Intakes, adequacy, food sources and biomarker status of iron, folate, and vitamin B_{12} in Māori and non-Māori octogenarians: Life and Living in Advanced Age: A Cohort Study in New Zealand (LiLACS NZ).

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

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Abstract

Background: Iron, folate and vitamin B_{12} are the three key nutrients associated with the development of anaemia and have also been associated with the dietary patterns linked to higher malnutrition risk in older adults. Octogenarians may be at increased risk for iron, folate and vitamin B_{12} deficiency due to reduced food intake. Dietary factors, cooking methods, medications, presence of inflammation, and impaired gastrointestinal absorption may affect the availability and bioavailability of these nutrients. There are currently no specific nutrient reference values (NRVs) or biomarker cut-offs for adults in advanced age and little is known about the relationship between dietary intake and biomarkers for older adults.

Aim: To investigate the intake, adequacy, food sources and biomarker status of iron, folate and vitamin B_{12} and the relationship between dietary intake and biomarkers.

Methods: In the follow up assessment of LiLACS NZ, 216 Māori and 362 non-Māori participants completed a detailed dietary assessment using 2x 24-hr multiple pass recalls. Adequacy of iron, folate and vitamin B_{12} were determined by comparison to the Estimated Average Requirement (EAR) for adults aged 71+ years. Serum ferritin, serum iron, total iron binding capacity, transferrin saturation, red blood cell (RBC) folate, serum folate, serum vitamin B_{12} and haemoglobin were compared to recognised cut-offs for adults. Generalised linear models and binary regression estimated the association between dietary intake and biomarkers.

Results: Most participants had adequate dietary iron intakes (88% Māori; 95% non-Māori above EAR) and biomarkers for iron (>94% above cut-offs). The EAR for vitamin B_{12} was met by 74% Māori; 78% non-Māori and folate met by 42% Māori; 49% non-Māori. Māori versus non-Māori had higher intakes of vitamin B_{12} ($p=0.038$) and serum vitamin B_{12} ($p=0.026$). Increased dietary folate intake was associated with increased RBC folate for Māori ($p=0.001$) and non-Māori ($p=0.014$) and with increased serum folate for Māori ($p<0.001$). Folate intake >215µg/day was associated with reduced risk of deficiency in RBC folate for Māori ($p=0.001$).

Conclusions: Dietary intake and stores of iron are largely adequate in this population. Strategies to optimise the intake and bioavailability of foods rich in folate and vitamin B_{12} may be beneficial.

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Abbreviations

AMDR	Acceptable Macronutrient Distribution Range
BMI	Body Mass Index
CVD	Cardiovascular Disease
DFE	Dietary Folate Equivalents
EAR	*Estimated Average Requirement:* A daily nutrient level estimated to meet the requirements of half the healthy individuals in a particular life stage and gender group
EI:BMR	Energy Intake: Basal Metabolic Rate
FFQ	Food Frequency Questionnaire
Hb	Haemoglobin
HoloTC	Holotranscobalamin
H2RA	Histamine-2 receptor antagonist
IDA	Iron Deficiency Anaemia
IHD	Ischaemic Heart Disease
LiLACS NZ	Life and Living in Advanced Age: A Cohort Study in NZ
mg	Milligrams
MJ	Mega-joule
MMA	Methylmalonic acid
NHANES	National Health and Nutrition Examination Survey
NZANS	The New Zealand Adult Nutrition Survey
NZNNS	The New Zealand National Nutrition Survey
NRV	Nutrient Reference Value
NSAID	Non-Steroidal Anti-Inflammatory Drug
NZ Dep	*NZ Deprivation Index:* An index of socioeconomic deprivation in New Zealand
PHO	Primary Health Organisation
PPI	Proton Pump Inhibitor
RBC	Red Blood Cell
RDI	*Recommended Daily Intake:* The average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all healthy individuals in a particular life stage and gender group
SNP	Single Nucleotide Polymorphism
Tf-sat	Transferrin saturation
THF	Tetrahydrofolate
TIBC	Total Iron Binding Capacity
WHO	World Health Organisation
µg	Micrograms
µmol	Micromol
24-hr MPR	24-hour Multiple Pass Recall

Glossary

Whanaungataunga	Relationship, kinship, family connection
Kai	Food
Mana	Honour/prestige
Puha	New Zealand spinach
Hāngi	Food cooked in an earth oven (underground)
Boil-up	Traditional Māori food where vegetables and meat are boiled together in a stock pot
Kaimoana	Seafood
Hui	Gatherings
Octogenarian	Person aged between 80 and 90 years
Marae	A communal or sacred place that serves religious and social purposes

1. Introduction

One of the most profound demographic changes in New Zealand is the rapidly growing population of older adults, particularly the number of adults aged 85+years which is expected to increase six-fold by 2068 (Statistics New Zealand, 2016b). Although older Māori make up a small proportion of all adults aged 65+ and Māori life expectancy lags behind non-Māori by approximately seven years (Statistics New Zealand, 2015), projections indicate the older Māori population is likely to surge in the coming years at a higher rate than that of non-Māori (Ministry of Health, 2015b). Given these significant increases, there is a need to establish the determinants of successful ageing for octogenarians, and more specifically older Māori.

Ageing is associated with a decline in functionality, increase in chronic disease, disability, and demand for long term healthcare which has significant implications for support services (Cornwall & Davey, 2004). In particular, malnourished older adults have significant costs and implications for the healthcare system. Malnutrition is a more common occurrence among older adults due to reduced food and nutrient intake (Abizanda et al., 2016). Older Māori are five times more likely to be at nutritional risk compared to non-Māori (McElnay et al., 2012) and although the energy requirements for older adults are reduced due to their lower basal metabolic rate (BMR), their requirements for micronutrients generally remain the same or increase (NHMRC, 2006). Age-related factors such as declining oral health, impaired gastrointestinal absorption, and socioeconomic constraints may negatively affect the nutrient intakes of older adults and make it harder to reach adequate micronutrient intakes (Drewnowski & Darmon, 2005; Russell & Baik, 2001; Sheiham et al., 2001).

Up to 70% of Māori and 55.2% of non-Māori aged 65 and over have been shown to be at some degree of nutritional risk in a New Zealand study by McElnay et al. (2012). Similarly, in the LiLACS NZ feasibility study, up to 63% of Māori aged 75-79 were at high nutritional risk (Wham et al., 2015). A higher risk was associated with lower fruit and vegetable consumption, lower milk consumption, and lower meat consumption (McElnay et al., 2012). Similar dietary patterns have also been linked to lower dietary intakes and serum biomarkers of iron, folate, and vitamin B_{12} (Chen et al., 2005; Jackson et al., 2016; Mendonça et al., 2016). Iron, folate and vitamin B_{12} are largely intertwined in the production of red blood cells in the haematopoietic system, in particular, they are the most common nutrient deficiencies associated with anaemia (Frangos et al., 2016). The prevalence of anaemia among older adults living in developed countries reportedly ranged between 3% – 50% in a systematic review by Gaskell et al. (2008), translated into 1 in every 7 or 8 adults aged 65+ living in the community. Although the cause of anaemia may not be solely nutrition focussed, these nutrients may play a part in the incidence and treatment (Frangos et al., 2016). Aside from this, inadequate vitamin B_{12} and folate has also been associated with neurological complications, in particular, dementia and peripheral neuropathy (Doets et al., 2013; Mooijaart et al., 2005). Given this, it is imperative to understand the current status of these nutrients in older adults.

Older adults aged 71+ years were found to have lower dietary intakes of iron and vitamin B_{12} compared to other age groups in the NZANS 2008/09 (University of Otago & Ministry of Health, 2011a). Dietary folate intake was not examined in the NZANS 2008/09 due to unreliable methods to estimate dietary intake (University of Otago & Ministry of Health, 2011b) therefore current dietary folate intakes for older adults in New Zealand are not known. The prevalence of low iron and folate stores among adults aged 71+ was relatively low (<2% of men and women) in the NZANS

2008/09, however, vitamin B_{12} deficiency, defined as <148pmol/L, was much higher among New Zealand adults aged 65+ years with up to 12% not meeting the cut-off (Green et al., 2004). The Leiden 85+ study demonstrated a higher occurrence of low iron stores in octogenarians ranging between 4% – 15.9% depending on the biomarker investigated (den Elzen et al., 2013) and up to 17% of octogenarians in the Newcastle 85+ study were shown to be deficient in vitamin B_{12} (Mendonça et al., 2016). However, folate stores were mostly adequate in these two cohorts (99.8% – 96.4% with adequate RBC folate concentrations) (den Elzen et al., 2013; Mendonça et al., 2016).

Dietary intakes may not reflect biomarker status given that various processes, often augmented by increasing age, may alter the bioavailability of these nutrients. These include impaired gastrointestinal absorption (Green et al., 2004), use of certain medications and polypharmacy (Allen, 2008), and the food matrix (EFSA NDA Panel, 2015; Fairweather-Tait & Teucher, 2002). However, several studies indicate that higher dietary intakes of iron, folate and vitamin B_{12} may be associated with higher stores of these nutrients, indicated by favourable serum biomarker levels (Mendonça et al., 2016; Milman et al., 1990; Milman et al., 2004).

Not all foods are equally bioavailable and the relationship between food group intake and biomarker status among older adults has not been extensively researched. However, it is well known that iron from meat and meat products is more bioavailable than that from plant sources and fortified foods (Fairweather-Tait & Teucher, 2002; Russell, 2001). Whereas, folic acid and synthetic vitamin B_{12} used in fortification is more bioavailable and less affected by age-related impaired gastrointestinal absorption compared to naturally occurring food-folate and vitamin B_{12} (Allen, 2008; Chernoff, 2013). Therefore, an investigation of the current status of these nutrients and contributing food sources among older New Zealanders may provide insights into the relationship between dietary intake and serum biomarker status and could inform strategies to prevent deficiency among older adults.

As it stands, New Zealand does not have specific nutrient reference values (NRVs) for octogenarians as the current NRVs combine all older adults into a 70+ age group (NHMRC, 2006). Given that the NRVs are likely to be based on small numbers of older adults or extrapolation of data from younger age groups (NHMRC, 2006) these NRVs may not represent positive health outcomes for older adults (Wham et al., 2016a). When assessing the nutrient intake and status of the population, the New Zealand Adult Nutrition Survey (NZANS) 2008/09 aggregated all older adults into a 71+ age category and all older Māori into a 51+ age category (NHMRC, 2006; University of Otago & Ministry of Health, 2011b). Therefore, it is unclear what the specific nutrient intakes are for adults aged 80+ years. An investigation into the nutrient profile of older adults, including serum biomarkers, is warranted and may help to determine whether these NRVs are adequate for this population.

Therefore, the aim of the present study is to determine the dietary intakes, adequacy, food sources and biomarker status of iron, folate and vitamin B_{12} among Māori and non-Māori octogenarians and to investigate the association between dietary intake and biomarker status.

1.1. Aims and objectives

Aim:

To investigate the dietary intake, adequacy, food sources and biomarker status for iron, folate and vitamin B_{12} and to investigate the association between biomarker status and dietary intake using recognised cut-offs in Māori and non-Māori octogenarians.

Objectives:

1. To determine the dietary intake of iron, folate and vitamin B_{12} among Māori and non-Māori octogenarians.
2. To investigate the dietary adequacy of iron, folate and vitamin B_{12} in relation to nutrient reference values (EAR) for older adults >70 years.
3. To determine the main food sources that contribute to the dietary intakes of iron, folate and vitamin B_{12}.
4. To investigate iron status using haemoglobin, serum iron, transferrin saturation and total iron binding capacity.
5. To investigate folate status using serum folate and red blood cell folate.
6. To investigate vitamin B_{12} status using serum vitamin B_{12}.
7. To investigate the association between dietary intake and biomarkers for iron, folate and vitamin B_{12} in relation to recognised cut-offs.

1.2. Structure of thesis

This thesis includes four key chapters with additional sections for references and appendices. Chapter one, the introduction, outlines the scope and justification of this research regarding iron, folate and vitamin B_{12} in octogenarians. This chapter also outlines the study aims, objectives, and researcher contributions. Chapter 2 provides a review of current literature regarding the health and nutritional status of the ageing population, factors relating to intakes, adequacy and biomarkers for iron, folate and vitamin B_{12}, with particular focus on literature relating to older adults. Chapter 3 provides the research study manuscript including abstract, introduction, methods, results, discussion, and conclusion. The research manuscript chapter was written with the intention of publication in the Journal *Nutrients* (See appendix C for journal requirements). Chapter 4 outlines the conclusions and recommendations based on the findings of the present research including the strengths and limitations of the study. Chapter 5 includes the appendices with supplementary methods relating to supplementary results including demographic and health characteristics of the study population and their association with dietary intake and biomarkers for iron, folate and vitamin B_{12}.

1.3. Researcher's contributions

Table 1.1: Researchers contributions

Danika Pillay	Master's student; - Primary researcher/writer - Literature review - Statistical analysis
Associate Professor Carol Wham	Supervisor; Investigator Life and Living in Advanced Age: a Cohort Study in New Zealand (LiLACS NZ) - Research design overview - Thesis guidance and assistance - Research manuscript overview
Simon Moyes	Senior Data Analyst; - Data handling - Assistance with statistical analysis
Professor Ngaire Kerse	Principle Investigator LiLACS NZ; - Provision of data - Data analysis overview - Research manuscript overview
Karen Hayman	Research Fellow - Methodology for biomarker collection and analysis

2. Literature Review

2.1. Ageing in New Zealand

The proportion of New Zealanders aged 65+ is increasing at an accelerated rate. Despite lower population numbers, the group aged 85+ is expected to have the most rapid growth with an expected 575% increase from 2014 to 2068 (Statistics New Zealand, 2016b). This projected increase comes as a result of the steady increase in population life-expectancy. Since the 1970s, men and women are now expected to live 10.5 and 7.7 years longer, respectively (Statistics New Zealand, 2015). However, Māori life-expectancy still lags behind non-Māori by approximately 7 years (Ministry of Health, 2015b; Statistics New Zealand, 2015). In 2013, Māori made up 15.6% of the New Zealand population and it was estimated that this proportion of Māori will increase to 19.5% of the total population by 2038 with the proportion of Māori aged 65+ expected to almost double by 2038 (Ministry of Health, 2015b; Statistics New Zealand, 2013). Ageing is associated with a decline in functional capacity, increase in chronic disease, disability and demand for long term healthcare which has led to potential concerns over the impact and the demand for healthcare and disability services (Cornwall & Davey, 2004). Therefore, it is important to understand the older adult population in order to promote independent living and successful ageing.

2.2. Health of older adults

Advancing age is a risk factor for chronic diseases such as cardiovascular disease (CVD), stroke and cancer. Cardiovascular diseases such as ischaemic heart disease (IHD) and stroke are the leading causes of mortality worldwide and those aged 80+ years old have the highest mortality rates of any age group (Finegold et al., 2013). In New Zealand, CVD is the second leading cause of mortality followed by stroke, and Māori are 1.8 times more likely to develop IHD and 1.3 times more likely to have had stroke than non-Māori (Ministry of Health, 2015a). Debilitating effects of stroke include functional disability and dysphagia, both of which may negatively impact the nutritional status of older adults. There can also be age-related changes in the brain resulting in cognitive impairments (Chen et al., 2010). In New Zealand dementia is one of the top four leading causes of health loss for older people aged 75+ (Ministry of Health, 2016b). The LiLACS NZ study reported 15% of all participants were cognitively impaired, similar to that in the Newcastle 85+ study where the prevalence of cognitive impairment and dementia was 12.5% and 6.7%, respectively (Collerton et al., 2009; Teh et al., 2014).

2.2.1. Body composition changes with ageing

Ageing is also associated the loss of lean body mass, strength and function, termed sarcopenia, and is usually associated with an increased fat accumulation in the muscle and increase in connective tissue (Morley, 2012). It has been estimated that 11-50% of octogenarians have sarcopenia resulting from inactivity and inadequate dietary intake (Morley, 2012). Sarcopenia is associated with increased frailty, risk of falls and disability (Faulkner et al., 2007; Morley, 2012). It has been shown that younger Māori have different body compositions to non-Māori, including a higher proportion of lean body mass (Rush. et al., 2009) which may continue into older age (Wham et al., 2015). However, Māori aged over 65 years are also more likely to be overweight and obese in comparison to non-Māori (Ministry of Health, 2015b).

2.3. Nutrition status of older adults

Maintaining good nutrition is vital for healthy ageing and poor nutrition increases the risk of hospitalisations, disability, mortality, and decreases independence and quality of life (McElnay et al., 2012). Although older adults have reduced energy requirements due to their reduced basal metabolic rate, their requirements for micronutrients generally remain the same or increase

(NHMRC, 2006; Wham et al., 2016b). The current estimated average requirements (EARs) and recommended daily intakes (RDIs) for micronutrients set by the National Health and Medical Research Council (NHMRC) (NHMRC, 2006) are aggregated for adults over 70 years which may not reflect the needs of adults 80 years and over. Micronutrient intakes are usually achieved when consuming a healthy well-balanced diet which meets the energy and acceptable macronutrient distribution range (AMDR) recommendations. However, macronutrient intakes were investigated in LiLACS NZ which showed that less than half of the Māori and non-Māori octogenarians met the AMDR for protein and less than 55% met the AMDR for carbohydrate, suggesting that further investigation into micronutrient status may be warranted (Wham et al., 2016a).

2.3.1. Cultural eating practices

The eating practices of Māori differ to that of non-Māori in that food (kai) is more than just nourishment for the body, it is a part of upholding the mana (honour/prestige) of the people of the marae and a way of expressing welcome. Before colonisation, the traditional Māori diet was high in protein and fibre and low in fat, however, post-colonisation and the rise in Western diets among these indigenous people led to a change in traditional foods and introduction to new foods that were generally higher in fat and refined carbohydrates, which may explain the increased burden of chronic disease in Māori compared to non-Māori (Rush et al., 2010). Traditional foods for Māori include kaimoana (seafood), puha, watercress, hāngi and boil-up. Frequent consumption of kaimoana and meat suggests that traditional Māori meals are generally based around these protein foods (Rush et al., 2010). Surveys suggest that while main food sources of energy are similar for Māori and non-Māori (bread, potatoes / kumara / taro, and milk) some differences occur whereby Māori consume more energy from butter and margarine, and fish and seafood whereas non-Māori consume more energy from fruit (University of Otago & Ministry of Health, 2011a).

2.3.2. Factors affecting nutrition status

Physiological changes associated with ageing and changes in health status and lifestyle can compromise energy intake and make it difficult for older adults to achieve their energy and nutrient requirements (Ministry of Health, 2013). There are numerous age-related factors that affect nutritional intake in older adults including poor oral health or ill-fitting dentures, decreased production of saliva, and changes in sensory perceptions such as taste and smell (Doty et al., 1984; Mahan et al., 2012; Murphy et al., 2002; Sheiham et al., 2001). Neurological diseases and old-age are also commonly associated with dysphagia and it is estimated that 7-22% of community dwelling older adults have dysphagia in New Zealand (Australian and New Zealand Society for Geriatric Medicine, 2011; Mahan et al., 2012). The bioavailability of nutrients also change with ageing due to changes in the gastrointestinal tract as well as polypharmacy which can lead to malabsorption of both macronutrients and micronutrients (Heuberger & Caudell, 2011; Russell, 2001).

2.3.3. Risk of malnutrition in older adults

Older adults are at an increased risk for malnutrition and health problems due to inadequate food and nutrient intake. A New Zealand study by McElnay et al. (2012) found that 56.5% of community living older adults were at some degree of nutritional risk and Māori were 5.2 times more likely to be at nutritional risk compared to non-Māori. Similarly, the LiLACS NZ feasibility study found 63% of Māori participants aged 75-79 years were at high nutritional risk (Wham et al., 2015). Several dietary factors were related to higher nutritional risk including low intakes of milk products, meat and alternatives, and low intake of fruit and vegetables (McElnay et al., 2012; Wham et al., 2015) and may also be associated with lower serum levels of iron, folate and vitamin B_{12} in older adults (Chen et al., 2005; Jackson et al., 2016; Mendonça et al., 2016; Milman et al., 1990)

2.4. Iron, folate and vitamin B_{12}

Iron, folate and vitamin B_{12} are largely intertwined in the haematopoietic system. They are essential nutrients for the production of red blood cells and transport of oxygen around the body (Moll & Davis, 2017) and are also the most common nutrient deficiencies associated with anaemia, a condition prevalent in up to 50% of community living older adults (Gaskell et al., 2008). Anaemia, nutrient-related or otherwise, is associated with increased risk of morbidity and mortality and is increasingly prevalent with ageing (Gaskell et al., 2008). Vitamin B_{12} is also essential for normal neurological function (Moll & Davis, 2017) and the association between vitamin B_{12} and declining neurological function, particularly cognitive function and dementia, is unclear (Mooijaart et al., 2005). Therefore, there is a need to understand what the current status of these nutrients are among older adults, particularly octogenarians, given the changing demographic.

2.5. Iron status of older adults

Iron is essential for the transport of oxygen around the body and plays an important role in electron transport, respiration and hormone synthesis (Fairweather-Tait et al., 2014). These processes are vital for physical performance, immunity, cognitive function, thermoregulation and thyroid metabolism (Fairweather-Tait et al., 2014). Approximately 3-4g of iron is stored in the body, of which, 70% is present in haemoglobin in red blood cells and myoglobin in muscle (Fairweather-Tait et al., 2014). The average Western diet contains approximately 10-15mg of iron per day and up to 10% is absorbed with the majority being used to synthesise haemoglobin (De Silva & Davis, 2013). Iron exists in two forms, inorganic non-haem iron and haem iron which are both absorbed in the small intestine (De Silva & Davis, 2013). Approximately 15% of haem-iron, found mainly in animal sources, is absorbed, compared to <5% of non-haem iron found mainly in plant sources (De Silva & Davis, 2013; López & Martos, 2004).

The NZANS 2008/09 (University of Otago & Ministry of Health, 2011a) reported that men and women aged 71+ years old had an intake of 11.4mg and 8.9mg of iron per day, respectively. They did not report any difference between Māori and non-Māori intakes of iron. The NHMRC (2006) recommends that the EAR for adults over 70 years is 6mg/d for men and 5mg/d for women and the majority of participants aged 71+ in the NZANS 2008/09 had adequate dietary intakes based on this (98.7% men; 97.7% women) (University of Otago & Ministry of Health, 2011a). The main contributors to iron intake for older adults in descending order were found to be bread, breakfast cereals, vegetables, and beef and veal suggesting that fortified food sources are an important contributor to iron intake in this age group.

2.5.1. Physiological mechanisms of absorption and iron homeostasis

Iron is absorbed in the acidic milieu of the duodenum and proximal jejunum (De Silva & Davis, 2013). However, the absorption of haem-iron and non-haem iron differ significantly. Non-haem iron must first be reduced from Fe^{3+} to Fe^{2+} before it can be transported into the cell and absorption can be disrupted by other dietary factors. Polyphenols, phytates found in grains, nuts and legumes, as well as the zinc and calcium content of the meal can inhibit or compete with non-haem iron for absorption thereby reducing its bioavailability (De Silva & Davis, 2013). The absorption of non-haem iron is also inhibited at a pH greater than 5 which can be an issue in the presence of atrophic gastritis, a condition that reduces gastric acid secretion (Kassarjian & Russell, 1989; Russell, 2001). However, consumption of vitamin C containing foods have been shown to increase the absorption of non-haem iron (Russell, 2001). In contrast, haem iron is able to be taken up by the enterocytes without modification and is not affected by the lack of acid so normal absorption can still occur in individuals with reduced gastric acid secretion making animal sources of iron particularly important in this demographic (Russell, 2001).

Iron is regulated in the body by multiple transporters and homeostatic mechanisms that ensure free iron is limited due to the potential for free-radical damage and sequestering by pathogens (Fairweather-Tait et al., 2014). Therefore, in the case of inflammation, the availability of iron is reduced and must be taken into account when assessing biochemical data, particularly in this age group where a chronic low-grade inflammatory state may be present (den Elzen et al., 2013).

2.5.2. Factors affecting iron intake and status

2.5.2.1. Oral health

Oral health is an important contributor to a person's ability to consume adequate nutrition for health and well-being. In the British National Diet and Nutrition Survey, older adults with fewer teeth or those who used dentures were shown to consume significantly less haem and non-haem iron than those who did not use dentures and had more teeth (Sheiham et al., 2001). Problems associated with oral health such as loose/painful teeth, ill-fitting dentures and decreased production of saliva can compromise chewing and swallowing ability and can lead to avoidance of some nutritionally dense foods such as wholegrains, fruits, vegetables, and meat, in favour of soft and easily chewable alternatives (Mahan et al., 2012; Sheiham et al., 2001). In New Zealand, approximately 40% of adults over the age of 75 years have had all their teeth removed which may lead to less dietary variety and increased risk of micronutrient deficiencies (Ministry of Health, 2015a; Sheiham et al., 2001).

2.5.2.2. Cooking methods

Traditional foods or foods eaten at a hui (traditional gathering) were examined by Rush et al. (2010) and meat was frequently consumed by Māori, however, upon investigation, the meat consumed was more likely to be pork or fatty cuts of lamb and beef. Pork has been shown to be lower in total iron and low in highly absorbable haem-iron in comparison to beef and lamb (Boccia-Lombardi et al., 2002). Also consumed frequently by Māori were boil-ups with vegetables and meat, however, this method of cooking may also decrease the total amount of iron available (Rush et al., 2010). It has been found that raw meats including beef and lamb have higher concentrations of haem iron compared to their fully cooked counterparts, with boiling leading to approximately 53% loss of haem iron in the meat (Boccia-Lombardi et al., 2002; Pourkhalili et al., 2013). This is particularly concerning for older adults who are less likely to eat "risky" foods such as rare or raw beef which can be much higher in iron than its fully cooked counterpart (Anderson et al., 2011; Boccia-Lombardi et al., 2002).

2.5.2.3. Economic constraints

Economic constraints may also play a role in micronutrient intake. Older people are vulnerable to poor diets stemming from a lower income, particularly Māori who are more likely to be socioeconomically deprived and have low food security (Ministry of Health, 2015b). In New Zealand, food costs are the second highest contributor to household expenditure following housing and household utilities (Statistics New Zealand, 2016a). Therefore, food expenditure or food quality is likely to decline as income declines. In particular, a systematic review of diet quality and food prices found that important iron sources such as meat, fish and vegetables have been shown to decrease in favour of cereals, added sugars and fat when cost minimisation is the goal (Drewnowski & Darmon, 2005). This may also highlight the importance of fortified cereals and bread to ensure older adults are consuming enough iron despite financial constraints.

2.5.2.4. Iron fortification

In New Zealand, manufacturers are able to voluntarily fortify foods with iron. These foods include biscuits, bread, cereal flours, pasta, meat-alternatives and formulated supplementary foods

(FSANZ, 2007). Interestingly, most New Zealanders derive their iron from bread and breakfast cereals (University of Otago & Ministry of Health, 2011a). However, the bioavailability of this iron depends on the structure of the food and is often influenced by the same dietary factors as non-haem iron which includes phytates, calcium and polyphenols (Fairweather-Tait & Teucher, 2002; Hurrell & Egli, 2010). Although ferrous sulphate is commonly used as an iron fortifier and is highly bioavailable, the vehicle, often bread and cereals which are high in phytates, may lead to reduced absorption of this iron (De Silva & Davis, 2013; Fairweather-Tait & Teucher, 2002; FSANZ, 2007). Therefore, although most New Zealanders are consuming diets adequate in iron, the bioavailability of this iron, particularly for older adults remains to be understood.

2.5.3. Biomarkers for iron status and relationship to dietary intake

2.5.3.1. Serum iron

Serum iron is the fraction of iron that circulates around the body bound to transferrin and has a very rapid turnover (WHO & CDC, 2007). The World Health Organisation (WHO) defines a normal serum iron concentrations ranging between 50-120µg/dl (equivalent to approximately 9-22µmol/L), however, a range of cut-offs between 50-60µg/dl have been used to define insufficiency. In the Leiden 85+ study, 4.8% of octogenarians had inadequate serum iron when a cut-off of <10µmol/L was used (den Elzen et al., 2013). In adults aged over 85 years, serum iron does not seem to be affected by dietary intake of iron, even in cases of inadequate intake (Milman et al., 1990; Milman et al., 2004). However, serum iron is particularly influenced by infection and inflammation due to iron sequestering as well as meal timing, and is also subject to diurnal variations (De Silva & Davis, 2013; WHO & CDC, 2007). Serum iron increases are often observed following a meal therefore fasting samples may be a better indicator of the true serum iron concentration of an individual (WHO & CDC, 2007).

2.5.3.2. TIBC and transferrin saturation

Total iron binding capacity (TIBC) is the total number of binding sites for iron atoms on transferrin and is a more stable measurement of iron status, however, it does not appear to change until iron stores are depleted (WHO & CDC, 2007). Normal levels of TIBC range between 45-71µmol/L and will increase above this level when iron stores are depleted (Mahan et al., 2012). It has been shown to significantly increase when dietary intake is inadequate (Milman et al., 1990). Serum iron and TIBC are used to calculate transferrin saturation which is an indication of the adequacy of the iron supply for developing red blood cells. A value <15% is indicative of deficiency (WHO & CDC, 2007). In the Leiden 85+ study, up to 15.9% of octogenarians had inadequate transferrin saturation levels when a 20% cut-off was used (den Elzen et al., 2013). Transferrin saturation has been shown to significantly decline based on low dietary iron intakes in adults over 85 years (Milman et al., 1990; Milman et al., 2004). However, both TIBC and transferrin saturation are influenced by inflammation and should be interpreted in light of this (WHO & CDC, 2007).

2.5.3.3. Serum ferritin

Serum ferritin is a more commonly used marker of iron assessment in the literature and correlates closely with iron stores (den Elzen et al., 2013). In a systematic review of iron status and dietary intake among adults (aged >18 years) in developed countries by Jackson et al. (2016), serum ferritin was shown to have a promising positive association with a higher dietary iron intake from animal foods. Although, several limitations exist when interpreting this biomarker as it is often raised during inflammation and inflammatory conditions, and has also been shown to increase with age, possibly due to the low-grade inflammatory state found in older adults (den Elzen et al., 2013; WHO & CDC, 2007). A value <12µg/L usually indicates that liver stores of iron have been depleted (WHO & CDC, 2007), however, it has been proposed that a higher cut-off be used in the older adult

population, in particular, the use of 22µg/L as a cut-off has been shown to be a more sensitive and specific indicator of iron deficiency in community dwelling older adults (Choi et al., 2005). In the Leiden 85+ study, two separate cut-offs were used for men (<20µg/L) and women (<15µg/L) and up to 4% of octogenarians had low serum ferritin levels.

2.5.3.4. Haemoglobin

Haemoglobin is used as a measure of anaemia but is not specific. The cause of anaemia may be iron-deficiency, vitamin B_{12} deficiency, folate deficiency or otherwise therefore it may not necessarily be correlated with dietary intake (Fairweather-Tait et al., 2014). Haemoglobin has been shown to decline with age from 70-80 years in both men and women and is correlated with decreased muscle strength, poorer physical performance and higher mortality risk (Milman et al., 2008). The NHANES III and NZANS 2008/09 used a lower cut-off for men and women aged 70+ when assessing iron status to account for the natural decline with age (Looker et al., 1997; University of Otago & Ministry of Health, 2011b).

Table 2.1: Iron status measures and cut-offs

Status	Measures and cut-offs
Low iron stores	Serum ferritin <12µg/L[1] Serum iron <10µmol/L[1] Total iron binding capacity >71µmol/L[2] Transferrin saturation <15%[1]
Low haemoglobin	Haemoglobin (NHANES III)[3, 4] <124g/L (70+ years, men); <118g/L (70+ years, women) Haemoglobin (WHO)[5]: <130g/L (men); <120g/L (women)

[1] WHO and CDC (2007)
[2] Mahan et al. (2012)
[3] Looker et al. (1997)
[4] University of Otago and Ministry of Health (2011b)
[5] WHO (2001)

2.5.4. Iron deficiency and iron-deficiency anaemia in older adults

Iron deficiency is defined as a condition where there are no moveable stores of iron in the body and where there are signs of compromised supply of iron to tissues and erythron (WHO, 2001). It begins with the depletion of iron stores in the body and proceeds to iron-deficient erythropoiesis, manifested by morphologically abnormal red blood cells, which is followed by iron-deficiency anaemia (IDA) if the negative iron balance continues (De Silva & Davis, 2013). Between 3% and 50% of older adults living in developed countries have been shown to be anaemic (Gaskell et al., 2008; Guralnik et al., 2004). In an Italian study of 8744 older adults (>65 years) approximately 16% of all anaemia was attributed to iron deficiency (Tettamanti et al., 2010). This is similar to the findings from NHANES III where iron deficiency accounted for up to 16% of all anaemia (Guralnik et al., 2004). IDA in older adults is sometimes related to diet but is more often related to gastrointestinal bleeding caused by non-steroidal anti-inflammatory drugs, colonic cancer or polyps, gastric cancer or inflammatory bowel disease (Mukhopadhyay & Mohanaruban, 2002).

Iron deficiency and anaemia has been implicated in impaired cognitive and mental health (Yavuz et al., 2012), falls and fractures (Toxqui & Vaquero, 2015) and mortality (Mukhopadhyay &

Mohanaruban, 2002) in older adults. Dementia and depressive symptoms may be higher in people with anaemia and iron-deficiency (Stewart & Hirani, 2012; Yavuz et al., 2012), however, these conditions are also associated with poorer nutrition in older adults which may also result in iron depletion.

2.5.5. Elevated iron

It has also been proposed that iron stores increase with age. The Framingham Heart Study found that older adults (aged 67–96 years) had a very low prevalence of iron-deficiency (2.7%) and iron-deficiency anaemia (1.2%) whereas 12.9% of the study population had elevated iron stores (Fleming et al., 2001). Serum ferritin was used as a marker of abnormally high iron stores, although it has not yet been validated, values >300µg/L for men and >200µg/L for women were used to define elevated iron (Fleming et al., 2001). Several dietary factors were associated with elevated iron stores in the Framingham Heart Study including the use of supplemental iron (>30mg/d), having >21 servings of fruit per week or >3 servings of fruit or fruit juice per day, and consuming >4 servings of red meat per week whereas intake of whole grains was negatively associated with iron stores (Fleming et al., 2002).

Mutations in the HFE gene, namely mutations in C282Y and H63D, also known as hereditary haemochromatosis may also lead to elevations in iron stores. The prevalence of haemochromatosis in the general population is very low and the same penetrance rate is seen in older adults (Van Aken et al., 2002). Although elevated levels of iron have been implicated in cardiovascular disease and mortality, adults are still able to live to old age with the mutation. In the Leiden 85+ study, the prevalence of haemochromatosis was around 0.2% which was compatible with what was seen in younger age groups (Van Aken et al., 2002).

2.6. Folate status of older adults

Folate is the term comprising all the different active forms of the vitamin, including synthetic folic acid used in supplements and fortification (Devalia et al., 2014; Moll & Davis, 2017). Naturally occurring folate (food-folate) is found mainly in liver and green leafy vegetables and is usually, but not exclusively, present as polyglutamates which must first be reduced to monoglutamates in order to be absorbed (De Silva & Davis, 2013). Folate comprises of several different forms, therefore, estimation of folate intake is complex as food composition databases may not take into account the different forms. Further, the bioavailability of folate is much higher in folic acid supplements (~100%) and fortified foods with folic acid (~85%) in comparison to food folates (~50%) (Allen, 2008). Therefore, nutrient reference values are now based on Dietary Folate Equivalents (DFE) which account for the differing bioavailability between food folate, folic acid added to foods, and folic acid supplements (NHMRC, 2006; Suitor & Bailey, 2000), expressed as:

1µg DFE = 1.0µg food folate = 0.6µg folic acid added to foods = 0.5µg folic acid supplements

Metabolically active folate derivatives play an important role in single-carbon transfers for the metabolism of nucleotides and amino acids (De Silva & Davis, 2013). It also plays a key role in the methylation of homocysteine to produce methionine, a reaction key for DNA synthesis, repair and methylation, therefore without folate, living cells would not be able to divide (De Silva & Davis, 2013; NHMRC, 2006).

Dietary folate intake was not assessed in the NZANS 2008/09 due to limitations in the ability to estimate folate content of foods from the New Zealand Food Composition Database (University of Otago & Ministry of Health, 2011b). However, dietary folate intake was assessed in the New Zealand National Nutrition Survey (NZNNS) 1997 where they found that approximately 7% of the

population aged 65+ had inadequate intakes, and it was generally higher in women than in men (Ministry of Health, 1999). In the NZNNS 1997, approximately 26% of Māori women aged 45+ were found to have an inadequate intake, much higher than that of non-Māori women aged 65+ (9.2%) (Ministry of Health, 1999). However, contrary to current recommendations, the EAR used in the NZNNS 1997 was 150µg/day which has now been updated to 320µg/day (NHMRC, 2006). Biochemical analyses were performed in the population that participated in the NZANS 2008/09 where they found that those aged 71+ had the highest mean red blood cell (RBC) folate compared to their younger counterparts with the prevalence of low levels being <2.0% (University of Otago & Ministry of Health, 2011a). Similar data was found when they looked at serum folate levels where <2% of men and women were found to be low (University of Otago & Ministry of Health, 2011a).

2.6.1. Physiological mechanisms of folate absorption, transport and storage

Dietary folates are first absorbed in the acidic milieu in the duodenum and proximal jejunum via active and passive transport (Milman, 2012; Moll & Davis, 2017). Absorption of food-folate may be influenced by several factors including the food matrix where incomplete liberation from plant cellular structures may lower bioavailability whereas ascorbic acid and milk may enhance the stability of folate and increase bioavailability (Brouwer et al., 2001; EFSA NDA Panel, 2015). The active absorption mechanism is also pH dependant with optimal absorption at a pH around 5 (EFSA NDA Panel, 2015). Dietary folates and folic acid must first be converted to the circulating form, methyl-THF, before being transported to tissues or stored in the liver and kidney. Approximately 50% of the body stores of folate are found within the liver (Devalia et al., 2014).

Once transported to the tissues, methyl-THF is converted to the active form, tetrahydrofolate (THF), (De Silva & Davis, 2013; Moll & Davis, 2017). THF can be converted to other forms including 5,10-methyl-THF which is the co-enzyme for the rate-limiting step in DNA synthesis (De Silva & Davis, 2013; Moll & Davis, 2017). This process is also linked to several B-vitamins, including vitamin B_{12} which is used to convert homocysteine to methionine, a reaction needed for conversion of THF to 5,10-THF, without which DNA synthesis and repair would fail (De Silva & Davis, 2013). However, folates can be reduced to their active form prior to absorption into the circulation, therefore, with large doses of folate, red blood cells can continue to be produced normally, masking the deficiency in vitamin B_{12} (Mahan et al., 2012).

2.6.2. Factors affecting folate intake and status

2.6.2.1. Fruit and vegetable consumption

Consumption of folate-rich fruits and vegetables has been shown to decline with ageing. In a study of 1430 Taiwanese older adults aged 69-90 years, the men and women aged 80+ years were been shown to have significantly lower vegetable and fruit intake, respectively, compared to their younger counterparts (Chen et al., 2005). Vegetables are the most commonly consumed food at a hui and the second most common traditional Māori food consumed, however, these vegetables include root vegetables, watercress and pumpkin which are more likely to be lower in folate (Rush et al., 2010). Fruits and vegetables are likely to be an important source of folate in this age group given that liver is not frequently consumed (Chen et al., 2005). However, in New Zealand, more than a third of adults aged over 75 years do not consume at least 3 servings of vegetables each day and adults in the most socioeconomically deprived areas were less likely to consumed these serves compared to those in the least deprived areas (Ministry of Health, 2016a).

2.6.2.2. Oral health and cooking methods

Oral health plays an important role in food consumption as older adults with poor chewing ability or ill-fitting dentures have been shown to consume lower amounts of vegetables and overall lower

nutrient intakes (Holmes & Roberts, 2009). This may be a factor in the cooking methods employed by older adults to make vegetables more palatable and softer which in turn affects the folate content of the food eaten (Allen, 2008). Food folates are relatively unstable to oxidation and heat and large losses can occur during processing and cooking, particularly in green-leafy vegetables where up to 80% of folate can be lost through boiling and up to 50% lost from legumes (Allen, 2008). Boiling is a traditional way of cooking for Māori and for older adults, which may put them at further risk for inadequate dietary intakes (Allen, 2008; Rush et al., 2010).

2.6.2.3. Smoking and alcoholism

It has also been suggested that smoking impaired folate absorption, however, more recent research is now showing that the link between smoking and folate absorption is not causal, rather that smokers tended to have a diet poorer in folate-rich foods such as fruit and vegetables and as a result had lower folate levels (Ministry of Health, 2013; Vardavas et al., 2008). However, it has been proposed that alcoholism may affect folate intake since alcoholics have been shown to have poorer folate status. This may be due to poor folate absorption, reduced uptake and storage, or low folate intake due to poorer diet quality (Allen, 2008). In New Zealand, Māori are 1.5 times more likely to have hazardous drinking patterns in comparison to non-Māori which may put Māori more at risk for folate inadequacy (Ministry of Health, 2015a).

2.6.2.4. Folate fortification

The folate status of the population may further be influenced by fortification of flour used to make bread. In New Zealand, folate fortification is voluntary and a limited variety of fortified foods are available, however bread remains a household staple for many New Zealanders and is the top contributor to energy intake in Māori aged 51+ years and non-Māori aged 71+ years (University of Otago & Ministry of Health, 2011a). It does appear that folate status has improved between the pre- and post-fortification periods in New Zealand where women had a significantly higher RBC folate concentration compared to the pre-fortification studies (Ministry for Primary Industries, 2012). Similar changes in RBC folate concentrations among participants in the U.S National Health and Nutrition Examination Surveys were observed between the period of 1988 - 2006 where mandatory bread fortification was implemented (McDowell et al., 2008). This may be the reason that there are very low prevalence rates of folate insufficiency in older adults in New Zealand and suggests that folate-fortified food sources are an important contributor to total folate intake.

2.6.2.5. Genetics

Single-nucleotide polymorphisms (SNPs) in the MTHFR gene have been significantly associated with a lower folate status, in particular, homozygosity with the T allele (TT) results in reduced 5-methyl-THF production and high homocysteine concentrations, consistent with low folate status (Zinck et al., 2015). Several variants of the MTHFR gene are associated with lower RBC folate status and some variants are associated with higher RBC folate status. In the Newcastle 85+ study, participants heterozygous for the A allele of the MTHFR gene had higher concentrations of RBC folate than those homozygous for the G allele (Mendonça et al., 2016) suggesting that the genetic profile of individuals may also influence folate status.

2.6.2.6. Medications

Several medications have also been shown to impair folate status including methotrexate, anti-convulsants such as phenytoin and phenobarbital, and high doses of non-steroidal anti-inflammatory drugs (>3,900mg/d) (Allen, 2008). Given that polypharmacy is more common among older adults and is associated with impaired nutritional status, older adults may be more at risk for folate insufficiency (Heuberger & Caudell, 2011; Maher et al., 2014)

2.6.3. Biomarkers of folate status and association with dietary intake

2.6.3.1. Serum folate

Serum folate concentration usually reflects recent folate intake and short-term status. Lower consumption of fruit and vegetables has been significantly associated with lower serum folate concentrations in Taiwanese octogenarians (Chen et al., 2005). However, there are several limitations in assessing folate status using serum folate as recent folate intake can raise serum folate concentrations and mask underlying deficiencies, therefore, one measurement is not sufficient for assessing folate status (Devalia et al., 2014). Further, there is no clear consensus on the level of serum folate that indicates deficiency and it has been proposed that serum folate <7nmol/L be used as a guideline due to the increased risk of developing megaloblastic anaemia below this level (Devalia et al., 2014). The Leiden 85+ study found that 0.2% of octogenarians had inadequate serum folate using this cut-off (den Elzen et al., 2013). The NZANS 2008/09 used a slightly lower cut-off point of <6.7nmol/L, as per the NHANES III criteria to indicate deficiency (University of Otago & Ministry of Health, 2011b; Wright et al., 1998).

2.6.3.2. Red blood cell folate

RBC folate can be used as a measure of long-term folate status as it gives an assessment of the folate status over the lifetime of the red blood cells (Wright et al., 1998). The association between dietary folate intake and RBC folate concentration was investigated in the Newcastle 85+ Study where they found that higher dietary folate intake was significantly associated with higher concentrations of RBC folate and the likelihood of having low RBC concentration was more than halved when dietary folate intake was >264µg/day compared to when intake was <157µg/day in octogenarians (Mendonça et al., 2016). Typically a RBC folate level <340nmol/L has been associated with clinical folate deficiency (Devalia et al., 2014) and the Newcastle 85+ study reported that 3.6% of octogenarians were deficient in RBC folate according to this threshold (Mendonça et al., 2016). However, New Zealand and England have used a lower cut-off <317nmol/L in the national nutrition surveys as per the NHANES III criteria (Bates et al., 2015; University of Otago & Ministry of Health, 2011b; Wright et al., 1998).

Table 2.2: Folate measures and cut-offs

Status	Measures and Cut-offs
Low folate stores	RBC folate <317nmol/L [1, 2, 3] Serum folate <6.7nmol/L [1, 2, 3]

[1] Bates et al. (2015)

[2] University of Otago and Ministry of Health (2011b)

[3] Wright et al. (1998)

2.6.4. Folate insufficiency and anaemia

Folate plays a key role in DNA synthesis and due to the rapid turnover of red blood cells, the clinical signs of folate deficiency will usually appear first in the haematopoietic system with morphological changes in the megaloblasts and macrocytosis of the red blood cells as a result of disrupted DNA synthesis and repair, an indication of the onset of megaloblastic anaemia (Moll & Davis, 2017; Wright et al., 1998). Inadequate intake is the leading cause of folate insufficiency and body stores of folate only last up to 2-3 months meaning that deficiency can occur rapidly with insufficient intake (Bailey et al., 2015; Moll & Davis, 2017).

In the Leiden 85+ study, low serum folate was associated with lower haemoglobin levels and anaemia (den Elzen et al., 2008). In the Italian study by Tettamanti et al. (2010) approximately 10% of all anaemia was attributed to vitamin B_{12} and folate deficiencies, collectively. However, in the study by Frangos et al. (2016) looking at anaemia in octogenarians, 4.6% of participants with anaemia had folate deficiency, however, it was not the sole cause of anaemia for any of the patients. Data from these prospective studies and also from the NZANS 2008/09 suggest that the prevalence of folate deficiency and its association with anaemia in older adults is small, some of which may be attributed to voluntary and mandatory folate fortification.

2.7. Vitamin B_{12} status of older adults

Vitamin B_{12} is the term used to describe the group of compounds that exhibit the biological activity of cyanocobalamin. There are many types of cobalamin compounds that are required for the synthesis of fatty acids in myelin and, together with folate, for DNA synthesis (NHMRC, 2006). Adequate intake is essential for normal blood function and neurological function. Vitamin B_{12} can be synthesised by microorganisms and exist in food products from an animal origin including milk, cheese and eggs (De Silva & Davis, 2013). A typical Western diet contains 5 – 30µg of vitamin B_{12} per day which covers the average requirement of 1 – 4µg lost per day through normal physiological processes (De Silva & Davis, 2013). Vitamin B_{12} can also be stored in the liver and be sufficient for up to 4 years without further supply (De Silva & Davis, 2013; NHMRC, 2006).

The NZANS 2008/09 reported the median intakes of vitamin B_{12} in men and women aged 71+ to be 4.2µg/d and 2.7µg/d, respectively (University of Otago & Ministry of Health, 2011a). This correlates with the NHANES 2001-2002 where men aged 60+ years had larger vitamin B_{12} intakes compared to women of the same age group (Hinds et al., 2011). Currently the EAR for adults aged 70 years and over is set at 2.0µg/d for men and women (NHMRC, 2006) and approximately 3.8% of men and 27% of women aged 71+ were found to have an inadequate intake in the NZANS 2008/09 (University of Otago & Ministry of Health, 2011a). Similarly, in the Newcastle 85+ study, more women (12.1%) did not meet the EAR compared to men (4.5.%), despite a lower EAR being used (1.0µg/day) (Mendonça et al., 2016). This may be because older women tend to have lower energy intakes in comparison to men and subsequently lower micronutrient intakes, particularly from animal products where vitamin B_{12} is sourced (Hinds et al., 2011; University of Otago & Ministry of Health, 2011a). Principle food sources of vitamin B_{12} among men and women aged 71+ in the NZANS 2008/09 in descending order were found to be milk, beef and veal, fish and seafood, and egg and egg dishes (University of Otago & Ministry of Health, 2011a).

2.7.1. Physiological mechanisms of vitamin B_{12} absorption, transport, and storage

Vitamin B_{12} is bound to food proteins where it serves as a coenzyme. There are many events that need to occur for successful vitamin B_{12} absorption and any interruption results in malabsorption. Inside the stomach, vitamin B_{12} is liberated from the protein by the action of stomach acid and pepsin, allowing vitamin B_{12} to then bind to small proteins secreted by the stomach called R-binders (Russell & Baik, 2001). Vitamin B_{12}, bound to the R-binders, is then transported to the proximal small intestine where the R-binders are removed from the vitamin through the action of pancreatic proteases (Russell & Baik, 2001). Vitamin B_{12} is then bound to a small glycoprotein secreted by the stomach called intrinsic factor which transports vitamin B_{12} to the terminal ileum where it can be actively absorbed (Russell & Baik, 2001).

The overall bioavailability of Vitamin B_{12} is said to be around 50%, yet, interestingly, the efficiency of absorption is lower from sources containing higher amounts of vitamin B_{12} such as liver and meat (Gille & Schmid, 2015). Therefore, it may be better for older adults to have multiple low-vitamin B_{12}

dietary sources such as milk and dairy rather than one large source of dietary vitamin B_{12} from liver or meat (Gille & Schmid, 2015). The absorption of vitamin B_{12} can be reduced due to impaired gastrointestinal absorption as a result of decreased gastric acid and intrinsic factor, a common phenomenon in older adults (Stover, 2010). However, synthetic crystalline vitamin B_{12} is not impaired by these processes and therefore has a higher rate of absorption than naturally occurring vitamin B_{12} (Chernoff, 2013). Given this, fortified foods may have the potential to improve vitamin B_{12} intakes and status among older adults.

2.7.2. Factors affecting vitamin B_{12} intake and status

2.7.2.1. Atrophic Gastritis

In older adults there is often a decreased secretion of intrinsic factor, stomach acid and pepsin that occurs due to atrophic gastritis which decreases the absorption of food-bound cobalamin and accounts for 30-70% of all vitamin B_{12} deficiencies (Kassarjian & Russell, 1989; Stover, 2010). Although vitamin B_{12} is secreted in bile and reabsorbed via the enterohepatic circulation, it also requires intrinsic factor therefore, where there is lack of intrinsic factor, there is both malabsorption from the diet and increased losses from inability to reabsorb vitamin B_{12} (O'Leary & Samman, 2010). The prevalence of atrophic gastritis in older adults (aged >65 years) living in New Zealand was relatively low (6.7%) in a study by Green et al. (2004), however, the prevalence among octogenarians is unclear.

2.7.2.2. Vegetarianism and veganism

Vitamin B_{12} is exclusively found in foods derived from animal origin and intake is dependent on the amount of animal-origin and fortified foods that exist in the diet (Allen, 2008). The content of vitamin B_{12} in meat can ranges between 0.7 – 5.2µg/100g depending on the animal it was derived from, cooking method, and cut of meat with animal liver containing up to 110µg/100g of vitamin B_{12} (Gille & Schmid, 2015). Whereas the vitamin B_{12} content of milk and milk products are much lower (ranging between 0.07 – 3.1µg/100g). This is a potential issue for older adults who may have vegetarian or vegan diets. In the EPIC-Oxford UK study, approximately 35% of the adults aged 80+ were vegetarian or vegan and tended to have the lowest dietary intake of vitamin B_{12}, ranging between 0.41 – 2.57µg/day. Unsurprisingly, the intake of vitamin B_{12} progressively got higher as animal-sourced food intake increased with meat-eaters having the highest intake (Davey et al., 2003). The prevalence of veganism in older New Zealand adults is unknown.

2.7.2.3. Cooking methods

Vitamin B_{12} concentrations seem to stay similar or even increase after cooking depending on the method of cooking. Vitamin B_{12} concentrations in meat products can be concentrated when using dry-methods of cooking, but can also be decreased if using moist-heat methods, such as boiling since vitamin B_{12} is water soluble (Gille & Schmid, 2015). Vitamin B_{12} in milk and dairy products is also influenced by thermal processing where 10-20% of vitamin B_{12} can be lost after processing milk, 25% lost in the fermentation of yoghurt, and up to 60% removed in the cheese-making process (Gille & Schmid, 2015).

2.7.2.4. Smoking status

Smoking also appeared to be positively correlated with vitamin B_{12} levels, however this is not a causal relationship, rather, the dietary patterns of smokers are more likely to favour meat consumption over fruit and vegetables (Vardavas et al., 2008). In New Zealand, although adults over the age of 75 are the less likely to be current smokers, Māori are almost three times more likely to be current smokers than non-Māori (Ministry of Health, 2015a).

2.7.2.5. Genetics

Genetic variants in exon 2 of the FUT2 gene are significantly associated with a lower risk of deficiency in vitamin B_{12} and are also associated with higher serum vitamin B_{12} (Zinck et al., 2015). It has been hypothesised that the FUT2 variants reduces the risk of Helicobacter pylori infection and related gastritis-induced vitamin B_{12} malabsorption, or that FUT2 may increase the secretion of intrinsic factor, essential for vitamin B_{12} absorption (Zinck et al., 2015). The findings are consistent in the Newcastle 85+ where women with FUT2 GG genotype had higher concentrations of serum vitamin B_{12} suggesting that genetics have a role to play in vitamin B_{12} status (Mendonça et al., 2016).

2.7.2.6. Medications

Medications are also a key contributor to the absorption of vitamin B_{12}, in particular are the drugs that impair gastric acid and pepsin secretion which inhibits the liberation of food-bound vitamin B_{12} (Allen, 2008). These medications include H_2 receptor antagonists (H2RAs) and proton pump inhibitors (PPIs). However, the effect of H2RAs are dose-related where a dose of cimetidine >1000mg/d is sufficient to disrupt the absorption whereas <400mg/d is not (Allen, 2008). However, proton pump inhibitors such as omeprazole can reduce vitamin B_{12} absorption by up to 70% at doses as low as 20mg/d and a dose >40mg/d is likely to lead to a 90% reduction in absorption (Allen, 2008). The effect is more likely to be seen in long-term users of these drugs, particularly older adults given that up to 44% of New Zealanders aged over 75 years use more than five medicines continuously over a nine month period with omeprazole being the third most prescribed medicine in New Zealand (bpacnz, 2012a, 2012b, 2013).

2.7.3. Biomarkers of Vitamin B_{12} status and relationship to dietary intake

2.7.3.1. Serum vitamin B_{12}

In the Newcastle 85+ study, serum vitamin B_{12} was been shown to be weakly associated with dietary vitamin B_{12} intake, however, the risk of deficiency in serum vitamin B_{12} more than halved when dietary intake was >4.40µg/day compared to when dietary intake was <1.87µg/day (Mendonça et al., 2016). A higher intake of meat and milk was also significantly associated with a lower risk of deficiency (Mendonça et al., 2016). There is currently no universally accepted serum vitamin B_{12} cut-off to define deficiency, however, several have been proposed in the literature ranging from 148-250pmol/L (de Benoist, 2008; Devalia et al., 2014; O'Leary & Samman, 2010; Wong, 2015). The Leiden 85+ study found that 8.5% of octogenarians had low serum vitamin B_{12} based on the cut-off 150pmol/L (den Elzen et al., 2013). However, the 148pmol/L cut-off suggested by the World Health Organisation (de Benoist, 2008) has been debated suggesting that this level is too low and that clinical manifestations of a vitamin B_{12} deficiency may arise at or above this level.

2.7.3.2. Serum and urinary methylmalonic acid

Elevated MMA is a specific indicator of cobalamin metabolism and tissue storage and is considered the gold standard for laboratory diagnosis of vitamin B_{12} deficiency (O'Leary & Samman, 2010; Russell & Baik, 2001). No cut-off level exists for serum MMA and normal reference ranges vary between 100-750nmol/L with a value greater than 750nmol/L invariably indicating vitamin B_{12} deficiency (Devalia et al., 2014; Wong, 2015). However it has been proposed that even an MMA >300nmol/L would indicate a deficiency (O'Leary & Samman, 2010; Wong, 2015). Dietary intakes of vitamin B_{12} was not shown to affect serum MMA in adults (aged >19 years) part of the NHANES 1999 - 2004, however, total vitamin B_{12} exposure, including the use of supplements was been shown to have a significant inverse relationship with serum MMA concentrations (Bailey et al., 2011). MMA concentrations may also be elevated in patients with renal insufficiency, a condition that often increases with age (O'Leary & Samman, 2010).

2.7.3.3. Holotranscobalamin

Holotranscobalamin (holoTC) is the plasma vitamin B_{12} is bound to transcobalamin proteins for transport to the cells of the body and is perceived as a more suitable diagnostic test for vitamin B_{12} in comparison to serum vitamin B_{12} (Nexo & Hoffmann-Lücke, 2011). The current consensus is that reference ranges for holoTC are between 40 – 200pmol/L, however, there are several limitations in using this marker alone to assess vitamin B_{12} status. HoloTC may be influenced by diurnal variation, genetic variation, and chronic liver or kidney diseases (Nexo & Hoffmann-Lücke, 2011). Further, holoTC has not been reported as a more sensitive marker in the case of food-bound cobalamin absorption in older adults compared to serum vitamin B_{12}, and limited data exists on the association between holoTC and dietary intake (Golding, 2016).

Table 2.3: Vitamin B_{12} measures and cut-offs

Status	Measures and Cut-offs
Vitamin B_{12} Deficiency	Serum vitamin B_{12} <148pmol/L [1, 2] Serum MMA >750nmol/L [2, 3] *or* urinary MMA >4nmol/L [3] HoloTC <40pmol/L [4]

[1] de Benoist (2008)

[2] Devalia et al. (2014)

[3] Wong (2015)

[4] Nexo and Hoffmann-Lücke (2011)

2.7.4. Vitamin B_{12} deficiency in older adults

Older adults are particularly vulnerable to vitamin B_{12} deficiency due to inadequate dietary intakes and food-bound cobalamin malabsorption caused by age-related changes to the gastrointestinal tract. Pernicious anaemia is an autoimmune disease characterised by the destruction of the gastric mucosa resulting in neutral to slightly acidic gastric secretions with little or no intrinsic factor resulting in vitamin B_{12} malabsorption (Andrès et al., 2004; de Benoist, 2008). Lack of intrinsic factor, dietary inadequacy, and pernicious anaemia are the most common causes of vitamin B_{12} deficiency in older adults (Kassarjian & Russell, 1989; Russell & Baik, 2001).

Widespread vitamin B_{12} deficiency may increase the prevalence of anaemia in older adults, particularly those consuming low amounts of animal-sourced foods. In New Zealand, approximately 40% of those aged over 65 years were found to have an overt deficiency of vitamin B_{12} (<148pmol/L) or a marginal status (148-221pmol/L) (Green et al., 2004). Among 392 Swiss octogenarians in a study by Frangos et al. (2016), approximately 40% were shown to be anaemic, of which 20.8% were deficient in vitamin B_{12}. Manifestations of a vitamin B_{12} deficiency can range from mild to severe, however, there is conflicting evidence that vitamin B_{12} deficiency is associated with peripheral neuropathy and dementia in octogenarians (Mooijaart et al., 2005).

2.8. Summary

The older New Zealand population is growing rapidly, with an expected six-fold increase in those aged 80+ years by 2068. There is a need to understand the determinants of successful ageing in order to ensure the health of older adults and minimise costs to the healthcare system. Maintaining good nutrition is vital for healthy ageing given that poor nutrition increases the risk of hospitalisations, disability, and decreases quality of life. Therefore, there is a need to understand the current dietary intakes and factors that influence nutrient status. It particular, iron, folate and

vitamin B_{12} are largely intertwined in the production of red blood cells and are essential for normal blood and neurological function. The NZANS 2008/09 illustrated that older New Zealand adults aged 71+ years consume the least iron and vitamin B_{12} compared to their younger counterparts. Dietary folate was not assessed in the NZANS 2008/09 therefore current folate status of older New Zealanders is unknown.

Several factors may influence dietary intake of iron, folate and vitamin B_{12} in older adults. In particular, declining oral health and socioeconomic constraints may result in reduced intake of meat, fruit and vegetables, nuts and grains. Several studies in older adults have shown a positive correlation between dietary intake of iron, folate and vitamin B_{12} and their respective serum biomarkers. However, numerous factors including bioavailability, the food matrix, cooking methods, fortification, medications, genetics, and age-related changes to the gastrointestinal tract may hinder this association. Strategies to improve nutrient intake should be developed in light of these factors.

Further, iron, folate, and vitamin B_{12} are the three key nutrients associated with the development of anaemia. Predicted demographic changes highlight the need to understand more about anaemia in older adults given that anaemia is associated with cognitive dysfunction, falls and fractures, and mortality, and is prevalent among 3% - 50% of community dwelling older adults. As there are currently no NRVs and biomarker cut-offs for iron, folate and vitamin B_{12} that relate specifically to octogenarians, an investigation of these nutrients and biomarkers is warranted.

3. Research Study Manuscript

Intakes, adequacy, food sources and biomarker status of iron, folate, and vitamin B_{12} in Māori and non-Māori octogenarians: Life and Living in Advanced Age: A Cohort Study in New Zealand (LiLACS NZ).

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Abstract

Background: Octogenarians may be at increased risk for iron, folate and vitamin B_{12} deficiency due to reduced food intake. There are no specific nutrient reference values or biomarker cut-offs for these micronutrients for adults in advanced age and little is known about the relationship between dietary intake and biomarkers for older adults. The aim of this study was to investigate the intake, adequacy, food sources and biomarker status of iron, folate and vitamin B_{12} and the relationship between dietary intake and biomarkers.

Methods: In the follow up assessment of LiLACS NZ, 216 Māori and 362 non-Māori participants completed a detailed dietary assessment using 2x 24-hr multiple pass recalls. Adequacy of iron, folate and vitamin B_{12} were determined by comparison to the Estimated Average Requirement (EAR) for adults aged 71+ years. Serum ferritin, serum iron, total iron binding capacity, transferrin saturation, red blood cell (RBC) folate, serum folate, serum vitamin B_{12} and haemoglobin were compared to recognised cut-offs for adults. Generalised linear models and binary regression estimated the association between dietary intake and biomarkers.

Results: Most participants had adequate dietary iron intakes (88% Māori; 95% non-Māori above EAR) and biomarkers for iron (>94% above cut-offs). The EAR for vitamin B_{12} was met by 74% Māori; 78% non-Māori and folate met by 42% Māori; 49% non-Māori. Māori versus non-Māori had higher intakes of vitamin B_{12} ($p=0.038$) and serum vitamin B_{12} ($p=0.026$). Increased dietary folate intake was associated with increased RBC folate for Māori ($p=0.001$) and non-Māori ($p=0.014$) and with increased serum folate for Māori ($p<0.001$). Folate intake >215µg/day was associated with reduced risk of deficiency in RBC folate for Māori ($p=0.001$).

Conclusions: Dietary intake and stores of iron are largely adequate in this population. Strategies to optimise the intake and bioavailability of foods rich in folate and vitamin B_{12} may be beneficial.

Key words: Iron, folate, vitamin B_{12}, biomarkers, older adults, octogenarians, LiLACS NZ

Introduction

One of the most profound changes in New Zealand demographics is the rapidly growing population of older adults, particularly adults aged 85+ who are expected to increase six-fold by 2068 (Statistics New Zealand, 2016b). In particular, the older Māori population is likely to surge in the coming years at a rate higher than that of non-Māori (Ministry of Health, 2015b). Given this shift in demographics, it is essential to establish the determinants of successful ageing for octogenarians, and more specifically older Māori.

Ageing is associated with a decline in functionality and an increase in chronic disease, disability and the demand for long term healthcare which has significant implications for support services (Cornwall & Davey, 2004). Adequate nutrition is imperative to prevent malnutrition among older adults, a condition that has significant costs and implications for the healthcare system. Older Māori are five times more likely to be at high nutritional risk compared to non-Māori contributed to by low dietary intakes of fruit, vegetables, milk and meat (McElnay et al., 2012). Low intakes of these foods have also been linked to lower dietary intakes and serum biomarkers of iron, folate and vitamin B_{12} (Chen et al., 2005; Jackson et al., 2016; Mendonça et al., 2016). Iron, folate and vitamin B_{12} are largely intertwined in the production of red blood cells, and they are the most common nutrient deficiencies associated with anaemia (Frangos et al., 2016; Guralnik et al., 2004). They have also been implicated in impaired cognition and depression (Mooijaart et al., 2005; Yavuz et al., 2012), increased risk of falls (Toxqui & Vaquero, 2015), decreased quality of life and increased risk of mortality in older adults (Marian & Sacks, 2009; Mukhopadhyay & Mohanaruban, 2002; Pfisterer et al., 2016; Yildirim et al., 2015).

Older adults aged 71+ were found to have the lowest dietary intakes of iron and vitamin B_{12} compared to other age groups in the NZ ANS 2008/09 (University of Otago & Ministry of Health, 2011a). Dietary folate was not examined in the NZ ANS 2008/09 due to unreliable methods to estimate dietary intake therefore current dietary folate intakes for older New Zealanders is not known. The prevalence of low iron and folate stores among adults aged 71+ was relatively low (<2% of men and women) in the NZANS 2008/09 compared to the Leiden 85+ study where the occurrence of low iron stores in octogenarians ranged between 4% – 15.9% depending on the biomarker investigated (den Elzen et al., 2013). Vitamin B_{12} deficiency was much higher (12%) among New Zealand adults aged 65+ years (Green et al., 2004) which correlated with the findings from the Newcastle 85+ study where up to 17% of octogenarians were deficient in vitamin B_{12} (Mendonça et al., 2016). However, folate stores were mostly adequate in the Leiden and Newcastle 85+ cohorts (99.8% - 96.4% with adequate RBC folate concentrations) (den Elzen et al., 2013; Mendonça et al., 2016).

Several factors including impaired gastrointestinal absorption, medications, cooking methods, and genetics may affect iron, folate and vitamin B_{12} intake, absorption and biomarkers. Therefore, the relationship between dietary intake and serum biomarkers is complex. Not all foods are equally bioavailable, however, it is well known that iron from meat and meat products is more bioavailable than that from plant sources and fortified foods (Fairweather-Tait & Teucher, 2002; Russell, 2001) whereas, folic acid and synthetic vitamin B_{12} used in fortification is more bioavailable and less affected by age-related impaired gastrointestinal absorption compared to naturally occurring food-folate and vitamin B_{12} (Allen, 2008; Chernoff, 2013). Some studies have shown an association between increasing intakes of iron, folate and vitamin B_{12} and their respective biomarkers in older

adults (Chen et al., 2005; Jackson et al., 2016; Mendonça et al., 2016; Milman et al., 2004). However, this relationship has not been explored in older New Zealand adults, therefore, an investigation of the current status of these nutrients and contributing food sources among older New Zealanders may provide insights into the relationship between dietary intake and serum biomarker status and could inform strategies to prevent deficiency among older adults.

As it stands, New Zealand does not have specific nutrient reference values (NRVs) for octogenarians and the current NRVs combine all older adults into a 70+ age group therefore these NRVs may not represent positive health outcomes for octogenarians. Similarly, the NZANS 2008/09 aggregated all older adults into a 71+ age group and all older Māori into a 51+ age group. Therefore, the current status of iron, folate and vitamin B_{12} is not known for octogenarians. The aim of the current study was to investigate the intakes, dietary adequacy, food sources and biomarker status of iron, folate and vitamin B_{12}, and to determine whether there is a correlation between their respective biomarkers and dietary intakes.

Materials and Methods

Te Puāwaitanga o Ngā Tapuwae Kia ora Tonu also known as Life and Living in Advanced Age: A Cohort Study in New Zealand (LiLACS NZ) is a population-based longitudinal cohort study of Māori and non-Māori in advanced age. The study was set up in 2010 to determine the predictors of successful ageing among Māori and non-Māori in New Zealand.

This study is a sub-section of LiLACS NZ which aims to identify the dietary intake, adequacy, food sources and biomarker status of iron, folate and vitamin B_{12} as well as to investigate the association between dietary intake and biomarker status in Māori and non-Māori octogenarians. The data used in this study was collected over a two-year period from 2010-2012.

Participants and recruitment

Participants eligible for enrolment into LiLACS NZ were those born between 1st January 1925 and 31st December 1925 for non-Māori (85 years) and those born between 1920 and 1930 for Māori (80-90 years). Details of recruitment have been described elsewhere (Dyall et al., 2013; Hayman et al., 2012). Briefly, participants resided in the Bay of Plenty District Health Board or Lakes District Health Board in the central North Island of New Zealand. The NZ Māori Electoral roll and the NZ General Electoral Roll was used to identify and contact all eligible participants, in addition to GP practices, whanaungataunga, advertising, residential care networks and personal contacts. Seven local organisations were subcontracted by The University of Auckland to contact, recruit and enrol participants and to conduct the interviews and health assessments. This included three Primary Health Organisations (PHOs) and four Māori tribal organisations.

Ethics approval for Life and Living in Advanced Ages: A Cohort Study was granted by the Northern X Regional Ethics Committee (NXT 09/09/088) in December 2009 and written informed consent was obtained from all participants for each stage of the study (Dyall et al., 2013).

Comprehensive questionnaire and health assessment

At baseline, participants were asked to complete a comprehensive questionnaire, conducted by trained interviewers, and a health assessment conducted by a trained nurse. Details of the comprehensive questionnaire and health assessment are reported elsewhere (Hayman et al., 2012). Participant details including age, demographic information, physical and health characteristics were established at baseline and updated if necessary during Wave 2. Interviewers were required to view and record in detail the number of medications and supplements taken by participants. For those who did not wish to do the comprehensive questionnaire, a simplified version was made available (Hayman et al., 2012). Some questionnaires were incomplete and some questions were only asked in the comprehensive interview therefore rates of participation varied for certain measures.

Measures

Current living situation was self-reported and categorised as living alone, with spouse, or with others which included the spouse. Highest level of education was self-reported and categorised as primary schooling/none, secondary with and without qualification, trade/occupation, or tertiary. NZDep score was ascertained from the New Zealand Deprivation index obtained from the Ministry of Health to indicate socioeconomic deprivation (Salmond et al., 2007).

The use of dentures was categorised as none or dentures used which included full, partial, upper or lower dentures. Medications were characterised according to the number of medications taken and included the use of dietary supplements. Participants who used proton pump inhibitors (PPIs),

histamine 2 receptor antagonists (H2RAs), non-steroidal anti-inflammatory drugs (NSAIDs), and antacids were also reported. Smoking status was categorised as never smoked, former smoker or current smoker and was self-reported. Frequency of alcohol consumption was self-reported and reported as the number of times per week or month. Dietary supplement usage was reported as those who used vitamins or minerals and/or multivitamins/minerals and those who did not.

Weight was ascertained using a Tanita digital measuring scale (BC-541; Tanita Corporation) and height using a portable stadiometer (SECA 213). For participants unable to stand, height was estimated from demispan length. Weight and height were used to calculate BMI and reference ranges for BMI were taken from the Ministry of Health guidelines in accordance with the World Health Organisation (Ministry of Health, 2013).

Dietary assessment: 24-hour multiple pass recall

Diet was assessed using a 24-hour multiple pass recall (24-hr MPR). Two 24-hr MPR's were completed on separate days of the week conducted by trained interviewers (Figure 1). This method was validated for use in octogenarians as part of the Newcastle 85+ study and yielded more accurate estimates of energy and nutrient intake when compared with a food frequency questionnaire (FFQ) (Adamson et al., 2009). The 24-hr MPR consisted of 3 passes:

Pass 1: *Quick List*. An initial run through of what the participant had to eat and drink on the previous day recorded without interruption from the interviewer.

Pass 2: *Detailed record.* The participant was asked to provide more details including time and occasion for foods recorded in Pass 1. Portion sizes for each item were ascertained at this point. Prompts were given by the interviewer for additional details.

Pass 3: *Review.* Information recorded was reviewed to ensure the participant was confident that all items have been recorded.

Where possible, food weights were recorded from food packages or food labels or estimated from household measuring utensils. A "Photographic Atlas of Food Portion Sizes" used in the Newcastle 85+ 24hr MPR assessments (Adamson et al., 2009) was also adapted for use in the LiLACS NZ population after a pilot study in Māori and non-Māori found that it lacked several commonly eaten foods in this population (Wham et al., 2012). The Atlas included photographs of commonly eaten foods with eight different portion sizes and equivalent foods. This tool was used when portion sizes could not be ascertained from the participant, packaging, or nutrition labels. The information collected from this assessment was analysed using FOODfiles (2010), an electronic subset of data from the New Zealand Food Composition Database (The New Zealand Institute for Plant & Food Research Limited, 2011).

Iron, folate, and vitamin B_{12} intakes were assessed and dietary inadequacies were determined by comparison to the Nutrient Reference Values for Australia and New Zealand (NHMRC, 2006). Food items reported in the 24-hr MPR were allocated to food groups based on the thirty-three food groups used in the 2008/09 NZANS in order to calculate sources of nutrients by the type of food.

Nutritional Biomarkers

A blood test was collected in Wave 1 by a trained nurse after an overnight fast. Forty mL samples were collected between 7:30 a.m. and 10:30 a.m. from the antecubital fossa vein. Blood analysed for serum ferritin, serum iron, and total iron binding capacity were collected in plain tubes. Blood analysed for haemoglobin, RBC folate, serum folate and serum vitamin B_{12} were collected in ethylene diamine tetraacetic acid (EDTA) tubes. The samples were centrifuged locally within 3-4

hours of collection and sent immediately to the Tauranga PathLab where haemoglobin was tested within 6 hours of collection and RBC folate, serum folate and serum vitamin B_{12} were tested within 5 days. The rest of the samples were stored at -20°C until it could be transported to Auckland LabPlus where it was stored securely at -80°C until the remaining tests were conducted. Serum folate, RBC folate, and serum vitamin B_{12} were determined using chemiluminescence (UniCel DxI 800 Immunoassay System, Beckman Coulter, Inc.). Haemoglobin was determined using photometric measurement (UniCel DxH 800 Coulter Cellular Analysis System, Beckman Coulter, Inc.). Serum iron and unsaturated iron binding capacity (UIBC) was determined using colorimetric assay (Cobas 8000, c702, Roche Diagnostics, USA) and serum ferritin determined by chemiluminescence (Cobas 8000, c602, Roche Diagnostics, USA). Total iron binding capacity was calculated from the addition of the serum iron concentration to UIBC.

Serum ferritin (µg/L), serum iron (µmol/L), transferrin saturation (%), and total iron binding capacity (TIBC) (µmol/L) were used to assess iron status. The cut-off for serum ferritin was defined as <12µg/L, serum iron was defined as <10µmol/L and TIBC >71 µmol/L (Mahan et al., 2012; WHO & CDC, 2007). Transferrin saturation was calculated using the equation [(serum iron / TIBC) x 100] and a cut-off <15% was used (Mahan et al., 2012; WHO & CDC, 2007).

Serum vitamin B_{12} (pmol/L) was used to assess vitamin B_{12} status and the cut-off defined as <148pmol/L (de Benoist, 2008). Red blood cell (RBC) folate (nmol/L) and serum folate (nmol/L) were used to assess folate status. Cut-offs for RBC folate was classified as <317nmol/L and serum folate <6.7nmol/L (de Benoist, 2008; University of Otago & Ministry of Health, 2011b).

Haemoglobin (g/L) was assessed to determine possible anaemia which was defined as <125g/L for men and <118g/L for women based on NHANES III data (Looker et al., 1997).

Statistical Analysis

Most statistical analyses were performed using IBM SPSS statistics package version 23 (SPSS Inc., Chicago IL, USA). Basic descriptive analyses were completed for the number of participants, gender, ethnicity, living situation, housing situation, highest level of education, NZDep score, BMI, denture use, smoking status, alcohol intake, medication usage, and dietary supplement usage. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to assess age, dietary intake of energy, iron, folate and vitamin B_{12} as well as biomarkers (serum ferritin, serum iron, TIBC, Tf-saturation, RBC folate, serum folate, vitamin B_{12}, and haemoglobin) for normality. Non-normally distributed data were log transformed and retested for normality. Log-transformed normal data was expressed as the geometric mean (95th% confidence interval). Non-normally distributed variables were expressed as a median [25th, 75th percentiles]. Mann-Whitney U tests and independent T-tests were used to determine ethnic and gender differences in Māori and non-Māori and men and women for energy intake, iron, folate and vitamin B_{12} and all biomarkers.

Binary logistic regression was used to determine the odds ratio (likelihood) of deficiency in biomarkers according to quartiles of dietary intake when controlling for age, gender and energy intake. Additional factors were added to the model based on the nutrient investigated. Estimated differences from the mean were calculated using generalised linear models controlling for age, gender, energy and supplement intake. Models were produced by SAS 9.3 (SAS Institute Inc., Cary, NC, USA). FOODfiles (2010) (The New Zealand Institute for Plant & Food Research Limited, 2011) was used to analyse energy and micronutrient intake and subsequently transferred to a Microsoft Excel 2013 (Microsoft Corporation) file to determine food sources. A *P* value <0.05 was considered to be statistically significant and all tests were two-tailed.

Results

Participant recruitment is described in Figure 3.1 In wave 1, baseline, 421 Māori and 516 non-Māori were enrolled into the study and blood samples were collected. In wave 2, at 12 month follow-up, a dietary assessment was completed by a total of 216 Māori and 362 non-Māori. Those who completed the dietary assessment did not differ from those who did not with respect to living arrangements, sex, age, or depression status (Hayman et al., 2012). Of those who completed the dietary assessment, haemoglobin was available for 142 Māori and 307 non-Māori; ferritin, serum iron, TIBC and transferrin saturation (Tf-sat) was available for 122 Māori and 260 non-Māori; RBC folate was available for 128 Māori and 282 non-Māori; serum folate was available for 130 Māori and 290 non-Māori; and serum vitamin B_{12} was available for 116 Māori and 242 non-Māori. Table 3.1 provides an overview of the wave 2 participant demographic, physical, and health characteristics and use of dietary supplements by ethnicity and gender.

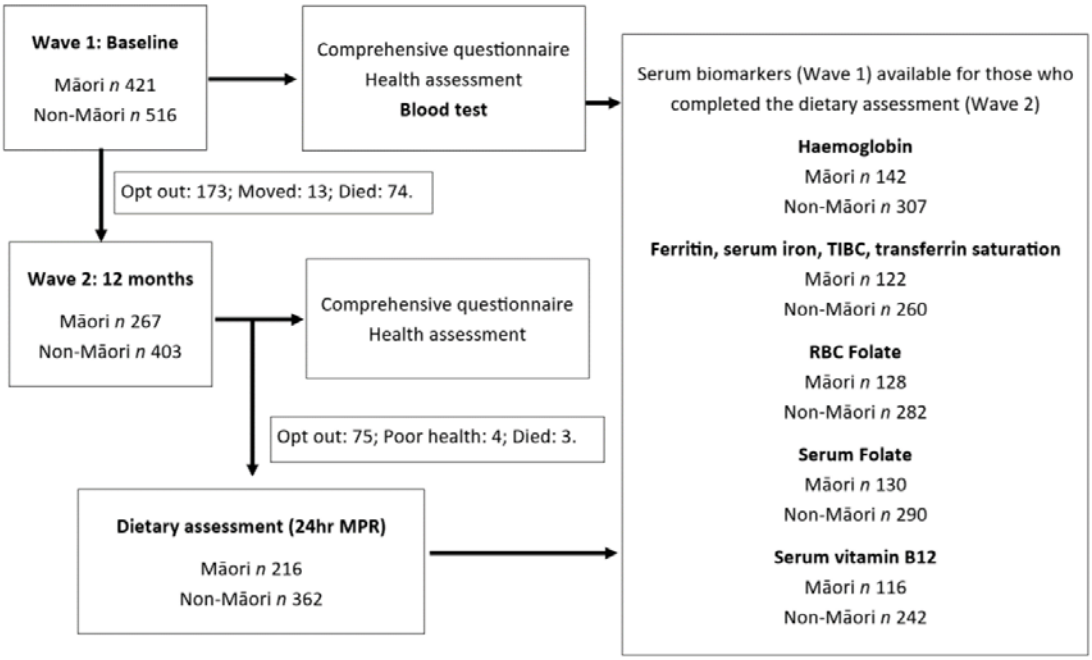


Figure 3.1: Flow chart of the number of participants at baseline, at 12 month follow-up, completion of dietary assessment and availability of serum biomarkers

Māori participants

There were 43% Māori men, median age 82 [81, 85] years and 57% Māori women, median age of 84 [81, 86] years. More Māori women lived alone (51%) compared to Māori men (25%). The majority of Māori lived in a private dwelling or unit (76%). The highest level of education was primary school or no education for more than a third of Māori men (37%) and a quarter of Māori women (25%), however, the majority of Māori men (53%) and Māori women (60%) had a secondary education with or without qualification. Over half of Māori men (59%) and Māori women (62%) had NZ Dep scores >8.

The majority of Māori men (46%) were in the overweight BMI category (25.0 – 29.9kg/m^2) compared to 27% of Māori women. The majority of Māori women (37%) fell into the obese BMI category (>30kg/m^2). Over half of Māori men (52%) and Māori women (62%) used dentures which included upper, lower, partial or full dentures. The majority of Māori men (60%) were former

smokers compared to just over a third (35%) of Māori women. More Māori women (53%) had never smoked compared to just over a third (32%) of Māori men. Almost half of Māori men (42%) and women (48%) reported that they never drank alcohol. Almost a quarter of Māori men (24%) drank alcohol >4 times a week compared to 15% of Māori women. The majority of Māori men (32%) and Māori women (31%) used between 4-6 medications. Proton pump inhibitors were used by over a third of Māori men (32%) and over a quarter of Māori women (26%). More Māori women (28%) reported consuming dietary supplements such as vitamins/minerals or multivitamins/minerals compared to Māori men (22%).

Non-Māori participants

Of non-Māori, 48% were men, median age 86 [85, 86] years and 52% were women, median age 86 [85, 86] years. More than half of all non-Māori women (65%) and over a third of non-Māori men (37%) lived alone. The majority of non-Māori men lived with their spouse (57%) and in a private dwelling or unit (74%). The majority of non-Māori men (51%) and non-Māori women (58%) had a secondary education/without qualification. However, more non-Māori men (19%) had a tertiary education compared to non-Māori women (12%). The majority of non-Māori men (42%) and women (44%) had NZ Dep scores between 5 and 7. Just over a third of non-Māori men (31%) and non-Māori women (33%) were in the most deprived category.

The majority of non-Māori men (47%) belonged to the overweight BMI category. The majority of non-Māori women (37%) belonged to the normal BMI category (18.5 – 24.9kg/m^2), however, almost a quarter of non-Māori women (24%) were also in the obese category (>30kg/m^2). A large proportion of non-Māori men (67%) and women (73%) used dentures. Over half of all non-Māori men (58%) reported that they were former smokers whereas the majority of women (68%) reported that they had never smoked. The majority of non-Māori men (45%) consumed alcohol >4 times per week compared to 17% of non-Māori women. The majority of non-Māori women (40%) reported that they never consumed alcohol. The majority of non-Māori men (35%) and non-Māori women (32%) used between 4-6 medications and a large proportion of non-Māori men (29%) and non-Māori women (36%) consumed at least one type of proton pump inhibitor. Supplements were used by 40% of all non-Māori participants with non-Māori women (49%) being the highest consumers of dietary supplements.

Table 3.1: Participant characteristics, demographics, and physical and health characteristics of Māori and non-Māori by gender.

Participant characteristics		Māori			Non-Māori		
		Total	Men	Women	Total	Men	Women
Number of participants, n (%)		216	92 (43%)	124 (57%)	362	172 (48%)	190 (52%)
Demographics							
Age (years)[1]		83 [81, 85]	82 [81, 85]	84 [81, 86]	86 [85, 86]	86 [85, 86]	86 [85, 86]
Living situation	Alone	73 (34%)	19 (25%)	54 (51%)	181 (50%)	61 (37%)	120 (65%)
	Spouse only	54 (25%)	35 (45%)	19 (18%)	127 (35%)	96 (57%)	31 (17%)
	With others[2]	56 (26%)	23 (30%)	33 (31%)	43 (12%)	10 (6%)	33 (18%)
Housing situation	Private dwelling/unit	165 (76%)	72 (78%)	92 (74%)	260 (72%)	128 (74%)	132 (69%)
	Retirement village	10 (5%)	3 (3%)	7 (6%)	64 (18%)	32 (19%)	32 (17%)
	Rest home/private hospital	5 (2%)	2 (2%)	3 (2%)	20 (6%)	6 (4%)	14 (7%)
	Other[3]	4 (2%)	0	4 (3%)	9 (3%)	3 (2%)	6 (3%)
Highest level of education	Primary or none	65 (30%)	34 (37%)	31 (25%)	63 (17%)	36 (21%)	27 (14%)
	Secondary/no qualification	123 (57%)	49 (53%)	74 (60%)	199 (55%)	88 (51%)	111 (58%)
	Trade/occupation	7 (3%)	4 (4%)	3 (2%)	42 (12%)	16 (9%)	26 (14%)
	Tertiary	16 (7%)	4 (4%)	12 (10%)	55 (15%)	32 (19%)	23 (12%)
NZDep score	1-4 (least)	37 (17%)	12 (13%)	25 (20%)	90 (25%)	46 (27%)	44 (23%)
	5-7	49 (23%)	26 (28%)	23 (19%)	157 (43%)	73 (42%)	84 (44%)
	8-10 (most)	130 (60%)	54 (59%)	76 (62%)	115 (32%)	53 (31%)	62 (33%)
Physical and health characteristics							
BMI (kg/m^2)	Underweight (<18.5)	1 (1%)	0	1 (1%)	6 (2%)	2 (1%)	4 (2%)
	Normal (18.5 – 24.9)	53 (25%)	17 (18%)	36 (29%)	123 (34%)	53 (31%)	70 (37%)
	Overweight (25.0-29.9)	75 (35%)	42 (46%)	33 (27%)	144 (40%)	80 (47%)	64 (34%)
	Obese (>30)	73 (34%)	27 (29%)	46 (37%)	79 (22%)	34 (20%)	45 (24%)
Dentures	None	36 (17%)	18 (20%)	18 (15%)	69 (19%)	37 (22%)	32 (17%)
	Dentures used[4]	125 (58%)	48 (52%)	77 (62%)	253 (70%)	115 (67%)	138 (73%)
Smoking status	Never	92 (43%)	29 (32%)	63 (53%)	192 (53%)	62 (36%)	130 (68%)
	Former	97 (45%)	55 (60%)	42 (35%)	153 (42%)	99 (58%)	54 (28%)
	Current	23 (11%)	8 (9%)	15 (13%)	17 (5%)	11 (6%)	6 (3%)
Alcohol intake	Never	83 (38%)	32 (42%)	51 (48%)	104 (29%)	31 (18%)	73 (40%)
	2-4 times per month or less	51 (24%)	19 (21%)	32 (26%)	103 (28%)	42 (24%)	61 (32%)
	2-3 times per week	14 (7%)	7 (9%)	7 (7%)	38 (11%)	20 (12%)	18 (10%)
	>4 times per week	34 (16%)	18 (24%)	16 (15%)	108 (30%)	76 (45%)	32 (17%)
Medications used[5]	None	36 (17%)	15 (16%)	21 (17%)	14 (4%)	9 (5%)	5 (3%)
	1-3	35 (15%)	14 (15%)	21 (17%)	53 (15%)	29 (17%)	24 (13%)
	4-6	68 (31%)	29 (32%)	39 (31%)	120 (33%)	60 (35%)	60 (32%)
	7-9	44 (20%)	21 (23%)	23 (19%)	97 (27%)	40 (23%)	57 (30%)
	10+	33 (15%)	13 (14%)	20 (16%)	78 (22%)	34 (20%)	44 (23%)
Types of Medications used	Antacids	3 (1%)	3 (3%)	0	3 (1%)	1 (1%)	2 (1%)
	H_2RAs	0	0	0	4 (1%)	1 (1%)	3 (2%)
	PPIs	61 (28%)	29 (32%)	32 (26%)	118 (33%)	50 (29%)	68 (36%)
	NSAIDs	18 (8%)	6 (7%)	11 (9%)	69 (19%)	26 (15%)	43 (23%)
Dietary supplements[6]	None	161 (75%)	72 (78%)	89 (72%)	217 (60%)	120 (70%)	97 (51%)
	Supplements used	55 (25%)	20 (22%)	35 (28%)	145 (40%)	52 (30%)	93 (49%)

Values expressed as number (percentage) unless otherwise specified. [1]Expressed as median [IQR]. [2] Includes spouse, children, relatives and non-relatives; [3] Includes Marae; [4] Includes upper, lower, full and partial dentures; [5]including dietary supplements; [6] includes intake of vitamins, minerals and/or multivitamins/minerals. **N.B.** Missing values indicate participants who completed the dietary assessment but did not answer the respective question in the comprehensive interview. Some questionnaires were incomplete therefore rates of participation will vary. Abbreviations: H_2RAs, Histamine-2 Receptor Antagonists; PPIs, proton pump inhibitors; NSAIDs, non-steroidal anti-inflammatory drugs.

Dietary intake and adequacy

Energy, iron, folate and vitamin B_{12} intake for Māori and non-Māori

Table 3.2 provides details on intake of energy, iron, folate and vitamin B_{12} in Māori and non-Māori. Overall, energy intake was significantly higher for Māori men (7.5 MJ/d) compared to Māori women (6.0 MJ/d) ($p<0.001$). Energy intake was also significantly higher for non-Māori men (7.9MJ/d) compared to non-Māori women (6.3 MJ/d) ($p<0.001$).

Folate intake per MJ was significantly higher for Māori women (44.37 µg/MJ) compared to Māori men (36.78 µg/MJ) ($p=0.019$). Intake of iron and vitamin B_{12} per MJ of energy did not differ significantly between men and women within their respective ethnic group.

Overall, Māori consumed significantly less energy (6.4 MJ/d) compared to non-Māori (7.1 MJ/d) ($p=0.001$) but had a significantly higher intake of vitamin B_{12} per MJ of energy (0.46 µg/MJ) compared to non-Māori (0.43 µg/MJ) ($p=0.038$). Intake of iron and folate per MJ of energy did not differ significantly between Māori and non-Māori.

Adequacy of iron, folate and vitamin B_{12} intake

The adequacy of dietary intake in relation to NRV's are also reported in Table 3.2. A small proportion of Māori men (12%) and women (11%) and non-Māori men (2%) and women (8%) did not meet the EAR for iron intake. A larger proportion of Māori men (58%), Māori women (58%), non-Māori men (43%) and non-Māori women (59%) did not meet the EAR for folate. Over a third of Māori women (37%) and non-Māori women (30%) did not meet the EAR for vitamin B_{12}, compared with 13% of Māori men and 12% of non-Māori men.

Biomarker status

Iron, folate and vitamin B_{12}

Biomarkers for iron, folate and vitamin B_{12} are reported in Table 3.2. Māori women had a significantly higher total iron binding capacity [57 (52, 67)] in comparison to Māori men [56 (51, 62)] (p=0.015). Serum ferritin was higher in Māori men [257 (127, 369)] compared to Māori women [172 (84, 304)] (p=0.015).

Haemoglobin levels were found to be significantly higher in non-Māori men [138 (127, 144)] compared to non-Māori women [129 (123, 138)] (p<0.001). Similarly, non-Māori men had a significantly higher ferritin level [179 (97, 361)] compared to non-Māori women [107 (71, 178)] (p<0.001). Non-Māori women had a significantly higher total iron binding capacity [57 (53, 65)] compared to non-Māori men [52 (48, 57)] (p<0.001). However, non-Māori men had a significantly higher transferrin saturation level [32 (24, 39)] compared to non-Māori women [26 (22, 32)] (p=0.001).

Overall, ferritin concentrations were significantly higher in Māori [188 (94, 340)] compared to non-Māori [122 (77, 224)] (p=0.002). Similarly, serum vitamin B_{12} concentrations were significantly higher in Māori (261, [95% CI=227, 299]) than in non-Māori (222 [95%CI= 207, 239]) (p=0.026). There were no other significant differences found between Māori and non-Māori for the other biomarkers assessed.

Biomarkers for iron, folate and vitamin B_{12} in relation to recognised cut-offs

Biomarkers in relation to recognised cut-offs are reported in Table 3.2. Approximately 11% of Māori men, 4% of Māori women, 16% of non-Māori men and 9% of non-Māori women were below their respective haemoglobin reference ranges. There were no Māori and only 1% of non-Māori below the cut-off for serum ferritin. Few participants had low serum iron (Māori: 3% men, 4% women; non-Māori: 5% men, 5% women) and transferrin saturation (Māori: 4% men, 5% women; non-Māori: 6% men, 6% women). Few participants exceeded the cut-off for TIBC (Māori: 2% men, 7% women; non-Māori: 3% men, 2% women).

Approximately a quarter of Māori men (24%) and up to a third of Māori women (29%), non-Māori men (31%) and non-Māori women (30%) were found to have a red blood cell folate level below the reference range (<317nmol/L). There was no serum folate deficiency among Māori men and women, and only 2% of non-Māori men and women were below the reference range (6.7nmol/L). Serum vitamin B_{12} deficiency was seen in 7% of Māori men and women, and 11% of non-Māori men and 8% of non-Māori women.

Table 3.2: Iron, folate and B_{12}: Dietary intakes and adequacy in relation to NRVs, nutritional biomarkers in relation to cut-offs, and top food contributors for Māori and non-Māori men and women.

	Māori			P-value¥	Non-Māori			P-value◊	P-value*
	Total	Men	Women		Total	Men	Women		
Energy Intake (MJ)[1]	6.4 [5.2, 8.2]	7.5 [6.1, 9.1]	6.0 [4.8, 7.2]	**<0.001**	7.1 [5.8, 8.7]	7.9 [6.7, 9.6]	6.3 [5.3, 7.5]	**<0.001**	**<0.001**
Iron									
Intake (mg/d)[1]	9.7 [7.1, 13.1]	11.2 [7.8, 14.4]	8.9 [6.7, 11.9]	-	10.6 [8.1, 13.3]	11.6 [9.9, 14.3]	9.3 [7.1, 11.7]	-	-
Intake per 1 MJ (mg)	1.47	1.43	1.54	0.376	1.44	1.44	1.42	0.692	0.391
<EAR (n, %)[a]	25 (12%)	11 (12%)	14 (11%)	-	18 (5%)	3 (2%)	15 (8%)	-	-
Haemoglobin (g/L)[1]	137 [128, 144]	137 [126, 146]	134 [127, 143]	0.197	136 [126, 143]	138 [127, 144]	129 [123, 138]	**<0.001**	0.167
Hb <125 g/L (men); or <118 g/L (women)	15 (7%)	10 (11%)	5 (4%)	-	45 (12%)	28 (16%)	17 (9%)	-	-
Ferritin	188 [94, 340]	257 [127, 369]	172 [84, 304]	**0.015**	122 [77, 224]	179 [97, 361]	107 [71, 178]	**<0.001**	**0.002**
<12 (µg/L)	0	0	0		1 (1%)	1 (1%)	0		
Serum iron (µmol/L)[1]	15 [11, 18]	14 [11, 17]	15 [12, 20]	0.544	15 [12,18]	18 [12, 19]	15 [12,17]	0.146	0.882
<10 µmol/L	8 (4%)	3 (3%)	5 (4%)	-	18 (5%)	8 (5%)	10 (5%)	-	-
TIBC (µmol/L)[1]	57 [52, 65]	56 [51, 62]	57 [52, 67]	**0.015**	56 [50, 63]	52 [48, 57]	57 [53, 65]	**<0.001**	0.080
>71 µmol/L	10 (5%)	2 (2%)	8 (7%)	-	9 (3%)	5 (3%)	4 (2%)	-	-
Tf-sat (%)[1]	28 [21, 34]	28 [22, 35]	28 [21, 35]	0.545	28 [22, 34]	32 [24, 39]	26 [22, 32]	**0.001**	0.544
<15%	10 (5%)	4 (4%)	6 (5%)	-	21 (6%)	10 (6%)	11 (6%)	-	-
Top Food Contributors	-	Cereals (18%), bread (12%), beef and veal (8%)	Cereals (17%), bread (15%), vegetables (8%)	-	-	Cereals (19%), bread (14%), beef and veal (9%)	Cereals (15%), bread (15%), vegetables (9%)	-	-
Folate									
Intake (µg/d)[1]	279 [191, 393]	298 [205, 391]	268 [186 ,397]	-	313 [224, 447]	341 [253, 479]	290 [202, 407]	-	-
Intake per 1 MJ (µg)	42.15	36.78	44.37	**0.019**	44.31	42.22	45.61	0.304	0.417
<EAR (n, %)[b]	125 (58%)	53 (58%)	72 (58%)	-	186 (51%)	74 (43%)	112 (59%)	-	-
Serum folate (nmol/L)[2]	18 (16 – 20)	18 (16 – 20)	19 (17 – 21)	0.110	19 (17 – 20)	17 (14 – 20)	19 (16 – 21)	0.480	0.430
<6.7nmol/L	0	0	0	-	6 (2%)	3 (2%)	3 (2%)	-	-
RBC folate (nmol/L)[2]	338 (302 – 379)	333 (298 – 372)	338 (315 – 363)	0.635	336 (317 – 357)	322 (261 – 398)	348 (303, 401)	0.866	0.228
<317nmol/L	58 (27%)	22 (24%)	26 (29%)	-	111 (31%)	54 (31%)	57 (30%)		-
Top Food Contributors	-	Cereals (20%), vegetables (15%), bread (14%)	Cereals (18%), bread (16%), vegetables (16%)	-	-	Cereals (19%), bread (16%), vegetables (14%)	Vegetables (16%), bread (15%), cereals (14%)	-	-
Vitamin B_{12}									
Intake (µg/d)[1]	3.0 [1.9, 4.4]	3.4 [2.5, 5.1]	2.7 [1.7, 3.9]	-	3.03 [2.1, 4.2]	3.6 [2.4, 4.8]	2.6 [1.8, 3.6]	-	-
Intake per 1 MJ (µg)	0.46	0.48	0.45	0.198	0.43	0.45	0.42	0.370	**0.038**
<EAR (n, %)[c]	55 (26%)	12 (13%)	43 (37%)	-	78 (22%)	21 (12%)	57 (30%)	-	-
Serum vitamin B_{12} (pmol/L)[2]	261 (227 – 299)	212 (188 - 239)	229 (209 – 251)	0.359	222 (207 – 239)	253 (196 – 363)	265 (224 – 315)	0.770	**0.026**
<148pmol/L	15 (7%)	6 (7%)	9 (7%)	-	34 (9%)	19 (11%)	15 (8%)	-	-
Top Food Contributors	-	Milk (20%), beef and veal (17%), fish and seafood (16%)	Milk (24%), fish and seafood (16%), beef and veal (15%)	-	-	Milk (23%), beef and veal (21%), fish and seafood (12%)	Milk (24%), beef and veal (16%), fish and seafood (9%)	-	-

[1] Expressed as median [IQR]; [2] Expressed as geometric mean and 95% confidence interval; [a] Based on an EAR 6mg/d for men and 5mg/d for women aged >70 years; [b] Based on EAR 320µg/d for men and women aged >70 years; [c] Based on an EAR of 2.0µg/d for men and women aged >70 years. ◊ Differences between Māori men and women (Independent samples T-test, Mann-Whitney U Test [two-tailed]); ¥ Differences between non-Māori men and women (Independent samples T-test, Mann-Whitney U Test); *Differences between Māori and non-Māori participants (Independent samples T-test, Mann-Whitney U Test). P-value <0.05 considered significant. Abbreviations: MJ, mega-joules; EAR, estimated average intake; Hb, haemoglobin; TIBC, total iron binding capacity; Tf-sat, transferrin saturation; RBC, red blood cell.

Food Sources

Food sources contributing to dietary iron intake

Food sources contributing to the majority of iron intake in Māori men included cereals (18%), bread (12%), and beef and veal (8%). For Māori women, the top three sources of iron included cereals (17%), bread (15%) and vegetables (8%) with beef and veal as the fourth highest source (8%). Non-Māori men had the majority of their iron from cereals (19%), bread (14%), and beef and veal (9%). Non-Māori women had the majority of their iron from cereals (15%), bread (15%) and vegetables (9%). Other dietary sources of iron includes non-alcoholic beverages, fish and seafood, fruit, pork, potato/kumara/taro, and savoury sauces and condiments (Figure 3.2).

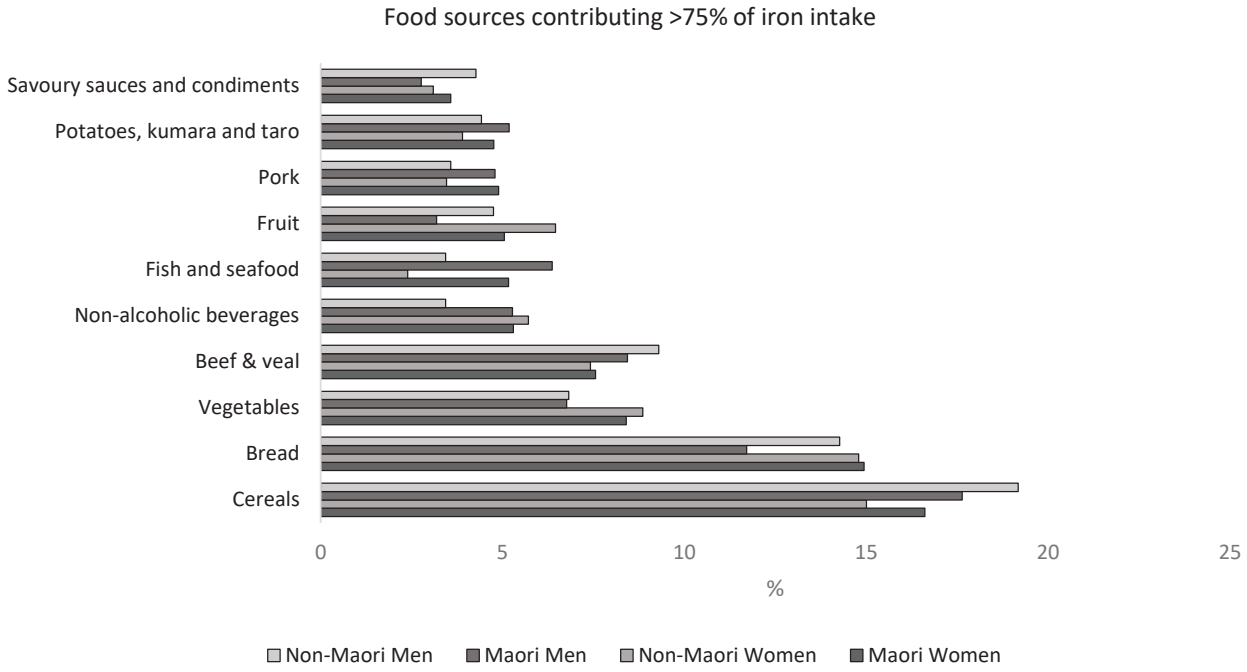


Figure 3.2: Percentage of food groups contributing to >75% of dietary iron intake according to ethnicity and gender.

Food sources contributing to dietary folate intake

Food sources contributing the greatest amount of dietary folate for Māori men and women, respectively, were cereals (20%; 18%), vegetables (15%; 16%) and bread (14%; 16%). Major sources for non-Māori men were cereals (19%), bread (16%) and vegetables (14%). Non-Māori women had the majority of their dietary folate from vegetables (16%), followed by bread (15%) and cereals (14%). Other food sources contributing to the majority of the dietary intake of folate included milk, fruit, potatoes, kumara and taro, and egg/egg dishes (Figure 3.3).

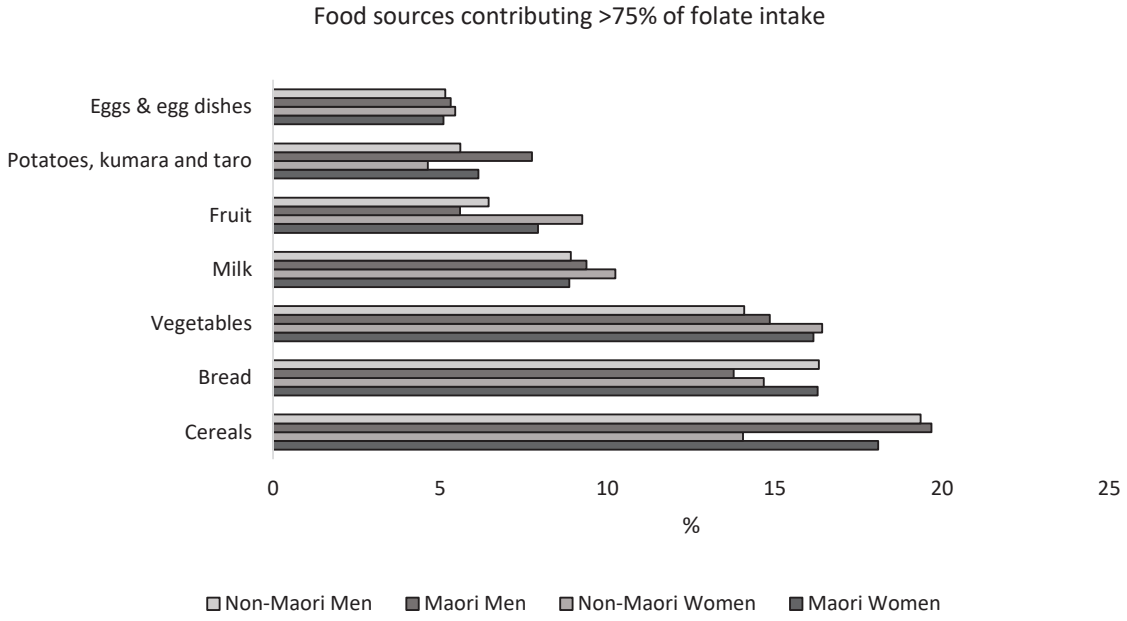


Figure 3.3: Percentage of food groups contributing to >75% of dietary folate intake according to ethnicity and gender.

Food sources contributing to dietary vitamin B_{12} intake

Food sources contributing the greatest amount of vitamin B_{12} for Māori men and women, respectively, were milk (20%; 24%), fish and seafood (16%; 16%), and beef and veal (17%; 15%). Similarly, the majority of vitamin B_{12} for non-Māori men and women, respectively, came from milk (23%; 24%), fish and seafood (12%; 9%), and beef and veal (21%; 16%). Other dietary sources contributing to the majority of vitamin B_{12} intake in this cohort included egg/egg dishes, lamb and mutton, poultry and cheese (Figure 3.4).

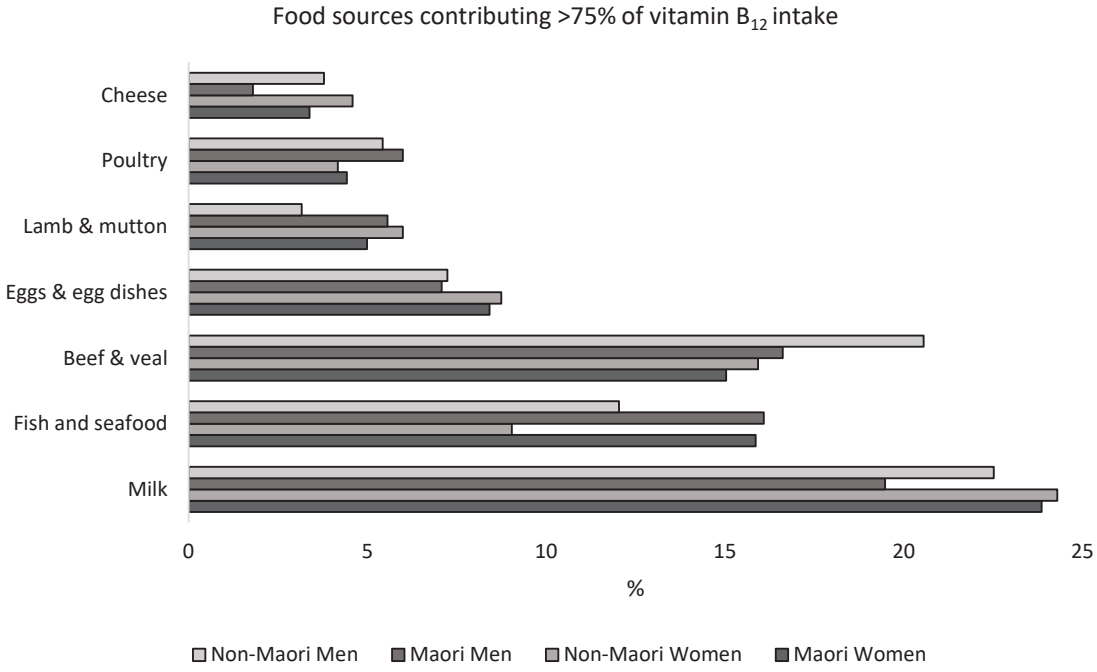
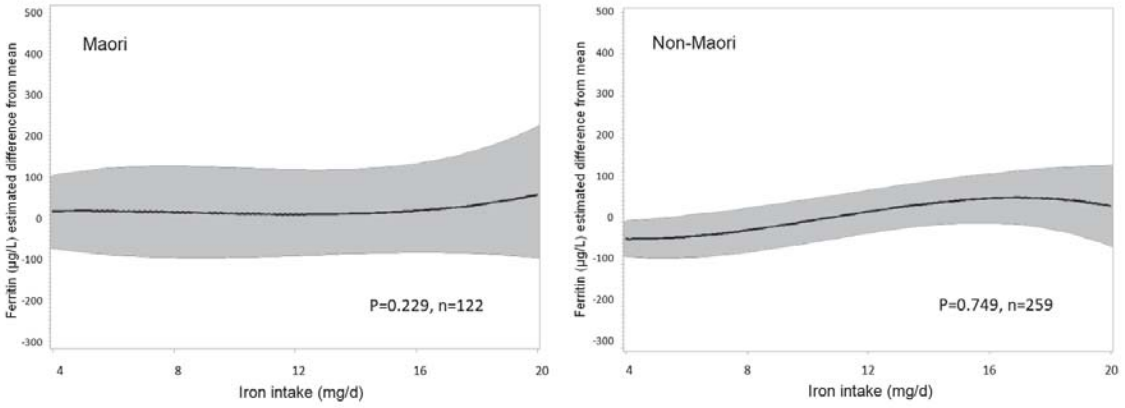


Figure 3.4: Percentage of food groups contributing to >75% of dietary vitamin B_{12} intake according to ethnicity and gender.

Association between dietary intake and biomarker status

Association between dietary iron intake and ferritin

Iron intake is not a significant predictor of serum ferritin concentration in Māori (p=0.229) (Figure 3.5). There is a positive trend for serum ferritin concentration with increasing iron intake in non-Māori participants, however, this result is not significant (p=0.749).

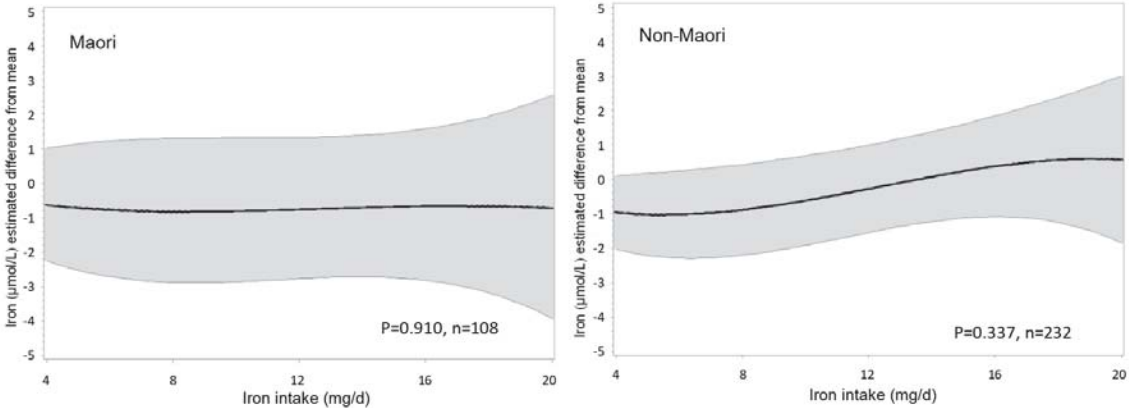


Estimated difference from the mean (and 95% CI) of serum ferritin concentration (µg/L) according to total iron intake for Māori and non-Māori. Estimates were calculated using generalised linear models controlling for age, gender, energy intake, and supplement intake. P-values are from the corresponding model on the ability of dietary intake to predict serum ferritin concentration. P-value <0.05 considered significant.

Figure 3.5: Association between dietary iron intake (mg/d) from all food sources including supplements and serum ferritin concentration (µg/L) in Māori and non-Māori.

Association between dietary iron intake and serum iron

Iron intake is not a significant predictor of serum iron levels in Māori (p=0.910) (Figure 3.6). There is a positive trend for serum iron with increasing iron intake in non-Māori participants, however, this result is not significant (p=0.337).

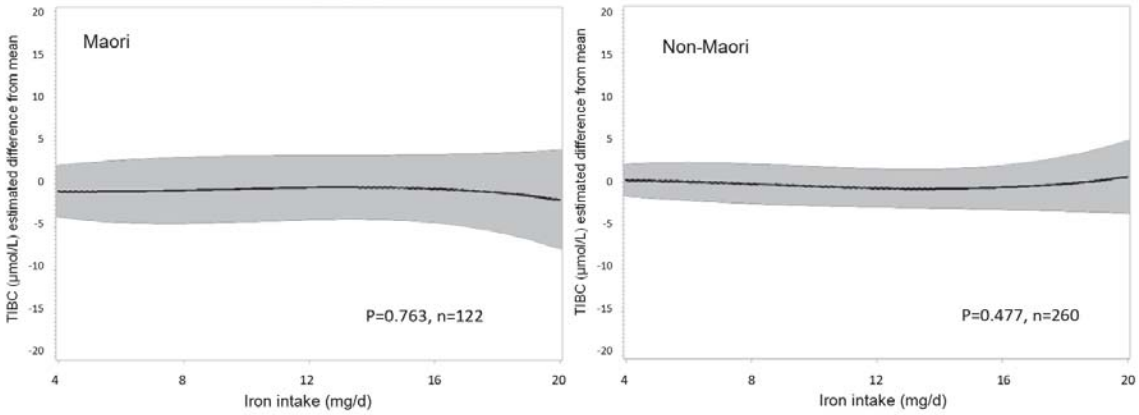


Estimated difference from the mean (and 95% CI) of serum iron concentration (µmol/L) according to total iron intake for Māori and non-Māori. Estimates were calculated using generalised linear models controlling for age, gender, energy intake, and supplement intake. P-values are from the corresponding model on the ability of dietary intake to predict serum iron concentration. P-value <0.05 considered significant.

Figure 3.6: Association between dietary iron intake (mg/d) from all food sources including supplements and serum iron concentration (µmol/L) in Māori and non-Māori.

Association between dietary iron intake and total iron binding capacity

Iron intake is not a significant predictor of total iron binding capacity in Māori (p=0.763) and non-Māori (p=0.477). The models do not show any obvious trends (Figure 3.7).

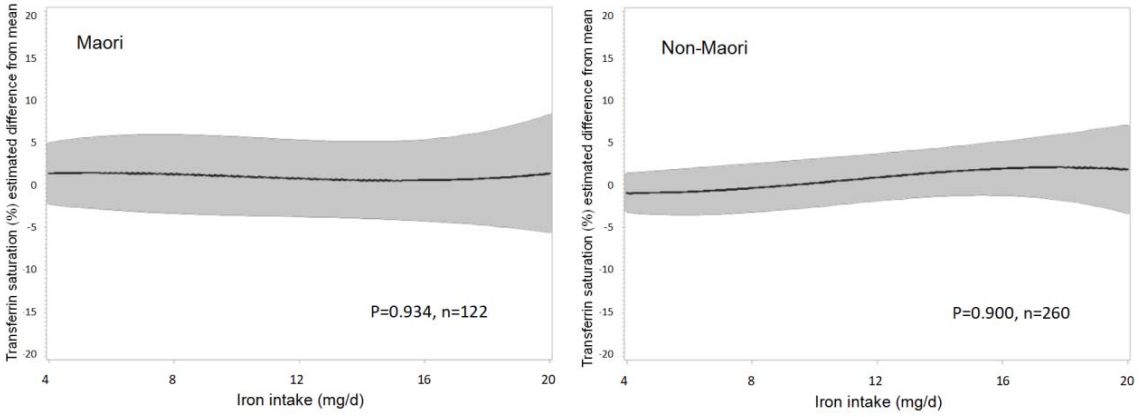


Estimated difference from the mean (and 95% CI) of total iron binding capacity (µmol/L) according to total iron intake for Māori and non-Māori. Estimates were calculated using generalised linear models controlling for age, gender, energy intake, and supplement intake. P-values are from the corresponding model on the ability of dietary intake to predict total iron binding capacity. P-value <0.05 considered significant.

Figure 3.7: Association between dietary iron intake (mg/d) from all food sources including supplements and total iron binding capacity (µmol/L) in Māori and non-Māori.

Association between dietary iron intake and transferrin saturation

Iron intake is not a significant predictor of transferrin saturation in Māori (0=0.934) and non-Māori (p=0.900). The models do not show any obvious trends (Figure 3.8).

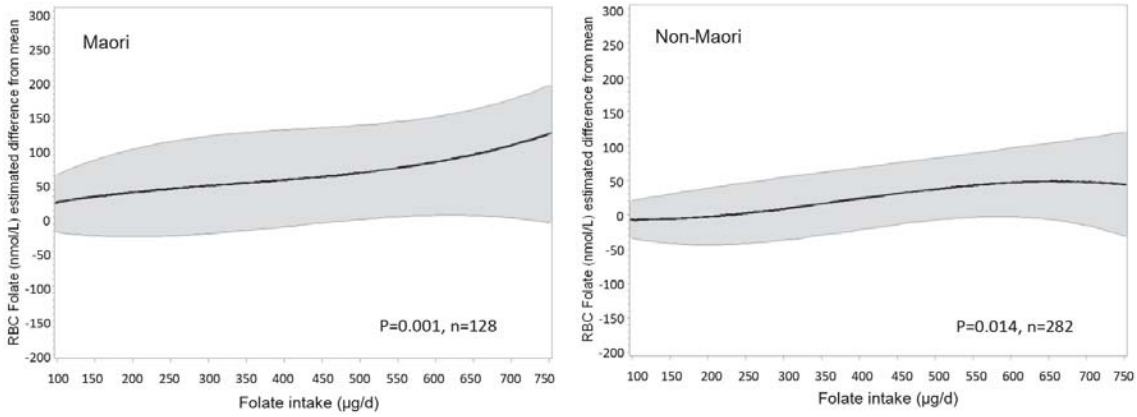


Estimated difference from the mean (and 95% CI) of transferrin saturation (%) according to total iron intake for Māori and non-Māori. Estimates were calculated using generalised linear models controlling for age, gender, energy intake, and supplement intake. P-values are from the corresponding model on the ability of dietary intake to predict serum transferrin concentration. P-value <0.05 considered significant.

Figure 3.8: Association between dietary iron intake (mg/d) from all food sources including supplements and transferrin saturation (%) in Māori and non-Māori.

Association between dietary folate intake and RBC folate

Dietary folate intake is significant predictor of RBC folate levels in both Māori (p=0.001) and non-Māori (p=0.014) when age, gender, energy intake, supplements and intake of folic acid are controlled for (Figure 3.9). Māori and non-Māori participants are more likely to have a higher RBC folate concentration if their folate intake is higher.

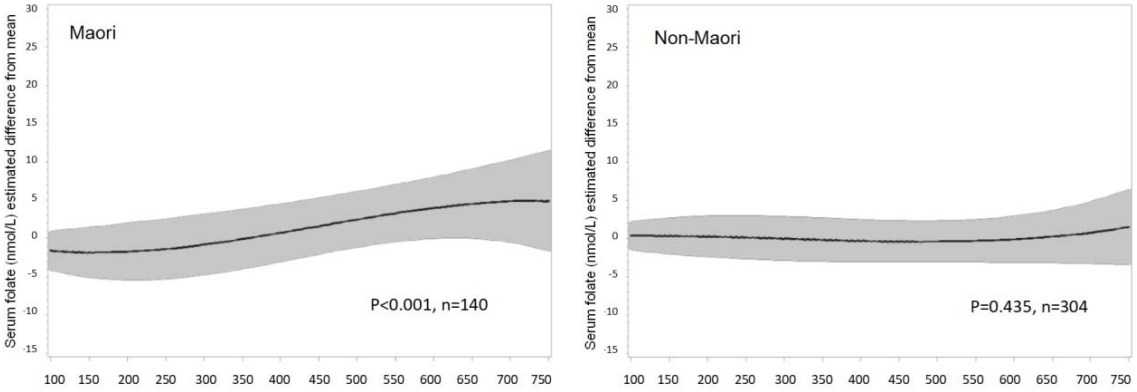


Estimated difference from the mean (and 95% CI) of RBC folate concentration (nmol/L) according to total folate intake for Māori and non-Māori. Estimates were calculated using generalised linear models controlling for age, gender, energy intake, supplement intake, and intake of folic acid. P-values are from the corresponding model on the ability of dietary intake to predict red blood cell folate. P-value <0.05 considered significant.

Figure 3.9: Association between dietary folate intake (µg/d) from all food sources including supplements and red blood cell folate concentration (nmol/L) in Māori and non-Māori.

Association between dietary folate intake and serum folate concentration

Dietary folate intake was a significant predictor of serum folate concentration in Māori (p<0.001) (Figure 3.10). Māori are more likely to have higher serum folate levels when dietary intake of folate is higher. The same significant trend was not seen for non-Māori (p=0.435).

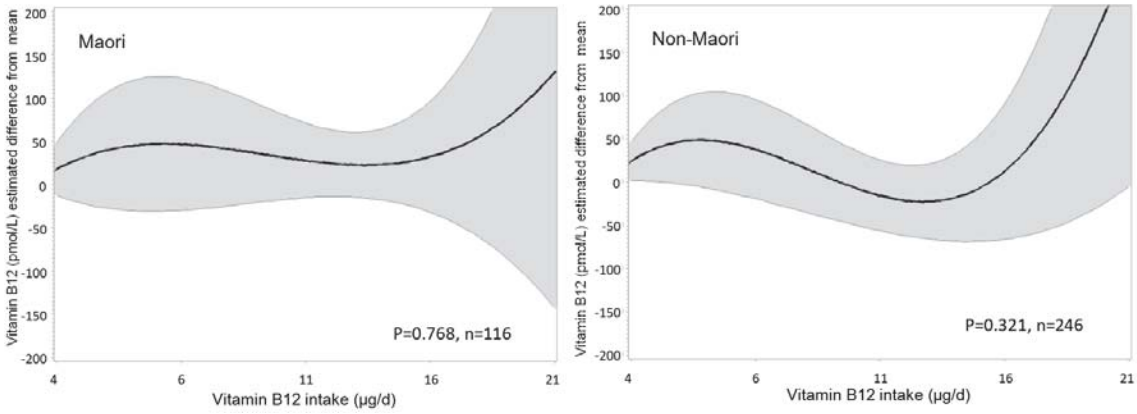


Estimated difference from the mean (and 95% CI) of serum folate concentration (nmol/L) according to total folate intake for Māori and non-Māori. Estimates were calculated using generalised linear models controlling for age, gender, energy intake, and supplement intake. P-values are from the corresponding model on the ability of dietary intake to predict serum folate concentration (nmol/L). P-value <0.05 considered significant.

Figure 3.10: Association between dietary folate intake (µg/d) from all food sources including supplements and serum folate concentration (nmol/L) in Māori and non-Māori.

Association between vitamin B_{12} intake and serum vitamin B_{12} concentration

Both the models for Māori and non-Māori show a trend of increasing serum vitamin B_{12} concentrations as vitamin B_{12} intake increases, however, the result is not statistically significant for Māori (p=0.768) and non-Māori (p=0.321) (Figure 3.11).



Estimated difference from the mean (and 95% CI) of serum vitamin B_{12} concentration (pmol/L) according to total vitamin B_{12} intake for Māori and non-Māori. Estimates were calculated using generalised linear models controlling for age, gender, energy intake, and supplement intake. P-values are from the corresponding model on the ability of dietary intake to predict serum vitamin B_{12} concentration. P-value <0.05 considered significant.

Figure 3.11: Association between dietary vitamin B_{12} intake (µg/d) from all food sources including supplements and serum vitamin B_{12} concentration (pmol/L) in Māori and non-Māori.

Risk of iron, folate or vitamin B_{12} deficiency by quartiles of dietary intake

Table 3.3 shows the odds ratio and 95% CI of deficient RBC folate (<317nmol/L) according to total folate intake quartiles. In Māori, as intake of folate increased, the odds of having low RBC folate decreased. This was significant at all intakes >215 µg /d. Similarly, when adjusted, the same significant trend was found. In the unadjusted model, non-Māori individuals with an intake >440 µg /d were significantly less likely to be deficient in RBC folate when compared to individuals with an intake <215µg/d. However, when adjusted for gender, energy intake, nutritional supplement usage and intake of folic acid, a similar but not significant trend was found.

There were no significant associations between the dietary intake of iron and vitamin B_{12} and their respective biomarkers, serum iron and serum vitamin B_{12}. (Refer to appendix B, table 5.4 for the association between dietary intake and other biomarkers of iron and folate)

Table 3.3: Binary logistic regression, odds ratio of deficiency: Likelihood of being deficient in serum iron, serum vitamin B_{12} or RBC folate in Māori and non-Māori according to quartiles of dietary intake of iron (mg/d), vitamin B_{12} (µg/d) or folate (µg/d).

	Māori				Non-Māori			
Iron intake (mg/d)	**Model 1 (unadjusted)** (n=8)	*p*	**Model 2 (adjusted)***	*p*	**Model 1 (unadjusted)** (n=18)	*p*	**Model 2 (adjusted)***	*P*
<7.75	1.00 (ref.)	-	1.00 (ref.)	-	1.00 (ref.)	-	1.00 (ref.)	-
7.75 – 10.42	0.43 [0.04, 4.39]	0.478	0.41 [0.04, 4.34]	0.458	0.63 [0.16, 2.45]	0.500	0.82 [0.19, 3.60]	0.789
10.43 – 13.27	0.75 [0.12, 4.80]	0.769	0.59 [0.08, 4.58]	0.617	0.29 [0.06, 1.58]	0153	0.49 [0.07, 3.32]	0.465
>13.27	1.11 [0.17, 7.20]	0.912	0.76 [0.07, 8.41]	0.820	1.17 [0.35, 3.90]	0.802	2.33 [0.46, 11.9]	0.311
Folate Intake (µg/d)	**Model 1 (unadjusted)** (n=58)	*p*	**Model 2 (adjusted)¥**	*p*	**Model 1 (unadjusted)** (n=111)	*p*	**Model 2 (adjusted)¥**	*p*
<215	1.00 (ref.)	-	1.00 (ref.)	-	1.00 (ref.)	-	1.00 (ref.)	-
215 – 304	0.16 [0.06, 0.47]	0.001	0.16 [0.05, 0.47]	0.001	0.91 [0.45, 1.81]	0.781	0.90 [0.44, 1.86]	0.774
305 – 440	0.19 [0.06, 0.55]	0.002	0.13 [0.04, 0.43]	0.001	0.61 [0.31, 1.23]	0.166	0.60 [0.27, 1.31]	0.201
>440	0.10 [0.03, 0.32]	<0.001	0.06 [0.01, 0.34]	0.001	0.45 [0.22, 0.91]	0.027	0.59 [0.20, 1.69]	0.325
Vitamin B_{12} intake (µg/d)	**Model 1 (unadjusted)** (n=15)	*p*	**Model 2 (adjusted)◊**	*p*	**Model 1 (unadjusted)** (n=34)	*P*	**Model 2 (adjusted)◊**	*p*
<2.07	1.00 (ref.)	-	1.00 (ref.)	-	1.00 (ref.)	-	1.00 (ref)	-
2.07 – 3.03	0.46 [0.08, 2.60]	0.378	0.39 [0.07, 2.28]	0.293	1.61 [0.56, 4.67]	0.379	1.41 [0.47, 4.21]	0.536
3.04 – 4.24	0.77 [0.18, 3.21]	0.717	0.57 [0.12, 2.65]	0.472	1.70 [0.58, 5.03]	0.338	1.33 [0.41, 4.29]	0.639
>4.24	0.62 [0.15, 2.56]	0.508	0.41]0.08, 2.14]	0.293	1.21 [0.38, 3.87]	0.743	0.89 [0.24, 3.31]	0.862

Values expressed as odds ratio [95% confidence interval]. P-value <0.05 considered to be significant.

Deficient serum iron concentration considered to be <10µmol/L; Deficient RBC folate concentration considered to be <317µg; Deficient serum vitamin B_{12} concentration considered to be <148µg.

*Model 2 adjusted for sex, age, energy intake and nutritional supplement intake.

¥Model 2 adjusted for sex, age, energy intake, nutritional supplement usage and intake of folic acid.

◊Model 2 adjusted for sex, age, energy intake, and intake of proton pump inhibitors, histamine-2 receptor antagonists, antacids and nutritional supplements.

Discussion

Iron

In the present study, most participants had an adequate dietary iron intake (88% Māori; 95% non-Māori met the EAR). Inadequate dietary iron intake was evident in a larger proportion of Māori (12% men; 8% women) and non-Māori (2% men; 8% women), compared to <3% of adults aged 71+ reported to have intakes below the EAR in the NZANS 2008/09 (University of Otago & Ministry of Health, 2011a). Given the dietary assessment method was similar in the NZANS 2008/09 (University of Otago & Ministry of Health, 2011b), and iron intake was similar between adults aged 71+ (men, 11.4mg/day; women, 8.9mg/day) and participants in the present study (Māori, 9.7mg/day; non-Māori, 10.6mg/day), the discrepancies may arise as a result of the probability analysis used in the NZANS 2008/09 to estimate inadequate dietary intake. This method may have led to an overestimation of dietary adequacy in older adults, as opposed to the direct comparison to the EAR as used in the present study (University of Otago & Ministry of Health, 2011b).

Only one participant had a low serum ferritin, similar to findings in the NZANS 2008/09 where less than 2% of adults aged 71+ had low ferritin (University of Otago & Ministry of Health, 2011a). As serum ferritin concentration may increase with age and inflammation, it has been proposed that a higher cut-off be used to assess deficiency (Babaei et al., 2017; Choi et al., 2005; Fairweather-Tait et al., 2014). Although not observed in the current study, previous reports have demonstrated a positive correlation between dietary iron intake and serum ferritin in octogenarians (Doyle et al., 1999; Milman et al., 2004). We found the majority of participants had adequate levels of serum iron (96% Māori; 95% non-Māori), TIBC (95% Māori; 97% non-Māori) and transferrin saturation (95% Māori; 94% non-Māori). Lower dietary iron intakes have been correlated with lower serum iron concentration and higher TIBC in this age group (Milman et al., 1990). However, dietary iron intake was not a significant predictor of serum ferritin, serum iron, TIBC, and transferrin saturation in the present study.

This may reflect the underlying limitations in assessment of iron biomarkers. Dietary iron intake is subject to issues with bioavailability (Fairweather-Tait & Teucher, 2002). In particular, haem-iron derived from animal sources is more bioavailable and less affected by dietary factors such as phytates, polyphenols, calcium and existing iron stores, compared to non-haem iron derived from plant sources and fortified foods (Fairweather-Tait & Teucher, 2002; López & Martos, 2004). It is estimated that >15% of haem iron is absorbed versus <5% of non-haem iron (López & Martos, 2004). Therefore, dietary iron intake may not be reflected in biomarker serum levels. Inflammation may also influence ferritin, serum iron, TIBC, and transferrin saturation (Fairweather-Tait et al., 2014; WHO & CDC, 2007); hence markers may be difficult to interpret in the absence of inflammatory status. This is particularly relevant to this age group where chronic low-grade inflammation may be present (den Elzen et al., 2013). As serum iron and transferrin saturation may also be subject to diurnal variation (WHO, 2001), this may further explain why the iron biomarkers were unrelated to dietary intake.

Men (both Māori and non-Māori) had significantly higher serum ferritin and lower TIBC concentrations compared to women which suggests the male participants in this study may have had larger iron stores than women. Men derived more iron from cereals, bread, and beef and veal whereas women had more iron from non-haem sources such as cereals, bread, and vegetables. Select cereals and bread have been fortified with iron, however, bioavailability may be reduced due

to the presence of phytates and fibre (Fairweather-Tait & Teucher, 2002; Hurrell & Egli, 2010). Therefore, the higher intake of haem-iron from beef and veal among men may explain why their iron stores were larger. This may also reflect men traditionally having higher iron stores and energy intakes compared to women (Garry et al., 2000; University of Otago & Ministry of Health, 2011a; Wham et al., 2016b).

Folate

Less than half of all participants met the EAR for folate (42% Māori; 49% non-Māori) and up to a third of participants were deficient in RBC folate (27% Māori; 31% non-Māori). Increased dietary folate intake was significantly correlated with increased RBC folate concentration for both Māori and non-Māori and with increased serum folate concentrations for Māori only. As RBC folate is a marker of long-term folate status and a large proportion of participants appeared deficient, it could indicate that consumption of folate-rich foods is inadequate, given that dietary intake is a major influencer of folate biomarkers (EFSA NDA Panel, 2015; Green, 2011). Food-folate bioavailability may also be a factor as it is well known that synthetic folic acid, used in the fortification of bread and cereals, is more bioavailable (~85%) than folate found naturally in foods (~50%) (Allen, 2008; Brouwer et al., 2001).

In the Newcastle 85+ study, higher intake of cereal and cereal products were significantly and positively correlated with RBC folate concentrations in octogenarians, however, the same was not seen for higher intakes of vegetables (Mendonça et al., 2016). Cereals and bread, subject to voluntary fortification in New Zealand, made up the majority of dietary folate intake for both Māori and non-Māori which may explain the significant association between dietary intake and RBC folate. Further, Māori individuals with a higher intake of folate (>215µg/day) were significantly less likely to be deficient in RBC folate compared to those who had intakes <215µg/day. This trend was also observed in the Newcastle 85+ study where the likelihood of deficiency in RBC folate was more than halved when dietary intake of folate was >264µg/day compared to dietary intakes of <157µg/day (Mendonça et al., 2016).

There may be a decline in dietary intakes of folate-rich fruit and vegetables with ageing (Chen et al., 2005) as a result of declining oral health and poor chewing ability (Holmes & Roberts, 2009). Furthermore, cooking methods employed to make food more palatable can negatively affect the folate content of the foods eaten where up to 80% of folate in vegetables can be destroyed through boiling (Allen, 2008; Holmes & Roberts, 2009). Incomplete liberation from cellular structures in the food matrix, and other dietary components, including ascorbic acid, have also been shown to influence absorption (Brouwer et al., 2001; Caudill, 2010; EFSA NDA Panel, 2015). These factors may explain the higher prevalence of inadequate dietary intake and RBC folate deficiency in the present study, compared to the 2008/09 NZANS where <3% of adults aged 71+ and Māori aged 51+ had deficient RBC folate concentrations (University of Otago & Ministry of Health, 2011a). Serum folate deficiency in the present study was rare (0% Māori; 2% non-Māori), similar to the findings from the 2008/09 NZANS where <2% of adults aged 71+ and Māori aged 51+ were below the cut-off (6.7nmol/L) (University of Otago & Ministry of Health, 2011a). However, serum folate is a measure of short-term folate status and can also be affected by recent dietary intake which may explain the discrepancies between RBC folate and serum folate (Devalia et al., 2014).

Dietary folate intake was assessed in the NZNNS 1997 where the prevalence of inadequate dietary intake among Māori aged 45+ years (1% men; 26% women) and non-Māori aged 65+ years (1%

men; 9.2% women) was lower, particularly for men, in comparison to the present analysis, although, the discrepancies may be because participants were aggregated into lower age categories and a lower EAR was used (150µg/day) than that in the present study (Ministry of Health, 1999).

Māori women tended to have significantly higher energy-adjusted folate intakes (44.37µg/MJ) compared to Māori men (36.78µg/MJ), yet, more Māori women were deficient in RBC folate (29%) versus Māori men (24%). Māori women were more likely to get their folate from fortified sources such as cereals and bread compared to cereals and vegetables for Māori men. Discrepancies may arise in the New Zealand Food Composition Database as folate-fortified products rely on manufacturers claims regarding folic acid content, which often may be higher than analytical values, and which can lead to an overestimation of dietary intake (University of Otago & Ministry of Health, 2011b). Generally men also tend to have higher energy intakes which may further explain that despite Māori women having higher energy-adjusted folate intakes, they still had a higher prevalence of RBC folate deficiency compared to men. Regardless, food sources fortified with folic acid appear to be the most important source of folate for older adults.

Vitamin B_{12}

In the present study, up to a quarter of participants did not meet the EAR for vitamin B_{12} (26% Māori; 22% non-Māori), yet, serum vitamin B_{12} was mostly adequate among the study participants (93% Māori; 91% non-Māori above the cut-off: 148pmol/L) which may reflect that vitamin B_{12} stores were largely adequate in this population. Men tended to have higher vitamin B_{12} intakes compared to women, although not significant, and more men met the EAR for vitamin B_{12} (87% Māori; 88% non-Māori) compared to women (63% Māori; 70% non-Māori). The results are similar to that reported in the NZANS 2008/09 where fewer women aged 71+ met the EAR (73%) compared to men (96.2%) (University of Otago & Ministry of Health, 2011a). Men tended to derive more vitamin B_{12} from sources such as beef and veal (17% Māori; 21% non-Māori) compared to women (15% Māori; 16% non-Māori) and women derived slightly more from sources lower in vitamin B_{12} such as milk (24% Māori and non-Māori) compared to men (20% Māori; 23% non-Māori).

The vitamin B_{12} present in meat products can range anywhere between 0.7 – 5.2µg/100g depending on the animal origin, cooking method, and cut of meat, and can be up to 110µg/100g for animal liver whereas the vitamin B_{12} content of milk is much lower and can range between 0.2 – 0.4µg/100g (Gille & Schmid, 2015) which may explain why more men had adequate dietary intakes compared to women. However, it has also been shown that the bioavailability of food-bound vitamin B_{12} is higher when the vitamin B_{12} content of the food is lower (Gille & Schmid, 2015) which may explain why fewer women met the EAR compared to men, but it was not reflected in their serum vitamin B_{12} concentrations. It may indicate that a more frequent intake of sources lower in vitamin B_{12} versus one dietary source high in vitamin B_{12} is better for maintaining adequate serum levels (Gille & Schmid, 2015).

Unsurprisingly, increasing intake of vitamin B_{12} was not significantly associated with serum vitamin B_{12} status in the present study and although the models showed a trend of increasing serum vitamin B_{12} as intake increased, the results were not significant and confidence intervals were very wide. Several studies indicate that serum vitamin B_{12} levels tend to saturate with dietary intakes of approximately 10µg/day and older adults with intakes >2.88µg/day may be half as likely to be deficient in serum vitamin B_{12} compared to those who have intakes <1.87µg/day (Allen, 2009; Doets & de Groot, 2016; Mendonça et al., 2016). Stores of vitamin B_{12} may last several years before

depletion and this may explain the observed lack of association between dietary intake and serum vitamin B_{12} in the present study (De Silva & Davis, 2013).

Māori had significantly higher energy-adjusted vitamin B_{12} intakes (0.46µg/MJ) compared to non-Māori (0.43µg/MJ) and this was reflected in their significantly higher serum vitamin B_{12} concentrations (Māori, 261pmol/L; non-Māori, 222pmol/L). Although older Māori tend to consume significantly less energy than non-Māori, protein foods make up a higher proportion of energy intake for Māori (16.3%) compared to non-Māori (15.4%) (Wham et al., 2016a). The 2008/09 NZANS also showed that Māori aged 51+ had higher intakes of vitamin B_{12} compared to the total population aged 71+ (University of Otago & Ministry of Health, 2011a). Traditionally Māori diets were high in protein and fibre, and the high intake of kaimoana (seafood) and meat suggests that protein foods are still an integral part of Māori meals, providing an important source of vitamin B_{12} for this population group (Rush et al., 2010).

As a result of atrophic gastritis, older adults may have decreased secretion of gastric acid and intrinsic factor, which impairs the absorption of food-bound vitamin B_{12} (Chernoff, 2013; Stover, 2010). However, the prevalence of atrophic gastritis in older New Zealanders (>65 years) has been reported to be relatively low (6.7%) (Green et al., 2004) and the prevalence among octogenarians is unclear. Therefore, it is not known how much of the serum vitamin B_{12} deficiency can be attributed to diet alone.

Strengths and Weaknesses

This study has provided the first detailed examination of iron, folate, and vitamin B_{12} intakes and biomarker status of Māori and non-Māori octogenarians. Robust methods of recruitment were undertaken as part of the comprehensive longitudinal study (LiLACS NZ) which led to an adequate representation of Māori and non-Māori (Dyall et al., 2013). The 24-hour multiple pass recall dietary assessment tool has been validated for use in octogenarians and provides more realistic estimates of energy and nutrient intake compared to a FFQ (Adamson et al., 2009).

Blood samples were rapidly processed after collection providing the most accurate estimates, and when blood could not be analysed, it was securely stored at the appropriate temperature ensuring its stability until it could be processed. However, a key limitation of the current study is that blood samples were collected in Wave 1 and dietary data was collected in Wave 2, 12 months later. Hence, the dietary intakes of these nutrients in Wave 2 may not be accurately representative of the dietary intakes in wave 1 during the blood collection. Further, given that blood test results were communicated to the participants' general practitioner, any action to correct deficiencies such as supplementation and dietary advice is not known.

The biomarker cut-offs and EARs used in the present analysis are based on adult studies and are not specific for adults aged 80+ years hence may not represent nutrition-related health outcomes for this age group. Additionally, there is no clear universal cut-off for serum vitamin B_{12} to define deficiency although several have been proposed ranging between 148pmol/L – 250pmol/L (de Benoist, 2008; Devalia et al., 2014; O'Leary & Samman, 2010; Wong, 2015) therefore, if a higher cut-off had been used there may have been a higher rate of deficiency observed.

In wave 2, a third (35%) of participants were found to be potential misreporters based on an $EI:BMR_{est}<0.9$ and $EI:BMR_{est}>2.0$ (Wham et al., 2016b) and, as this may influence some of the significant contrasts, interpretation should be made with caution.

In the assessment of biomarker status, participants who used supplements including vitamins/minerals and multivitamins/minerals were not excluded from the study. Therefore, dietary intake and supplement usage is intertwined and interpretation of biomarkers should be taken with caution. Further, the generalised linear models and binary logistic regression included participants using supplements which may have skewed the results given that the bioavailability of supplemental folic acid and vitamin B_{12} is higher than that found naturally in food. The results may have differed if these participants were excluded.

Conclusions

In summary, dietary intakes and stores of iron were largely adequate among the study participants. More men met the EAR for vitamin B_{12} compared to women, however vitamin B_{12} stores were not significantly different between genders and unsurprisingly dietary intake was not significantly correlated with serum vitamin B_{12}. Folate appeared to be the nutrient that most participants were lacking with more than half of participants not meeting the EAR and up to a third of participants deficient in RBC folate. Given that dietary folate intake was significantly correlated with RBC folate concentrations, it may suggest that dietary folate is lacking in the diets of octogenarians and strategies to improve this, including fortification and supplementations, may have positive impacts on the nutritional status of older adults and should be investigated further.

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Author contributions: D.P. analysed the data, performed statistical analysis and wrote the paper; S.M. assisted in data analysis; C.W. and N.K. are part of the LiLACS team that designed and conducted the study and critically reviewed the paper, commented, and approved the final manuscript.

Conflicts of interest: The authors report no conflict of interest.

4. Conclusion and recommendations

This study was the first to determine the dietary intakes, adequacy, food sources, and biomarker status of iron, folate and vitamin B_{12} in Māori and non-Māori octogenarians. These micronutrients are essential for the production of red blood cells and normal neurological functioning (Doets et al., 2013; Frangos et al., 2016). Understanding the current intakes, biomarkers and the association between the two is imperative for understanding ways of treating deficiencies and optimising nutritional intake for older adults.

Dietary iron intakes and biomarkers of iron status were mostly adequate in this study population (>88%). Given that iron is the most common nutrient deficiency associated with anaemia, this result suggests that this may not be a nutrient of concern in octogenarians. However, current research is also debating whether the biomarker cut-offs for iron status are appropriate for older adults in the context of an inflammatory state and factors associated with ageing (Choi et al., 2005; WHO & CDC, 2007). Therefore, dietary adequacy of iron may shift in light of these factors. Iron fortified foods such as cereals and bread were the largest contributors to overall iron intake suggesting that these foods are an important staple source of iron among octogenarians.

The more apparent issue in this population appeared to be folate, given that more than half of all participants had inadequate dietary folate intakes and up to a third were RBC folate deficient. This finding differs from previous studies which suggest that folate deficiency among octogenarians is rare (<5%) (den Elzen et al., 2013; Mendonça et al., 2016) Folate deficiency may lead to morphological changes in the red blood cells which can result in megaloblastic anaemia (Moll & Davis, 2017). As dietary folate intake was significantly and positively correlated with RBC folate concentrations in the present study, there may be an opportunity to correct deficiencies through diet, fortification and supplements.

Fortified cereals and bread were the top contributors to folate, and, given that foods fortified with folic acid are more bioavailable than naturally occurring folates, we can conclude that these are important sources of folate. These findings may also contribute to the body of evidence which suggests that folate fortification of foods in New Zealand should be mandatory, particularly as a way to positively impact the folate status of older adults. However, encouraging older adults to consume more fruits and vegetables is an important strategy to improve folate status especially given the valuable nutrients and dietary fibre these foods provide. The Newcastle 85+ study showed that folate from fruit was significantly and positively associated with RBC folate concentrations and a higher vegetable intake was associated with a lower risk of deficiency (Mendonça et al., 2016). In the present study, a higher dietary folate intake was also associated with a lower risk of RBC folate deficiency in both Māori and non-Māori.

Up to a quarter of participants did not meet the EAR for vitamin B_{12}, yet <11% of participants were deficient in serum vitamin B_{12}. This suggests there is a need to improve the dietary intake of vitamin B_{12} among older adults by promoting vitamin B_{12}-rich foods. Women (>30%) were more likely to have inadequate dietary intakes of vitamin B_{12} compared to men (<13%), however, serum vitamin B_{12} biomarkers were not significantly different between genders possibly due to the large storage capacity of vitamin B_{12} in the liver which can last several years before depletion. A lower vitamin B_{12} status in women has previously been reported in the literature (Mendonça et al., 2016) possibly related to the lower consumption of animal foods by women compared to men. In the current study women tended to have lower intakes of 'beef and veal' and 'fish and seafood' compared to men which may be an area that can be impacted through education and health promotion. Encouraging

older adults to consume more animal-sources of food such as milk, meat and eggs may provide a valuable source of vitamin B_{12}.

Further, a higher consumption of vitamin B_{12} from meat and meat products has been shown to reduce the risk of serum vitamin B_{12} deficiency in octogenarians (Mendonça et al., 2016). However, traditional Māori foods should also be considered, for example, kaimoana (seafood) is commonly consumed in Māori culture (Rush et al., 2010) and contributed more to vitamin B_{12} for Māori compared to non-Māori. Although dietary vitamin B_{12} intake did not correlate with serum biomarkers, similar to the findings from the Newcastle 85+ study (Mendonça et al., 2016), further investigation into the reasons behind this may provide insights into the ways we are able to influence vitamin B_{12} status.

Research related to the dietary intakes of iron, folate and vitamin B_{12} is limited in octogenarians, especially for older Māori. Findings from the present study adds to the body of knowledge which may help to inform dietary guidelines for older adults. Overall, we can conclude from this study that dietary iron intake and status was mostly adequate among the participants, however, there was a high prevalence of inadequacy of dietary folate in more than half of the participants. Encouraging foods such as dark green leafy vegetables which provides valuable nutrients and fibre and legumes such as chickpeas which can contribute to protein intake may help to ameliorate this finding. Strategies to reduce the losses of folate in cooking water may be an important and practical way to improve dietary intakes. Vitamin B_{12} intakes were inadequate in approximately a quarter of the participants and especially among women compared to men. This may reflect a lower intake of animal products rich in vitamin B_{12}. Encouraging the consumption of animal-sourced foods rich in vitamin B_{12} such as meat, fish, eggs and milk may help to improve the dietary intakes of vitamin B_{12}.

4.1. Strengths

This study has provided the first detailed examination of iron, folate, and vitamin B_{12} intakes and biomarker status of Māori and non-Māori octogenarians. Robust methods of recruitment were undertaken as part of the comprehensive longitudinal study (LiLACS NZ) which led to an adequate representation of Māori and non-Māori (Dyall et al., 2013).

The 24-hour multiple pass recall dietary assessment tool has been validated for use in octogenarians and provides more realistic estimates of energy and nutrient intake compared to a FFQ (Adamson et al., 2009). Further, the "Photographic Atlas of Food Portion Sizes" was adapted for use in the LiLACS NZ study population which allowed for the inclusion of more commonly eaten and tradition foods (Wham et al., 2012).

Blood samples were rapidly processed after collection providing the most accurate estimates, and when blood could not be analysed, it was securely stored at the appropriate temperature ensuring its stability until it could be processed.

4.2. Limitations

A key limitation of the current study is that blood samples were collected in Wave 1 and dietary data was collected in Wave 2, 12 months later. Therefore, the dietary intakes of these nutrients in Wave 2 may not be accurately representative of the dietary intakes in Wave 1 during the blood collection. Further, given that blood test results were communicated to the participants' general practitioner, any action to correct deficiencies such as supplementation and dietary advice is not known. Although biomarkers for iron have been shown to be relatively stable over a period of 5 years in men and women aged 40-65 years (Al-Delaimy et al., 2006), the same is not known for folate and vitamin B_{12}.

The biomarker cut-offs used in the present analysis are based on adult studies and are not specific for older adults. Additionally, there is no clear universal cut-off for serum vitamin B_{12} to define deficiency and several have been proposed ranging between 148pmol/L – 250pmol/L (de Benoist, 2008; Devalia et al., 2014; O'Leary & Samman, 2010; Wong, 2015). If a higher cut-off was used, a higher rate of deficiency may have been observed and the results of the binary regression analysis may have differed.

The EARs used in the present study was set for adults aged 71+, possibly based on low numbers of older adults and/or extrapolation from younger age groups (NHMRC, 2006; Wham et al., 2016b). Therefore, they may not represent nutrition-related health outcomes for older adults.

The potential for misreporting was not assessed in the present study and interpretation of dietary intake should be taken in light of this. In wave 2, a large proportion of participants (35%) were found to be potential misreporters based on an EI:BMR_{est}<0.9 and EI:BMR_{est}>2.0 and a higher prevalence was reported among Māori (Wham et al., 2016b) which may influence some of the significant contrasts between Māori and non-Māori.

In the assessment of biomarker status, participants who used supplements including vitamins/minerals and multivitamins/minerals were not excluded from the study. Hence, dietary intake and supplement usage will be intertwined and interpretation of biomarkers should be taken with caution. Further, the generalised linear models and binary logistic regression included participants using supplements which may have skewed the results given that the bioavailability of supplemental folic acid and vitamin B_{12} is higher than that found naturally in food. The results may have differed if these participants were excluded.

4.3. Recommendations

- Further investigation into the iron, folate and vitamin B_{12} status of octogenarians living outside of the Bay of Plenty and Lakes DHB may help to validate the findings of the present study and allow comparisons between different geographic areas of New Zealand.
- Implementing regular screening for folate deficiency using the RBC folate biomarker, as it is a more accurate reflection of folate status over a period of time, can identify those who are most at risk and need supplementation or referrals to dietetic services for dietary advice.
- Encouraging dietary variety through GP services, social events, and social/cultural networks may positively impact both folate status and overall nutrient intake.
- Supplementation may be an appropriate avenue for those who are at a state of deficiency or are more at risk of inadequate folate intakes due to decreased dietary variety, or decreased food intake overall.
- Further investigations into the determinants of low iron, folate, and vitamin B_{12} may help to inform the strategies needed to improve the status of these nutrients in older adults given that these nutrients are largely intertwined with several non-diet related factors.
- In the development of dietary guidelines for older adults, it is important to take into account socioeconomic factors, dietary variety, cooking methods, medication intake, inflammation, and impaired gastrointestinal absorption. All of which may influence the availability and bioavailability of iron, folate and vitamin B_{12}.
- In the present study, the prevalence of low haemoglobin was investigated, however, the cause was not. Future investigations to determine whether iron, folate, vitamin B_{12} and/or another reason is associated with low haemoglobin in this population may help to inform preventative measures to avoid anaemia.
- Given that elevated iron stores are commonly seen in older adults, in particular, elevated serum ferritin (Choi et al., 2005; Fleming et al., 2001), an investigation into high iron stores may explain the relatively low prevalence of low iron stores in the present study and could further inform whether the biomarker cut-offs for serum ferritin, serum iron and transferrin saturation are appropriate for older adults.

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5. Appendices

Appendix A: Supplementary methods

Appendix B: Supplementary results

Appendix C: Journal requirements: *Nutrients*

Appendix A: Supplementary Methods

Additional Statistical Analyses

Multivariate generalised linear models controlling for age, gender and energy intake were used to determine associations between demographic and health characteristics and the dietary intakes (Table 5.2) and biomarkers (Table 5.3 and 5.4) relating to iron, folate and vitamin B_{12}. Binary logistic regression was used to determine the odds ratio (likelihood) of deficiency in serum folate, Tf-saturation and TIBC according to quartiles of dietary folate or iron intakes when controlling for age, gender and energy intake. Additional factors were added to the model based on the nutrient investigated. A p-value <0.05 was considered significant.

Appendix B: Supplementary Results

Distribution of dietary intake

Distribution of dietary iron intake for Māori and non-Māori by gender

Figure 5.1 shows the distribution of intakes for iron for Māori and non-Māori by gender with the EAR and recommended daily intake (RDI) indicated. The majority of Māori and non-Māori men and women had intakes greater than the EAR. A large proportion of Māori and non-Māori women had intakes below the RDI, approximately 40% and 36%, respectively, compared with 26% Māori men and 11% non-Māori men. A higher proportion of Māori did not meet the EAR compared to non-Māori, approximately 12% Māori men and women compared with 2% non-Māori men and 8% non-Māori women.

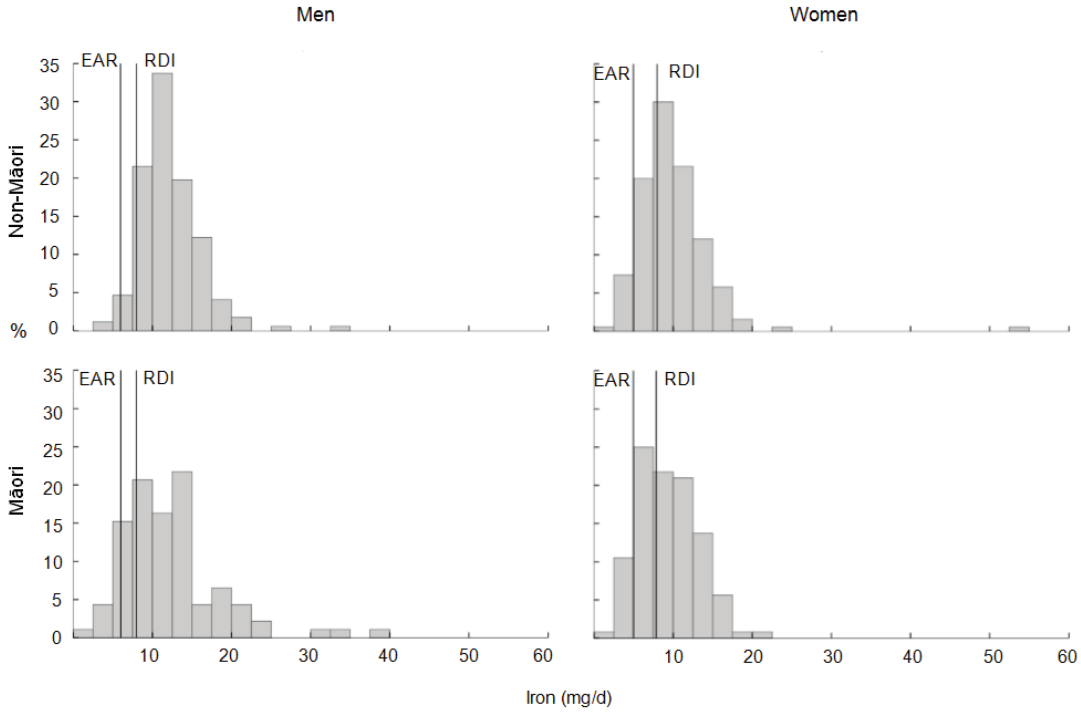

Figure 5.1: Intake distribution of iron with the estimated average requirement (EAR) and recommended daily intake (RDI) marked for Māori and non-Māori by gender.

Distribution of dietary folate intake for Māori and non-Māori by gender

Figure 5.2 shows the distribution of intakes for folate for Māori and non-Māori by gender with the EAR and recommended daily intake (RDI) indicated. The majority of Māori and non-Māori men and women had intakes below the EAR and RDI. Over half of Māori men (58%) and women (58%) had intakes less than the EAR. Similarly, 43% of non-Māori men and 59% of non-Māori women had intakes less than the EAR. Approximately 77% Māori men, 76% Māori women, 61% non-Māori men and 72% non-Māori women had intakes below the RDI.

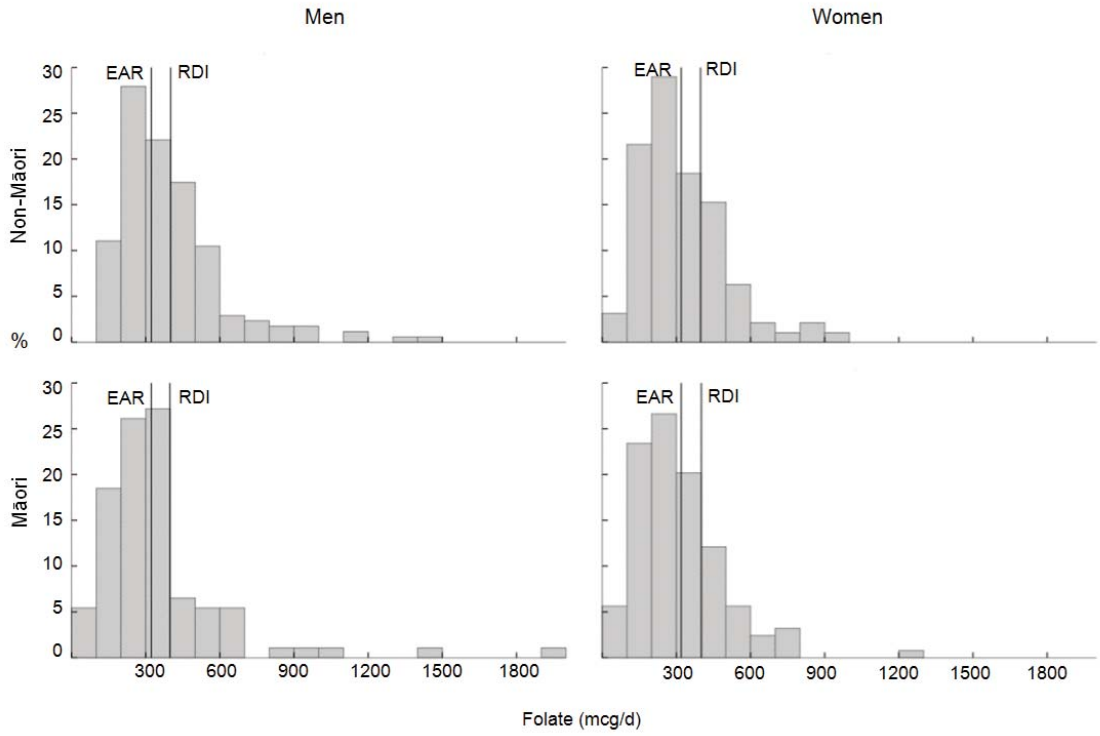

Figure 5.2: Intake distribution of folate with the estimated average requirement (EAR) and recommended daily intake (RDI) marked for Māori and non-Māori by gender.

Distribution of dietary vitamin B_{12} intake for Māori and non-Māori by gender

Figure 5.3 shows the distribution of intakes for vitamin B_{12} for Māori and non-Māori by gender with the EAR and recommended daily intake (RDI) indicated. A higher proportion of Māori women (37%) and non-Māori women (30%) had intakes below the EAR compared with Māori men (13%) and non-Māori men (12%). The majority of Māori men (78%) and Māori women (57%) had intakes below the RDI. Almost half of non-Māori women (47%) and a quarter of non-Māori men (25%) had intakes below the RDI.

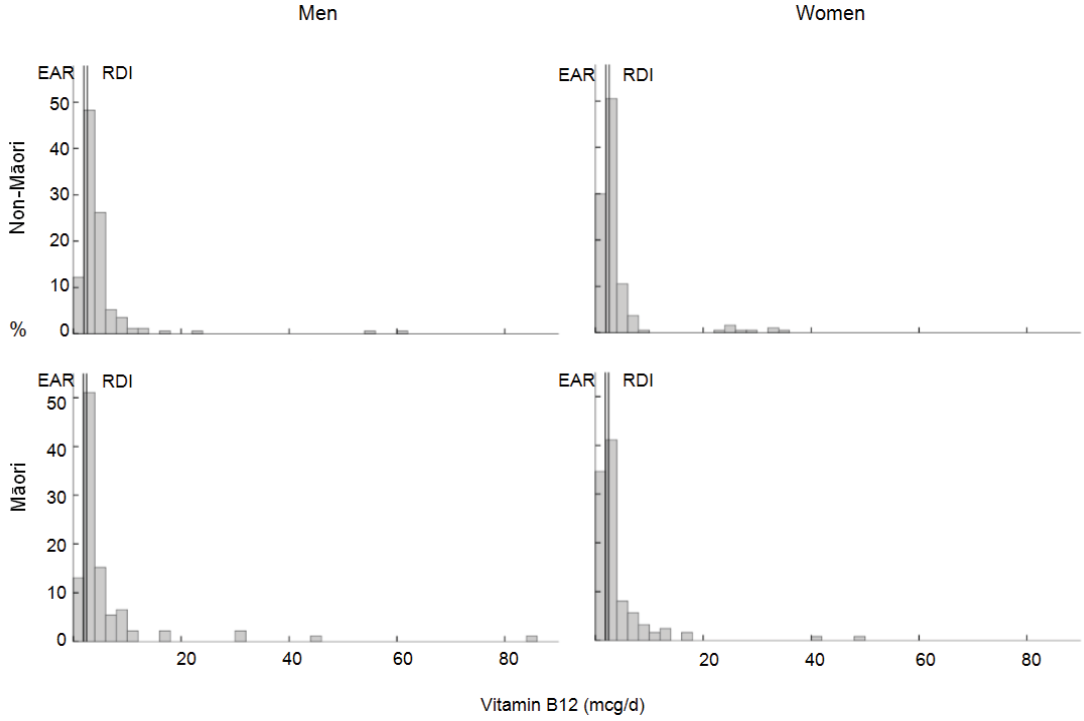

Figure 5.3: Intake distribution of vitamin B_{12} with the estimated average requirement (EAR) and recommended daily intake (RDI) marked for Māori and non-Māori by gender.

Dietary intake of iron, folate and vitamin B_{12} according to demographic and health characteristics for Māori and non-Māori

The daily intake of iron, folate and vitamin B_{12} by participant demographics, health and physical characteristics for Māori and non-Māori are reported in Table 5.1. Māori in the overweight category had significantly lower folate intake compared to Māori in the underweight category. Non-Māori that were classified as moderately deprived had a significantly higher vitamin B_{12} intake than non-Māori that were classified as most deprived. Non-Māori former smokers had a significantly higher iron intake compared non-Māori that had never smoked. Non-Māori who never consumed alcohol had a significantly lower iron intake compared to non-Māori who consumed alcohol >4 times per week. However, dietary iron was significantly higher in participants who drank 2-4 times a month or less and in those who consumed alcohol 2-3 times per week in comparison to those who drank >4 times a week.

Table 5.1: Daily iron, folate and vitamin B_{12} intake from food for Māori and non-Māori by demographics, physical and health characteristics.

Participant characteristics		Māori			Non-Māori		
		Iron (mg)	**Folate** (µg)	**Vitamin B_{12}** (µg)	**Iron** (mg)	**Folate** (µg)	**Vitamin B_{12}** (µg)
Living situation	Alone	9.5	287	3.2	10.2	304	2.8
	Spouse only	11.0	311	3.2	11.1	341	3.6
	With others[◊]	9.5	265	2.9	9.6	268	2.5
Housing situation	Private dwelling/unit	10.2	283	3.1	10.6	320	3.0
	Retirement village	9.3	249	3.6	10.8	341	3.3
	Rest home/private hospital	10.1	306	2.5	9.0	244	2.3
	Other[€]	14.1	395	5.7	8.6	263	3.0
Highest level of education	Primary or none	10.7	277	2.9	10.2	290	2.8
	Secondary/no qualification	9.5	282	3.0	10.5	306	2.9
	Trade/occupation	9.2	247	1.9	10.3	321	3.2
	Tertiary	10.0	254	3.3	11.2	381	3.7
NZDep score	1-4 (least)	11.7	355	3.0	10.5	313	2.8
	5-7	10.1	279	3.1	10.8	319	3.3
	8-10 (most)	9.3	266	3.0	10.1	303	3.0*[1]
BMI (kg/m^2)	Underweight (<18.5)	14.1	1275	12.9	9.7	229	2.2
	Normal (18.5 – 24.9)	9.1	269	3.1	10.5	313	2.7
	Overweight (25.0-29.9)	11.1	319	3.2	10.7	328	3.1
	Obese (>30)	9.4	256**[2]	2.8	10.1	295	3.0
Dentures	None	9.1	267	3.3	10.8	339	3.5
	Dentures used[Δ]	10.5	279	3.1	10.6	312	2.9
Smoking status	Never	9.5	281	2.8	10.1	300	2.9
	Former	9.9	279	3.0	10.8	332	3.1
	Current	10.5	269	3.4	11.4**[3]	339	3.2
Alcohol intake	Never	10.1	293	2.9	10.2	284	2.4
	2-4 times per month or less	9.7	279	3.4	10.7	313	3.2
	2-3 times a week	8.7	264	3.1	10.8	299	3.2
	>4 times a week	10.6	297	3.3	10.7*[4]	345	3.4
Medications[x]	None	8.9	268	2.5	10.8	295	2.7
	1-3	10.4	210	3.1	11.1	236	3.4
	4-6	9.6	211	3.1	10.5	230	3.2
	7-9	10.6	201	3.2	10.5	237	3.0
	10+	10.1	217	3.3	10.3	226	2.6
Dietary supplements[¥]	None	9.5	279	3.0	10.7	314	3.1
	Supplements used	11.1	279	3.1	10.4	313	2.8

Generalised linear model controlling for age, gender and energy intake. P-value <0.05 considered significant.

* p-value <0.05, **p-value <0.01

[◊] including spouse; [€] Includes Marae; [Δ] Includes full, partial, upper and lower dentures; [x] includes intake of dietary supplements; [¥] includes vitamins, minerals and multivitamins/minerals.

[1] Significant differences were found for non-Māori participants between vitamin B_{12} intake per day and NZDep score (p=0.042). Non-Māori in the NZDep category 5-7 had a significantly higher vitamin B_{12} intake compared to non-Māori in the most deprived category 8-10 (p=0.024).

[2] Significant differences were found for Māori participants between folate intake and BMI (p<0.001). Folate intake was significantly higher in Māori participants who were in the underweight category compared to those who were in the obese category (p<0.001).

[3] Significant differences were found for non-Māori participants between iron intake and smoking status (p=0.006). Iron intake was significantly lower in non-Māori who had never smoked compared to those who were former smokers (p=0.001).

[4] Significant differences were found for non-Māori participants between iron intake and alcohol intake (p=0.011). Dietary iron intake was significantly lower in non-Māori participants who never drank alcohol compared to non-Māori who drank >4 times a week (p=0.004). Dietary iron intake was significantly higher in participants who drank 2-4 times a month or less (p=0.020) or 2-3 times a week (p=0.010) compared to those who drank >4 times a week.

Biomarker status for iron, folate and vitamin B_{12} according to demographic and health characteristics for Māori

Biomarkers of iron status, folate status and vitamin B_{12} status by participant demographics, health and physical characteristics for Māori are reported in Table 5.2. Māori who lived in rest homes/private hospitals had a significantly higher TIBC and RBC folate compared to those who lived on a marae or with others. Serum iron and Tf-saturation was significantly higher in Māori participants who had a normal BMI compared to those who were in the obese BMI category. Māori who consumed alcohol 2-3 times a month or less had a significantly lower serum vitamin B_{12} concentration in comparison to Māori who consumed alcohol >4 times a week. Haemoglobin was significantly higher in Māori who consumed 4-6 medications compared to those who consumed 10+ medications. Ferritin was significantly lower in participants who consumed 10+ medications compared to participants who consumed no medications and between 4-6 medications. Similarly, Tf-saturation was significantly lower in participants who consumed 10+ medications compared to all other participants. TIBC was also significantly lower in in Māori who consumed between 1 and 9 medications compared to those who consumed 10+ medications. Māori participants who used dietary supplements had a significantly higher serum folate and RBC folate compared to Māori who did not use supplements.

Table 5.2: Biomarkers of iron status (haemoglobin, serum iron, total iron binding capacity, transferrin saturation), folate status (serum folate, RBC folate), and vitamin B_{12} status (serum vitamin B_{12}) for Māori by demographics, physical and health characteristics.

Participant characteristics		Māori							
		Hb (g/L)	**Ferritin** (µg/L)	**S-iron** (µmol/L)	**TIBC** (µmol/L)	**Tf-sat** (%)	**S-folate** (nmol/L)	**RBC folate** (nmol/L)	**S-B_{12}** (pmol/L)
Living situation	Alone	136	137	16	57	28	17	335	256
	Spouse only	137	192	16	55	28	16	326	254
	With others◊	136	243	14	57	23	19	332	303
Housing situation	Private dwelling/unit	136	188	16	57	28	17	331	253
	Retirement village	137	130	15	54	27	18	335	277
	Rest home/private hospital	136	28	11	86	13	28	759	390
	Other€	133	260	16	65**0	27	26	329**1	336
Highest level of education	Primary or none	136	174	15	57	28	18	320	241
	Secondary/no qualification	136	184	16	56	27	16	334	269
	Trade/occupation	140	98	16	62	25	20	310	246
	Tertiary	139	243	16	57	30	16	335	312
NZDep score	1-4 (least)	141	175	17	58	29	16	344	296
	5-7	136	191	15	57	27	19	372	241
	8-10 (most)	136	191	16	57	28	17	306	259
BMI (kg/m^2)	Underweight (<18.5)	.	.	.	.	.	.	.	.
	Normal (18.5 – 24.9)	135	182	18	57	31	17	365	294
	Overweight (25.0-29.9)	136	192	14	56	25	16	326	241
	Obese (>30)	138	175	15*2	59	25*3	17	329	253
Dentures	None	138	190	16	57	28	17	294	244
	Dentures usedΔ	136	182	16	56	28	17	333	254
Smoking status	Never	137	183	16	57	28	17	335	284
	Former	137	192	14	57	25	17	334	241
	Current	141	257	19	57	31	16	278	328
Alcohol intake	Never	136	185	15	57	26	18	333	318
	2-4 times a month or less	138	244	16	56	28	16	327	208
	2-3 times a week	136	210	17	55	29	17	311	252
	>4 times a week	135	127	14	56	23	19	365	292*4
Medicationsχ	None	143	323	18	62	33	15	233	244
	1-3	134	236	17	57	31	18	311	248
	4-6	138	199	16	55	27	16	304	264
	7-9	137	244	16	57	28	18	377	250
	10+	134**5	115**6	12	65**7	23**8	18	335	231
Dietary supplements¥	None	137	205	16	57	28	17	310	259
	Supplements used	137	142	15	57	27	19**9	405**10	250

Generalised linear model controlling for age and gender. P-value <0.05 considered significant.

* p-value <0.05, **p-value <0.01

◊ including spouse; € Includes Marae; Δ Includes full, partial, upper and lower dentures; χ includes intake of dietary supplements; ¥ includes vitamins, minerals and multivitamins/minerals.

Abbreviations: Hb, haemoglobin; s-iron, serum iron; TIBC, total iron binding capacity; Tf-sat, transferrin-saturation; s-folate, serum folate; RBC, red blood cell; s-B_{12}, serum vitamin B_{12}; BMI, body mass index; NZDep, NZ deprivation index score.

[0] Significant differences were found for Māori participants between TIBC and housing situation (p=0.006). Māori who lived in rest homes/private hospitals had a significantly higher TIBC compared to Māori who lived on a Marae/other (p=0.038).

[1] Significant differences were found for Māori participants between RBC folate and housing situation (p=0.005). Māori who lived in rest homes/private hospitals had a significantly higher RBC folate compared to Māori who lived in a Marae/other housing (p=0.007).

[2] Significant differences were found for Māori participants between serum iron and BMI (p=0.019). Serum iron was significantly higher in Māori classified as having a normal BMI compared to Māori who were classified as obese (p=0.018).

[3] Significant differences were found for Māori participants between Tf-saturation and BMI (p=0.015). Tf-saturation was significantly higher in Māori who were classified as having a normal BMI compared to Māori who were obese (p=0.015).

[4] Significant differences were found for Māori participants between serum vitamin B_{12} and alcohol consumption (p=0.030). Māori who consumed alcohol 2-3 times a month or less had a significantly lower serum vitamin B_{12} concentration when compared to Māori who consumed alcohol >4 times a week (p=0.011).

[5] Significant differences were found for Māori participants between haemoglobin and medication usage (p=0.007). Haemoglobin was significantly higher in Māori who consumed no medications (p=0.003) and Māori who consumed 4-6 medications (p=0.014) when compared to Māori who consumed 10+ medications.

[6] Significant differences were found for Māori participants between ferritin and medication usage (p=0.009). Serum ferritin was significantly higher in Māori who consumed no medications (p=0.008) and Māori who consumed 4-6 medications (p=0.002) compared to Māori who consumed 10+ medications.

[7] Significant differences were found for Māori participants between TIBC and medication usage (p=0.003). TIBC was significantly lower in Māori who consumed 1-3 medications (p=0.001), Māori who consumed 4-6 medications (p<0.001) and Māori who consumed 7-9 medications (p=0.025) when compared to Māori who consumed 10+ medications.

[8] Significant differences were found for Māori participants between Tf-saturation and medication usage (p=0.017). Tf-saturation was significantly higher in Māori who consumed no medications (p=0.029), Māori who consumed 1-3 medications (p=0.005), Māori who consumed 4-6 medications (p=0.001) and Māori who consumed 7-9 medications (p=0.033) when compared to Māori who consumed 10+ medications.

[9] Māori participants who used dietary supplements had a significantly higher serum folate concentration compared to Māori participants who did not use dietary supplements (p=0.001).

[10] Māori participants who used dietary supplements had a significantly higher RBC folate concentration compared to Māori who did not use supplements (p=0.001).

Biomarker status for iron, folate and vitamin B_{12} according to demographic and health characteristics for non-Māori

Biomarkers of iron status, folate status and vitamin B_{12} status by participant demographics, health and physical characteristics for non-Māori are reported in Table 5.3. Serum folate concentration was significantly lower in non-Māori who lived alone and higher in non-Māori who lived with their spouse only when compared to non-Māori who lived with others. Non-Māori with primary or no education, secondary/without qualification and trade/occupation had a significantly lower serum ferritin and serum vitamin B_{12} in comparison to non-Māori who had tertiary education. Serum ferritin was significantly lower in non-Māori who never consumed alcohol and non-Māori who consumed alcohol 2-4 times a month compared to non-Māori who consumed alcohol >4 times a week. Haemoglobin was significantly higher in non-Māori who took 1-3 medications or 7-9 medications when compared to non-Māori who took 10+ medications. Similarly, serum ferritin was significantly higher in non-Māori who consumed between 1-3 medications compared to non-Māori who consumed 10+ medications. Serum folate and serum vitamin B_{12} concentrations were significantly higher in non-Māori participants who used dietary supplements compared to those who did not use supplements.

Table 5.3: Biomarkers of iron status (haemoglobin, serum iron, total iron binding capacity, transferrin saturation), folate status (serum folate, RBC folate), and vitamin B_{12} status (serum vitamin B_{12}) for non-Māori by demographics, physical and health characteristics.

		Hb (g/L)	**Ferritin** (µg/L)	**S-iron** (µmol/L)	**TIBC** (µmol/L)	**Tf-sat** (%)	**S-folate** (nmol/L)	**RBC folate** (nmol/L)	**S-B_{12}** (pmol/L)
Living situation	Alone	133	110	15	57	26	18	339	230
	Spouse only	140	150	17	55	30	19	367	214
	With others[◊]	136	145	15	55	26	14**[1]	321	194
Housing situation	Private dwelling/unit	136	122	16	57	28	18	360	219
	Retirement village	135	114	16	55	29	19	340	222
	Rest home/private hospital	121	145	15	53	27	15	238	282
	Other[€]	129	60	12	57	21	15	304	231
Highest level of education	Primary or none	137	110	15	58	28	17	353	199
	Secondary/no qualification	135	118	15	56	28	17	341	220
	Trade/occupation	135	121	15	54	28	17	362	211
	Tertiary	136	181**[2]	16	55	29	18	383	263*[3]
NZDep score	1-4 (least)	138	144	17	56	30	19	383	245
	5-7	136	121	15	56	26	17	357	220
	8-10 (most)	135	121	15	56	26	17	320	219
BMI (kg/m^2)	Underweight (<18.5)	148	286	23	61	38	21	469	253
	Normal (18.5 – 24.9)	135	123	16	55	28	17	343	216
	Overweight (25.0-29.9)	136	122	15	56	27	20	363	219
	Obese (>30)	134	115	15	56	28	16	330	249
Dentures	None	137	177	15	56	27	18	356	253
	Dentures used[Δ]	135	116	16	56	28	17	345	217
Smoking status	Never	135	110	15	56	27	18	347	221
	Former	136	153	17	55	30	17	357	219
	Current	138	164	15	55	25	15	298	285
Alcohol intake	Never	134	116	15	57	26	19	369	214
	2-4 times a month or less	135	112	15	56	28	17	358	235
	2-3 times a week	136	132	15	56	28	15	295	215
	>4 times a week	138	179*[4]	16	56	29	18	347	220
Medications[χ]	None	140	229	17	57	28	23	399	209
	1-3	142	167	17	55	33	15	293	207
	4-6	136	121	16	56	28	17	348	218
	7-9	135	105	15	58	27	18	379	231
	10+	131**[5]	117*[6]	15	57	26	19	331	245
Dietary supplements[¥]	None	137	125	16	56	28	16	345	205
	Supplements used[¥]	134	121	15	56	26	20**[7]	365	248**[8]

Generalised linear model controlling for age and gender. P-value <0.05 considered significant.

* p-value <0.05, **p-value <0.01

[◊] including spouse; [€] Includes Marae; [Δ] Includes full, partial, upper and lower dentures; [χ] includes intake of dietary supplements; [¥] includes vitamins, minerals and multivitamins/minerals.

Abbreviations: Hb, haemoglobin; s-iron, serum iron; TIBC, total iron binding capacity; Tf-sat, transferrin-saturation; s-folate, serum folate; RBC, red blood cell; s-B_{12}, serum vitamin B_{12}; BMI, body mass index; NZDep, NZ deprivation index score.

[1] Significant differences were found for participants between serum folate concentration and living situation (p=0.004). Serum folate was significantly lower in non-Māori participants who lived alone (p=0.009) and significantly higher in non-Māori who lived with only their spouse (p=0.001) when compared to non-Māori who lived with others.

[2] Significant differences were found for participants between ferritin concentration and highest level of education (p=0.002). Ferritin was significantly lower in participants who had primary schooling/no education (p=0.001), secondary/with qualification (p=0.001) and trade/occupation education (p=0.018) compared to participants who had a tertiary education.

[3] Significant difference were found for participants between serum vitamin B_{12} concentration and highest level of education (p=0.042). Non-Māori with a primary education (p=0.035), secondary/without qualification (p=0.045) and trade/occupation (p=0.007) had significantly lower serum vitamin B_{12} compared to participants with a tertiary education.

[4] Significant differences were found for participants between ferritin concentration and alcohol intake (p=0.034). Ferritin was significantly lower in participants who never consumed alcohol (p=0.016) and participants who consumed alcohol 2-4 times a month (p=0.018) compared to participants who consumed alcohol >4 times a week.

[5] Significant differences were found for participant between serum haemoglobin concentration and medication intake (p=0.013). Non-Māori who took 1-3 medications (p=0.001) or 7-9 medications (p=0.032) had a significantly higher haemoglobin than participants who took 10+ medications.

[6] Significant differences were found for participants between ferritin concentration and medication usage (p=0.028). Participants who consumed 1-3 medications had a significantly higher ferritin concentration compared to those who consumed 10+ medications (p=0.008).

[7] Participants using dietary supplements had a significantly higher serum folate concentration compared to those who did not use dietary supplements (p=0.005).

[8] Participants using dietary supplements had a significantly higher serum vitamin B_{12} concentration compared to those who did not use dietary supplements (p=0.002)

Risk of iron or folate deficiency by dietary intake using serum folate, Tf-saturation, and TIBC

The likelihood of being below the cut-offs for serum folate or transferring saturation, or exceeding the cut-off for TIBC, indicating iron or folate deficiency in Māori and non-Māori according to quartiles of iron or folate intake are reported in Table 5.5. No significant associations or trends were found between intakes of iron or folate and serum folate, transferrin saturation or total iron binding capacity in both the unadjusted and adjusted models.

Table 5.4: Likelihood of being below the cut-off for serum folate or transferrin saturation, or exceeding the cut-off for TIBC indicating iron or folate deficiency in Māori and non-Māori according to quartiles of dietary intake of iron (mg/d) and folate (µg/d).

		Non-Māori				Māori			
Serum folate	**Folate Intake (µg/d)**	**Model 1 (unadjusted)**		**Model 2 (adjusted)[¥]**		**Model 1 (unadjusted)**		**Model 2 (adjusted)[¥]**	
		(n= 6)	*p*		*p*	(n= 0)	*p*		*p*
	<215	1.00 (ref.)	-	1.00 (ref.)	-	-	-	-	-
	215 – 303	0.00 [0.00]	0.997	0.00 [0.00]	0.997	-	-	-	-
	304 – 440	0.35 [0.06, 1.99]	0.238	0.29 [0.02, 3.58]	0.332	-	-	-	-
	>440	0.00 [0.00]	0.997	0.00 [0.00]	0.997	-	-	-	-
Tf-saturation	**Iron intake (mg/d)[1]**	**Model 1 (unadjusted)**		**Model 2 (adjusted)[◊]**		**Model 1 (unadjusted)**		**Model 2 (adjusted)[◊]**	
		(n=21)	*p*		*p*	(n= 10)	*p*		*P*
	<7.75	1.00 (ref.)	-	1.00 (ref)	-	1.00 (ref.)	-	1.00 (ref.)	-
	7.75 – 10.42	0.65 [0.19, 2.25]	0.495	0.85 [0.22, 3.24]	0.816	0.32 [0.03, 2.98]	0.314	0.29 [0.03, 2.85]	0.287
	10.43 – 13.26	0.24 [0.05, 1.23]	0.092	0.35 [0.06, 2.22]	0.252	0.27 [0.03, 2.51]	0.247	0.21 [0.02, 2.23]	0.201
	>13.26	1.11 [0.36, 3.41]	0.859	1.90 [0.42, 8.53]	0.403	1.79 [0.41, 7.98]	0.446	1.29 [0.18, 9.27]	0.802
TIBC	**Iron intake (mg/d)[2]**	**Model 1 (unadjusted)**		**Model 2 (adjusted)[◊]**		**Model 1 (unadjusted)**		**Model 2 (adjusted)[◊]**	
		(n= 9)	*p*		*p*	(n= 10)	*p*		*P*
	<7.75	1.00 (ref.)	-	1.00 (ref.)	-	1.00 (ref.)	-	1.00 (ref.)	-
	7.75 – 10.42	0.53 [0.09, 3.26]	0.489	0.35 [0.05, 2.49]	0.297	0.90 [0.14, 5.76]	0.909	1.00 [0.15, 6.72]	1.000
	10.43 – 13.27	0.00 [0.00]	0.997	0.00 [0.00]	0.997	0.75 [0.12, 4.80]	0.746	0.71 [0.10, 5.23]	0.736
	>13.27	1.10 [0.24, 5.14]	0.903	0.30 [0.03, 3.05]	0.312	1.75 [0.32, 9.50]	0.517	1.91 [0.23, 16.1]	0.550

Binary regression model. Values expressed as odds ratio [95% confidence interval]. P-value <0.05 considered to be significant.

[¥]Model 2 adjusted for sex, age, energy intake, nutritional supplement usage and intake of folic acid

[◊]Model 2 adjusted for sex, age, energy intake and nutritional supplement intake.

Cut-offs indicating iron deficiency: TIBC >71µmol/L; Tf-saturation <15%.

Cut-offs indicating folate deficiency: s-folate <6.7nmol/L.

Abbreviations: Tf-saturation, transferrin saturation; TIBC, total iron binding capacity

Appendix C: Journal requirements: *Nutrients*

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

Manuscript Submission Overview

Types of Publications

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

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

- *Articles:* research manuscripts that report new evidence or new conclusions. The journal considers all original research manuscripts provided that the work reports scientifically sound experiments and provides a substantial amount of new information. Authors should not unnecessarily divide their work into several related manuscripts, although short *Communications* of preliminary, but significant, results will be considered. Replications of previous studies are permitted if clearly indicated as such.
- *Reviews:* review manuscripts provide concise and precise updates on the latest progress made in a given area of research. Systematic reviews should follow the PRISMA guidelines.
- *Case reports:* Case reports present detailed information on the symptoms, signs, diagnosis, treatment (including all types of interventions), and outcomes of an individual patient. Case reports usually describe new or uncommon conditions that serve to enhance medical care or highlight diagnostic approaches.
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Preparation of a Manuscript

General Considerations

- **Research manuscripts** should comprise:
  - Front matter: Title, Author list, Affiliations, Abstract, Keywords
  - Research manuscript sections: Introduction, Materials and Methods, Results, Discussion, Conclusions (optional).
  - Back matter: Supplementary Materials, Acknowledgments, Author Contributions, Conflicts of Interest, References.
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- **Abstract graphic:** Authors are encouraged to provide a graphical abstract as a self-explanatory image to appear alongside with the text abstract in the Table of Contents. Figures should be a high quality image in any common image format. Note that images displayed online will be up to 11 by 9 cm on screen and the figure should be clear at this size.
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Front Matter

These sections should appear in all manuscript types

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