70.3)
6	Breakfast cereals	8.5 (3.1, 13.9)	11 (35.5)	Potatoes, kumara, taro	5.2 (3.6, 6.8)	142 (64.8)
7	Milk	4.5 (0.5, 8.5)	3 (9.7)	Bread-based dish	5.2 (4.2, 6.2)	69 (31.5)
8	Bread-based dish	4.5 (0.2, 8.8)	2 (6.5)	Savoury sauce, condiments[4]	5.1 (3.6, 6.7)	155 (70.8)
9	Potatoes, kumara, taro	4.4 (2.2, 6.5)	8 (25.8)	Eggs, egg dishes	4.1 (3.0. 5.2)	59 (26.9)
10	Nuts, seeds	2.9 (1.2, 4.6)	1 (3.2)	Milk	3.4 (2.7, 4.1)	140 (63.9)

Abbreviations: CI, 95% Confidence Interval.[1] Total mean percentage of folate consumed from the food group, [2] includes rolls and speciality breads, [3]includes those that provide energy, [4]Including yeast and vegetable extracts.

5 Discussion and Conclusion

The findings from the present study suggest that vegetarian diets are higher in overall dietary total folate among the sample population, with vegetarian participants consuming nearly double the intake of folic acid compared to non-vegetarians. Likewise, the prevalence of inadequate folate intakes was substantially lower among vegetarians compared to non-vegetarians. While the major food contributors to folate intakes were similar between vegetarian and non-vegetarian participants, the ranking of foods differed with savoury sauces (includes yeast extracts) in particular contributing a higher percentage of dietary folate among vegetarians. Overall, the high prevalence of inadequate folate intakes (>50%) among the whole sample population was concerning, with a risk of inadequacy being even greater among participants from higher deprivation levels.

Folate intakes among the whole sample population

When comparing the dietary folate intakes in the present study with data obtained in last national survey reporting folate intakes in 1997, we observed that overall total folate intakes were approximately 100 μg/d higher (307 vs 203 μg DFEs per day among females aged 15–24 years) (Russell et al., 1999). Nonetheless, the major food group contributors of folate intakes were similar, with both naturally rich folate foods and fortified food products such as breads (13%) and breakfast cereals (11%) ranked as major contributor to overall intakes. The 2008 – 09 Adult Nutrition Survey did not report folate

intakes due to an unreliable food composition database, and as such, the latest nationally representative survey providing folate intakes – the New Zealand 1997 survey – is now over 20 years old. It is likely that food consumption patterns have changed since the 1997 survey, including fortification practices such as increased voluntary folic acid fortification of bread products. In comparison to countries with similar fortification practices to New Zealand such as the UK, the dietary folate intakes reported in the present study are substantially higher (178 vs 307 μg DFEs) (Roberts et al., 2018).

More recent studies albeit smaller, non-representative surveys of reproductive age New Zealand women have reported similar daily folate intakes compared to the present study (307 μg DFEs). In a survey of 125 young adult females conducted in 2009, mean daily folate of 362 μg DFEs per day were reported, with 37% of participants not achieving adequate dietary folate intake compared to the present study where 57% of participants were at risk of folate inadequacy (Evans et al., 2014). In addition, a smaller cross-sectional study on 47 adolescent New Zealand female ballet dancers in 2015 found 62% of participants fell below the EAR of with a mean daily intake of 302 μg/day (Beck et al., 2015).

Folic acid intakes in the present study were fairly low at 33 μg/day as compared to the estimated mean folic acid intakes per day among Australian adolescents aged 14-16 years of 110 μg/day reported prior to mandatory folic acid fortification. Of note, a fairly large proportion of our participants consumed no folic acid in their reported diets (30% of vegetarian and 37.4% of non-vegetarians). Following mandatory fortification of Australian bread, estimated mean folic acid intakes increased substantially to 323 μg/day.

None of our participants in the present study had intakes above the UL whereas 4% of Australian adolescents were above the UL post-fortification (Food Standards Australia New Zealand, 2016).

Folate intakes among vegetarian and non-vegetarian female adolescents

Green leafy vegetables, legumes, and fruits are richer food sources of folate compared to meat products (except some offal including liver). As such, it was not surprising to observe a higher intake of folate among vegetarians as compared to non-vegetarians, with vegetables ranking as the highest contributor of folate among vegetarians compared to the second highest contributor of folate among non-vegetarians. In addition, recommended meat alternatives such as legumes and beans were classified in the present study within the vegetable food group category, and likely making a meaningful contribution to the folate intakes of our vegetarian participants.

Similar to our findings, previous published literature of female populations, including adolescent females, have reported higher folate intakes among those consuming vegetarian diets compared to those with meat in their diets. (Houghton et al., 1997; Larsson & Johansson, 2002; Segovia-Siapco et al., 2019; Sivaprasad et al., 2016). Interestingly, savoury sauces were ranked as the third highest contributor of folate to diets of vegetarian participants (12%) in the present study likely due to inclusion of folate-rich yeast-based extract spreads in this food group. In contrast, savoury sauces were ranked eighth (5%) among the top ten major contributors of folate among non-vegetarian participants. Lastly, breakfast cereals were also a major contributor of folate to the diets of both vegetarian and non-vegetarian participants – although this food group

was ranked as the third highest contributor in non-vegetarians and sixth highest contributor in vegetarians. A recent labelling survey report in 2017 revealed that breakfast cereals had the highest number of folic acid fortified products (37%) followed by bread (10%) and breakfast beverages (8%) (Ministry for Primary Industries, 2018).

Despite higher folate intakes among vegetarians compared to non-vegetarians in the present study, the prevalence of inadequacy (32%) remains a concern. In addition, among those reporting consuming a vegetarian diet, Maori participants compared with NZEO ethnicities, and those from higher deprivation areas, had significantly lower intakes of folate and a higher prevalence of inadequate intakes. Likewise, healthy weight vegetarian participants appeared to have higher folate intakes compared to overweight and obese participants. The sample size, however, in each of these subgroups at higher risk of inadequate intakes was very small, therefore reducing the power of these results. For non-vegetarian participants, folate intakes were also higher among participants from low deprivation areas compared to moderate and high deprivation areas, however, unlike vegetarians, folate intakes did not differ between ethnic groups and BMI categories.

Strengths and Limitations of the study

The results of this study are limited by the non-representative nature of the study design and small sample size, particularly among vegetarian participants and various socio-demographic subgroups. As such, our findings cannot be generalised to the population. However, careful protocol and methodology was employed in the collection of dietary data including two 24-hour recalls with statistical adjustment for usual intakes. In addition, our food composition table had values for both natural food folate and folic

acid, however, in most cases these were manufacturer reported values rather than analysed. We did not, however, collect accurate data on fortified bread values and the supplementation use among the participants. Thus, creating a level of uncertainty which may lead to an over- or underestimation of both folic acid and total folate (DFE) for the sample population. An analysis of blood folate status would have contributed to a better understanding of the folate health of the participants.

Conclusion

Overall, the findings from the present study suggest adolescent females are not consuming adequate intakes of folate, although participants consuming vegetarian diets are at lower risk of inadequacy. Nonetheless, certain socio-demographic subgroups such as those from high deprivation areas may be at even greater risk of inadequacy. A proposal to mandate the fortification of bread with folic acid was withdrawn in 2012 although results from the present study lend support to re-visit this public health. Regardless, national dietary survey data are needed to confirm our findings and better understand changing food patterns, particularly given the increased adoption of vegetarian diets among young adults.

6 Application of Research to Dietetic Practice

The present study results suggest that over 50% of female adolescent New Zealanders are currently falling below the estimated average requirement for folate in this lifecycle group. Adolescence is a vulnerable period of growth and development; it is therefore important to support reproductive health. Dietitians should be aware of the high risk of inadequate folate intakes and adjust their practice appropriately to ensure folate rich diets for female adolescents, including monitoring and evaluation of intakes.

Understanding the nutrients at risk within female adolescent diets is paramount to taking a more holistic approach to their care within a clinical setting. Having this information means folate, like calcium and iron (other at risk nutrients), can be treated within the diet in a more appropriate manner to identify any possible shortfalls. For example, suggesting more appropriate foods, knowing that the source of bread, breakfast cereal or juice a patient chooses is fortified, will give them the best opportunity to achieve an adequate diet.

Moreover, adolescence is the beginning of the reproductive age which alters requirements, and which brings on potentially new challenges like pregnancy. Rapid growth of mother and foetus requires a higher level of folate intakes, in particular, folic acid has shown to significantly reduce the risk of NTDs. Therefore, increased effort is needed to ensure folic acid intakes in this population are optimal as many women are unaware of their pregnancy in the early stages during critical time periods, reducing their opportunity to supplement with folic acid.

Changes in New Zealand's diet and food culture also increases the need for current research. Vegetarianism, new convenience foods and a change in cultural diversity means the evidence we have is outdated. It has been more than 10 years since a full scale nutrition survey has been produced (ANS'08-09) and over 20 years since folate data have been reported for the New Zealand public (NNS1997). This leaves small scale studies as the only current evidence of folate intakes for our population. Findings from these studies suggest that our voluntary fortification policy is unable to reduce the folate inadequacy of the female adolescent population as it stands. From a public health perspective this study also outlines the need for more up-to-date research and another look into the validity of current fortification processes, for where a new approach could be more effective.

Public health efforts among dietitians should be directed towards promoting new policy to meeting the current dietary shortfalls seen among adolescent females. An area of focus should be folate in the diet, whether that is promoting the current fortified foods through new marketing schemes or identifying food sources where the fortification could be adopted. This study presents an opportunity for more research to inform public health policy for the optimal health of New Zealanders.

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8 Appendices

Appendix 1

SuNDiAL 2019 Enrolment Questionnaire

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

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

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

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

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

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

Any questions?

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

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

Electronic consent
Click on the "agree" button below if:

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

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

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

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

If you don't want to take part in the SuNDiAL project, please click on the "disagree" button.

◯ AGREE
◯ DISAGREE

Thank you for agreeing to taking part in the SuNDiAL project! If you are female, aged 15-18 years of age and not pregnant, please answer the following two questions:

What age are you as of today?

- ◯ 15
- ◯ 16
- ◯ 17
- ◯ 18
- ◯ None of the above

What high school do you attend?

- ◯ Tauraroa Area School
- ◯ Mt Maunganui College
- ◯ Spotswood College
- ◯ Wellington Girls College
- ◯ Waimea College
- ◯ Hornby High School
- ◯ Columba College
- ◯ Kaikorai Valley College
- ◯ Queens High School
- ◯ Mt Aspiring College
- ◯ None of the above

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

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

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

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

Electronic consent

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

If you don't want to take part in these parts of the study, please click on the "DISAGREE" button.

BLOOD SAMPLE:

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

Click on the agree button below if:

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

◯ AGREE
◯ DISAGREE

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

◯ Yes
◯ No

URINE SAMPLE:

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

Click on the 'AGREE' button below if:

◯ AGREE
◯ DISAGREE

ACCELEROMETER:

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

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

◯ AGREE
◯ DISAGREE

Contact Information	
What is your name?	_____ (Preferred first name, Last name)
What is your date of birth?	_____
Age	_____
Phone number (mobile would be best - so we can text you reminders)	_____
What is your home address? (This will be the address where we will send your voucher)	_____ (number & street, suburb, city, postcode)
Do you live at this address during school term?	◯ Yes ◯ No
Do you live in a boarding house during school term? (Don't include private boarding)	◯ Yes ◯ No
Please put the name and/or address of the boarding house	_____ (number & street, suburb, city, postcode)
What is the address that you live at during school term?	_____ (number & street, suburb, city, postcode)

Health Information	
If you know your height, please write it here:	_____
What unit is this measurement in?	◯ centimetres ◯ metres ◯ feet and inches
If you know your weight (in kg) please write it here:	_____

Have you been diagnosed with diabetes?	◯ Yes ◯ No
If so, which type?	◯ Type 1 diabetes ◯ Type 2 diabetes ◯ Don't know
Do you avoid eating gluten?	◯ Yes ◯ No
Have you been diagnosed with either coeliac disease or gluten intolerance?	◯ Yes - coeliac disease ◯ Yes - gluten intolerant ◯ No diagnosis but suspected intolerance or coeliac ◯ No
Have you been diagnosed with a food allergy or intolerance? (not gluten)	◯ Yes ◯ No
Which foods are you allergic or intolerant to? (Select as many as apply)	☐ Eggs ☐ Dairy ☐ Nuts ☐ Shellfish ☐ Other
Other: please specify	_____
Are you vegetarian or vegan?	◯ Yes ◯ No
Which foods do you eat? (Select as many as apply)	☐ Egg ☐ Milk (not plant milk like soy milk) ☐ Fish or seafood ☐ Chicken or poultry ☐ Meat/red meat occasionally ☐ None of the above
Are you vegan?	◯ Yes ◯ No
How long have you been following this way of eating?	◯ Less than a month ◯ Between 1 and 6 months ◯ Between 6 months and 1 year ◯ Between 1 and 2 years ◯ More than 2 years ◯ My whole life

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

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

Other information

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

Thank you for enrolling in the SuNDiAL project!

What happens next?

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

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

Appendix 2

Dietary Habits Questionnaire

Fruit

On average how many servings of fruit - fresh, frozen, canned or stewed - do you eat per day or per week?
Do not include fruit juice or dried fruit.

A serving is the same as a medium piece of fruit like an apple or two small pieces of fruit like two apricots, or half a cup of stewed or canned fruit.

- ◯ Never I don't eat fruit
- ◯ Less than 1 serving a week
- ◯ 1 serving a week
- ◯ 2-4 servings a week
- ◯ 5-6 servings a week
- ◯ 1 serving a day
- ◯ 2 servings a day
- ◯ 3 servings a day
- ◯ More than 3 servings a day

Vegetables

On average how many servings of vegetables - fresh, frozen or canned - do you eat per day or per week?
Do not include vegetable juices.

A serving is the same as one potato, half a cup of peas or a cup of salad.

- ◯ Never I don't eat vegetables
- ◯ Less than 1 serving a week
- ◯ 1 serving a week
- ◯ 2-4 servings a week
- ◯ 5-6 servings a week
- ◯ 1 serving a day
- ◯ 2 servings a day
- ◯ 3 servings a day
- ◯ More than 3 servings a day

Bread

On average how often do you eat bread?

Include slices of bread, rolls, bagels, wraps, and gluten-free bread.

- ◯ Never I don't eat bread
- ◯ Less than once a week
- ◯ Once a week
- ◯ 2-4 times a week
- ◯ 5-6 times a week
- ◯ Once a day
- ◯ Twice a day
- ◯ 3 times a day
- ◯ More than 3 times a day

What type of bread, rolls or toast do you eat most of the time?

- ◯ White
- ◯ Wholemeal (brown colour)
- ◯ Light grain - has some grains but soft to eat (eg honey grain)
- ◯ Heavy grain - has some grains and a bit chewier (eg Vogels)
- ◯ Other (please specify)

If Other, please specify:

_____

Milk

How often do you have milk (cow's milk or plant milk)?

- ◯ I do not have any milk
- ◯ Rarely
- ◯ Monthly
- ◯ 2-3 times a month
- ◯ Once a week
- ◯ 2-4 times a week
- ◯ 5-6 times a week
- ◯ Once a day
- ◯ More than once a day

What type of milk do you use the most of?

- ◯ None
- ◯ Cow's milk
- ◯ Plant-based milk (eg soy, rice, almond, coconut)
- ◯ Other (such as goat or sheep milk)

What kind of milk do you usually have?

- ◯ Whole or standard milk (Dark blue or silver)
- ◯ Reduced fat (light blue)
- ◯ Skim or trim (green or yellow)
- ◯ Other (please specify)

If Other, please specify:

_____

What kind of milk do you usually have?

- ◯ Regular
- ◯ Lite
- ◯ Sweetened or flavoured

Spreads and Oils

What type of spread do you use the most of?

- ◯ None
- ◯ Butter (including semi soft)
- ◯ Margarine (eg Canola, Sunflower, Olive oil based, or table spread)
- ◯ Other (eg avocado, cream cheese), please specify
- ◯ I don't know

If other, please specify:

_____

What type of fat or oil is used most often in cooking in your household?

- ◯ None
- ◯ Butter
- ◯ Coconut oil
- ◯ Margarine
- ◯ Oil (eg Olive, Canola, or one in a bottle)
- ◯ Dripping or Lard
- ◯ I don't know

Nuts

How often do you eat the following types of nuts? (Include nuts in cooked foods, bars, cereals etc but don't include peanut butter or other nut butters)

	More than once a day	Once a day	5-6 times a week	2-4 times a week	Once a week	2-3 times a month	Monthly	Rarely	I do not eat these
Almonds	○	○	○	○	○	○	○	○	○
Brazil	○	○	○	○	○	○	○	○	○
Cashew	○	○	○	○	○	○	○	○	○
Hazelnut	○	○	○	○	○	○	○	○	○
Macadamia	○	○	○	○	○	○	○	○	○
Peanut	○	○	○	○	○	○	○	○	○
Pecan	○	○	○	○	○	○	○	○	○
Pine nut	○	○	○	○	○	○	○	○	○
Pistachio	○	○	○	○	○	○	○	○	○
Walnut	○	○	○	○	○	○	○	○	○
Other	○	○	○	○	○	○	○	○	○

How often do you eat nut butters?	More than once a day	Once a day	5-6 times a week	2-4 times a week	Once a week	2-3 times a month	Monthly	Rarely	I don't eat this type of nut butter
Almond butter	○	○	○	○	○	○	○	○	○
Cashew butter	○	○	○	○	○	○	○	○	○
Hazelnut butter	○	○	○	○	○	○	○	○	○
Peanut butter	○	○	○	○	○	○	○	○	○
Walnut butter	○	○	○	○	○	○	○	○	○
Other	○	○	○	○	○	○	○	○	○

Meat, Dairy and Eggs

How often do you eat each of the following foods:

	More than once a day	Once a day	5-6 times a week	2-4 times a week	Once a week	2-3 times a month	Monthly	Rarely	I do not eat this
Egg	○	○	○	○	○	○	○	○	○
Cow's milk	○	○	○	○	○	○	○	○	○
Dairy products excluding milk (eg cheese, yoghurt - don't include plant based)	○	○	○	○	○	○	○	○	○
Processed meat (eg ham, bacon, sausages, luncheon, canned corned beef, pastrami, salami)	○	○	○	○	○	○	○	○	○
Other red meat (including beef, lamb, venison etc don't include processed meat)	○	○	○	○	○	○	○	○	○
Pork	○	○	○	○	○	○	○	○	○
Poultry (including chicken, turkey etc)	○	○	○	○	○	○	○	○	○
Fish	○	○	○	○	○	○	○	○	○
Other seafood/shellfish (eg prawns, squid, crab)	○	○	○	○	○	○	○	○	○

Legumes

How often do you eat lentils, chickpeas, kidney beans or baked beans? (Don't include peas or peanuts)	◯ I do not eat legumes ◯ Rarely ◯ Monthly ◯ 2-3 times a month ◯ Once a week ◯ 2-4 times a week ◯ 5-6 times a week ◯ Once a day ◯ More than once a day

Other Foods	
How often do you eat tofu, tempeh and tofu products?	◯ I do not eat these ◯ Rarely ◯ Monthly ◯ 2-3 times a month ◯ Once a week ◯ 2-4 times a week ◯ 5-6 times a week ◯ Once a day ◯ More than once a day
How often do you eat vegetarian ingredients (like quorn, nut meat, vegetarian mince) that are used in other dishes?	◯ I do not eat these ◯ Rarely ◯ Monthly ◯ 2-3 times a month ◯ Once a week ◯ 2-4 times a week ◯ 5-6 times a week ◯ Once a day ◯ More than once a day
How often do you eat vegetarian sausages, nuggets, patties etc?	◯ I do not eat vegetarian meat alternatives ◯ Rarely ◯ Monthly ◯ 2-3 times a month ◯ Once a week ◯ 2-4 times a week ◯ 5-6 times a week ◯ Once a day ◯ More than once a day
How often do you eat vegetarian "meat alternatives" (like chicken-free chicken, vegetarian chicken schnitzel, meat-free bacon rashers etc)?	◯ I do not eat these ◯ Rarely ◯ Monthly ◯ 2-3 times a month ◯ Once a week ◯ 2-4 times a week ◯ 5-6 times a week ◯ Once a day ◯ More than once a day

Sweet Drinks	
How often do you drink diet or drinks labelled "sugar-free"?	◯ I do not drink diet or sugar-free drinks ◯ Rarely ◯ Monthly ◯ 2-3 times a month ◯ Once a week ◯ 2-4 times a week ◯ 5-6 times a week ◯ Once a day ◯ More than once a day
How often do you drink fizzy drinks? Don't include diet varieties. (eg Coca-cola, Pepsi, lemonade)	◯ I do not drink fizzy drinks ◯ Rarely ◯ Monthly ◯ 2-3 times a month ◯ Once a week ◯ 2-4 times a week ◯ 5-6 times a week ◯ Once a day ◯ More than once a day
How often do you drink fruit juices, drinks or cordials? (eg Just Juice, Fresh-up, Keri, Golden Circle, Ribena, Charlie's, Raro). Don't include diabetic, diet or sugar-free varieties.	◯ I do not drink juice or cordial ◯ Rarely ◯ Monthly ◯ 2-3 times a month ◯ Once a week ◯ 2-4 times a week ◯ 5-6 times a week ◯ Once a day ◯ More than once a day
How often do you drink energy drinks? (eg V, Lift plus, Red Bull, Powerade)	◯ I do not drink energy drinks ◯ Rarely ◯ Monthly ◯ 2-3 times a month ◯ Once a week ◯ 2-4 times a week ◯ 5-6 times a week ◯ Once a day ◯ More than once a day

Snacks

How often do you eat lollies, sweets, chocolate or confectionary?

- ◯ I do not eat these
- ◯ Rarely
- ◯ Monthly
- ◯ 2-3 times a month
- ◯ Once a week
- ◯ 2-4 times a week
- ◯ 5-6 times a week
- ◯ Once a day
- ◯ More than once a day

How often do you eat biscuits, cakes, slices, muffins, sweet pastries or muesli bars?

Include nut and other sweet snack bars.

- ◯ I do not eat these
- ◯ Rarely
- ◯ Monthly
- ◯ 2-3 times a month
- ◯ Once a week
- ◯ 2-4 times a week
- ◯ 5-6 times a week
- ◯ Once a day
- ◯ More than once a day

How often do you eat savoury snacks such as chips (crisps not hot chips) and crackers?

- ◯ I do not eat these
- ◯ Rarely
- ◯ Monthly
- ◯ 2-3 times a month
- ◯ Once a week
- ◯ 2-4 times a week
- ◯ 5-6 times a week
- ◯ Once a day
- ◯ More than once a day

Fast Food

How often do you eat fast food or takeaways from places like McDonalds, KFC, Burger King, Pizza shops or fish and chip shops?

- ◯ I do not eat fast food
- ◯ Rarely
- ◯ Monthly
- ◯ 2-3 times a month
- ◯ Once a week
- ◯ 2-4 times a week
- ◯ 5-6 times a week
- ◯ Once a day
- ◯ More than once a day

How often do you eat pies and other hot food that you buy ready-to-eat?

- ◯ I do not eat these
- ◯ Rarely
- ◯ Monthly
- ◯ 2-3 times a month
- ◯ Once a week
- ◯ 2-4 times a week
- ◯ 5-6 times a week
- ◯ Once a day
- ◯ More than once a day

Breakfast Consumption

How many days in an average week do you have something to eat for breakfast?

- ◯ I don't usually have breakfast
- ◯ 1 day a week
- ◯ 2 days a week
- ◯ 3 days a week
- ◯ 4 days a week
- ◯ 5 days a week
- ◯ 6 days a week
- ◯ 7 days a week

Supplement Use

Did you take any supplements during the last year?

- ◯ Yes
- ◯ No

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
- ☐ Echinachea
- ☐ 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.

_____

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:	_____
Oil: 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
Oil: 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?	◯ Daily ◯ More than once a week ◯ Once per week ◯ Monthly ◯ Regularly but for a limited time ◯ Not very often
Bran: 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.	

Lecithin: 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
Lecithin: 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.	_____
Lecithin: 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.	
LSA: 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
LSA: 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.	_____
LSA: 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.	
Kelp: 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
Kelp: 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.	_____

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.	
Spirulina: 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
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.	
Glucosamine and/or chondroitin: 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
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.	

Echinachea: 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
Echinachea: 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.	_____
Echinachea: 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.	

Do you have any comments about this survey?

Please add any comments you may have about this questionnaire here:

Appendix 3

Attitudes and Motivations for Food Choice

For the following questions please think about the foods that you usually eat and the foods that you usually avoid - this is called your "dietary pattern". Please choose how strongly you agree or disagree with the following statements:

	Strongly disagree	Disagree	Somewhat disagree	Neither agree nor disagree	Somewhat agree	Agree	Strongly agree
I can be flexible and sometimes eat foods that go against my dietary pattern	○	○	○	○	○	○	○
I view my dietary pattern as a way of making the world a better place for others	○	○	○	○	○	○	○
I feel that I have a moral obligation to follow my dietary pattern	○	○	○	○	○	○	○
My dietary pattern is an important part of how I would describe myself	○	○	○	○	○	○	○
Concerns about social issues motivate me to follow my dietary pattern	○	○	○	○	○	○	○

For the following questions please think about the foods that you usually eat and the foods that you usually avoid - this is called your "dietary pattern". Please choose how strongly you agree or disagree with the following statements:

	Strongly disagree	Disagree	Somewhat disagree	Neither agree nor disagree	Somewhat agree	Agree	Strongly agree
From time to time, I eat foods that go against my dietary pattern	○	○	○	○	○	○	○
My dietary pattern has a big impact on how I think of myself	○	○	○	○	○	○	○
I am motivated to follow my dietary pattern because eating foods that go against my dietary pattern is immoral	○	○	○	○	○	○	○
I follow my dietary pattern because I want to benefit society	○	○	○	○	○	○	○
A big part of my lifestyle revolves around my dietary pattern	○	○	○	○	○	○	○

For the following questions please think about the foods that you usually eat and the foods that you usually avoid - this is called your "dietary pattern". Please choose how strongly you agree or disagree with the following statements:

	Strongly disagree	Disagree	Somewhat disagree	Neither agree nor disagree	Somewhat agree	Agree	Strongly agree
I feel motivated to follow my dietary pattern because I am concerned about the effects of my food choices on other beings	○	○	○	○	○	○	○
I would eat a food product that goes against my dietary pattern if I were to hear that it tastes exceptionally good	○	○	○	○	○	○	○
My dietary pattern defines a significant aspect of who I am	○	○	○	○	○	○	○
I follow my dietary pattern because eating this way is good for the world	○	○	○	○	○	○	○
I follow my dietary pattern because eating this way is the morally right thing to do	○	○	○	○	○	○	○

For the following questions please think about the foods that you usually eat and the foods that you usually avoid - this is called your "dietary pattern". Please choose how strongly you agree or disagree with the following statements:

	Strongly disagree	Disagree	Somewhat disagree	Neither agree nor disagree	Somewhat agree	Agree	Strongly agree
Following my dietary pattern is an important part of who I am	○	○	○	○	○	○	○
I am motivated to follow my dietary pattern because I want to help others	○	○	○	○	○	○	○

Please choose how important it is to you that the food you eat on a typical day:				
	Not at all important	A little important	Moderately important	Very important
Contains a lot of vitamins and minerals	○	○	○	○
Helps me relax	○	○	○	○
Is cheap	○	○	○	○
Is what I usually eat	○	○	○	○
Has been produced in a way that animals have not experienced pain	○	○	○	○

Please choose how important it is to you that the food you eat on a typical day:				
	Not at all important	A little important	Moderately important	Very important
Is not forbidden in my religion	○	○	○	○
Has been prepared in an environmentally friendly way	○	○	○	○
Is easy to prepare	○	○	○	○
Is low in calories	○	○	○	○
Smells nice	○	○	○	○

Please choose how important it is to you that the food you eat on a typical day:

	Not at all important	A little important	Moderately important	Very important
Keeps me healthy	○	○	○	○
Makes me feel good	○	○	○	○
Has been produced in a way that animals' rights have been respected	○	○	○	○
Has been packaged in an environmentally friendly way	○	○	○	○
Helps me control my weight	○	○	○	○

Please choose how important it is to you that the food you eat on a typical day:				
	Not at all important	A little important	Moderately important	Very important
Is familiar	○	○	○	○
Looks nice	○	○	○	○
Is easily available in shops and supermarkets	○	○	○	○
Is high in fibre	○	○	○	○
Cheers me up	○	○	○	○

Please choose how important it is to you that the food you eat on a typical day:				
	Not at all important	A little important	Moderately important	Very important
Is not expensive	○	○	○	○
Has a pleasant texture	○	○	○	○
Is low in fat	○	○	○	○
Contains natural ingredients	○	○	○	○
Helps me cope with life	○	○	○	○

Please choose how important it is to you that the food you eat on a typical day:				
	Not at all important	A little important	Moderately important	Very important
Is in harmony with my religious views	○	○	○	○
Is nutritious	○	○	○	○
Can be cooked very simply	○	○	○	○
Tastes good	○	○	○	○
Helps me cope with stress	○	○	○	○

Please choose how important it is to you that the food you eat on a typical day:				
	Not at all important	A little important	Moderately important	Very important
Takes no time to prepare	○	○	○	○
Contains no artificial ingredients	○	○	○	○
Is like the food I ate when I was a child	○	○	○	○
Is high in protein	○	○	○	○
Is good value for money	○	○	○	○

Please choose how important it is to you that the food you eat on a typical day:				
	Not at all important	A little important	Moderately important	Very important
Can be bought in shops close to where I live	○	○	○	○
Is good for my skin/teeth/hair/nails etc	○	○	○	○
Has been produced in a way which has not shaken the balance of nature	○	○	○	○
Contains no additives	○	○	○	○
Keeps me awake/alert	○	○	○	○

Please let us know how strongly you agree or disagree with the following statements about eating meat:	Strongly disagree	Disagree	Somewhat disagree	Neither agree nor disagree	Somewhat agree	Agree	Strongly agree	I would prefer not to answer
It is only natural to eat meat	○	○	○	○	○	○	○	○
It is necessary to eat meat in order to be healthy	○	○	○	○	○	○	○	○
Not eating meat is socially unacceptable	○	○	○	○	○	○	○	○
Meat is delicious	○	○	○	○	○	○	○	○
It is unnatural to eat an all plant-based diet	○	○	○	○	○	○	○	○

Please let us know how strongly you agree or disagree with the following statements about eating meat:	Strongly disagree	Disagree	Somewhat disagree	Neither agree nor disagree	Somewhat agree	Agree	Strongly agree	I would prefer not to answer
You cannot get all the protein, vitamins and minerals you need on an all plant-based diet	○	○	○	○	○	○	○	○
It is abnormal for humans not to eat meat	○	○	○	○	○	○	○	○
Meat adds so much flavour to a meal it does not make sense to leave it out	○	○	○	○	○	○	○	○
Our human ancestors ate meat all the time	○	○	○	○	○	○	○	○
Human beings need to eat meat	○	○	○	○	○	○	○	○

Please let us know how strongly you agree or disagree with the following statements about eating meat:	Strongly disagree	Disagree	Somewhat disagree	Neither agree nor disagree	Somewhat agree	Agree	Strongly agree	I would prefer not to answer
Most people I know eat meat	○	○	○	○	○	○	○	○
The best tasting food is normally a meat based dish	○	○	○	○	○	○	○	○
Human beings naturally crave meat	○	○	○	○	○	○	○	○
A healthy diet requires at least some meat	○	○	○	○	○	○	○	○
It is normal to eat meat	○	○	○	○	○	○	○	○
Meals without meat would just be bland and boring	○	○	○	○	○	○	○	○

Please answer the following questions:	
Are you currently trying to do any of the following?	◯ Lose weight ◯ Stay the same weight ◯ Gain weight ◯ No, not trying to do anything about my weight
How often do you usually brush your teeth?	◯ More than 3 times a day ◯ 3 times a day ◯ Twice a day ◯ Once a day ◯ 3-6 times a week ◯ 1-2 times a week ◯ Less than once a week ◯ Less than once a month ◯ Never
How often do you use dental floss?	◯ More than 3 times a day ◯ 3 times a day ◯ Twice a day ◯ Once a day ◯ 3-6 times a week ◯ 1-2 times a week ◯ Less than once a week ◯ Less than once a month ◯ Never
When you brush your teeth, how much toothpaste do you use? (see image below)	◯ I don't use toothpaste when I brush my teeth ◯ A smear ◯ A pea-sized amount ◯ More than a pea-sized amount

Smear


Pea-sized


More than a pea-sized amount


After you brush your teeth, do you:

- ◯ Spit into the basin, then swallow (straightaway or later on)
- ◯ Spit into the basin, rinse with water, and then spit into the basin again, then swallow (straightaway or later on)
- ◯ I don't spit into the basin

What brand of toothpaste do you use?

- ◯ Colgate
- ◯ Macleans
- ◯ Aim
- ◯ Oral B
- ◯ Ecostore
- ◯ Red Seal
- ◯ Sensodyne
- ◯ Other

If other, please specify:

Please answer the following questions:	
Do you take PE as a subject at school?	◯ Yes ◯ No
Do you play a school sport?	◯ Yes ◯ No
Do you play a sport out of school?	◯ Yes ◯ No
What is the most common way that you get to and from school?	◯ By car ◯ By bus ◯ Bike ◯ Scooter/skateboard ◯ Walk ◯ Other (please specify)

If other, please specify: _____

Do you have any comments about the questions in the previous section?

Please add any comments you may have here:

What happens next?

We have one more online questionnaire for you to complete about your dietary habits. If you would like to take a break, please click the "Save & Return Later" button (be sure to take note of your code so you can return to this survey). Or, if you would like to continue, please click the "Submit" button.

You're nearly there!

Appendix 4

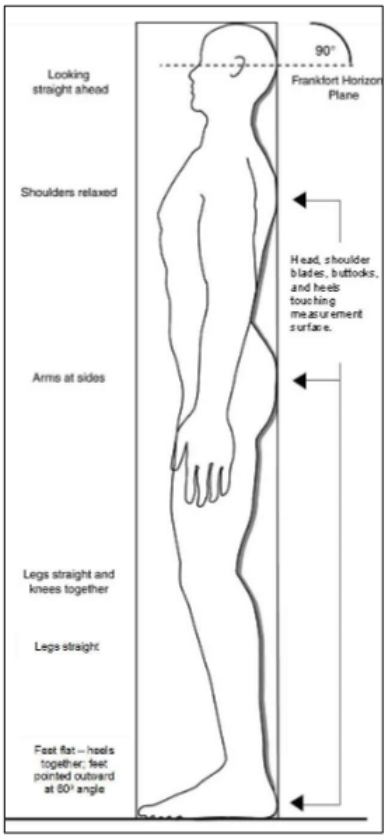
ANTHROPOMETRIC MEASUREMENTS

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

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

HEIGHT

1. Ask the participant to remove their shoes, as well as any hair ornaments or buns/braids on the top of the head.
2. If the participant is taller than the investigator, use a step tool to take the measurements. Errors can be minimised by the investigator being parallel to the participant and the headpiece.
3. Tell the participant to stand with their heels together and toes apart pointing outward at approximately a 60-degree angle.
4. Make sure the back of the head, shoulder blades, buttocks, and heels of the participant are touching the backboard/stadiometer.
5. Make sure the participant's head is aligned in the Frankfort horizontal plane, where a horizontal line connects from the ear canal to the lower border of the orbit of the eye.
6. Lower the headpiece to rest firmly on the top of the participant's head and ask the participant to stand as tall as possible and take a deep breath.
7. Record the result to the nearest 0.1 cm in the HEIGHT 1 box on the recording sheet without informing the participants.



WEIGHT

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

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

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

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

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

ULNA LENGTH:

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

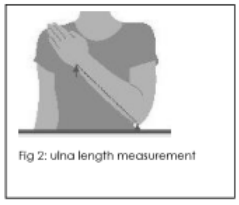


Fig 2: ulna length measurement

REPEAT ALL MEAUREMENTS

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

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

Appendix 5

sundial PROJECT

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)

sundial
PROJECT

Tips Sheet

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

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

Where possible for packaged foods collect the brand name

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

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

Do not

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

Do

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

Useful Prompts for Specific Food Groups

FRUIT

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

VEGETABLES

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

DAIRY

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

NUTS

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

BREAD

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

MARGARINE/BUTTER/TABLE SPREAD

- People often use the term butter and margarine interchangeably so collect the brand name (do not comment on the fact they might not have used the correct description)
- Low fat or standard
- Phytosterols (cholesterol reducing)
- Use pictures to help indication of thickness of spread

DRINKS

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

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

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

PACKAGED FOODS

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

MIXED DISHES

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

Appendix 6

Phase 1 fortified bread list	**Phase 2 fortified bread list**
Burgen multigrain	Countdown essential
Tiptop bread (9 grains & seeds)	Molenberg
Molenberg	Tiptop
Vogels GF bread	Freya's multigrain
Sunny crust bread	Burgen multigrain
Freya's multigrain	Vogels mixed grain
Budget -white	
Value wheat meal	
Pams bread	