The consequences of avoiding bread: the effect of bread avoidance on selenium status and thyroid function.

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Abstract

Selenium is an important nutrient for the conversion of inactive thyroxine (T4) to the active hormone triiodothyronine (T3) and therefore is vital for thyroid health. Unfortunately the New Zealand food supply is generally low in selenium due to the poor content of the soil. However, bread is often made using selenium-rich Australian wheat and therefore is a good dietary source of the nutrient. The avoidance of bread is becoming popular with the rise of lower carbohydrate diets, increasing the risk of low status and subsequent impaired thyroid function. This study aimed to investigate if avoidance of bread by mid-life women in New Zealand results in low selenium status and subsequent impaired thyroid function, as well as investigating alternate dietary sources. This study is an extension of a previous study which investigated iodine status in women who avoid bread. This study investigated selenium intake (estimated from 3-day-diet-diaries, urinary excretion and plasma concentration), status (plasma) and thyroid hormone biomarkers (T3, T4, TSH, T3:T4 ratio, Tg, TPOAb) in 37 mid-life women based in Auckland, New Zealand.

Median intake estimated from 3-day diet diaries and urinary excretion was 52µg/day (36, 84) and 51 µg/day (38, 72) respectively which is below the recommended daily intake (60µg/day). It was found that 49% of participants had intakes below the estimated average requirement (50µg/day), indicating deficiency. Median plasma concentration was 118.9µg/L (107.2, 131.3) suggesting optimal status, however nearly a third of participants had low plasma concentrations below 110µg/L. Impaired thyroid function was found in 2 participants, both of whom demonstrated subclinical hyperthyroidism. Major alternate dietary sources of selenium were found to be seafood, meat, poultry and nuts. Participants who did not regularly eat these foods did not manage to meet their daily requirements for selenium intake.

Overall, a high prevalence of inadequate intake was observed in women who avoid bread. Subsequently, almost a third of participants had suboptimal plasma concentrations indicating low status. Only two participants were found to have impaired thyroid function, potentially due to the lower requirements of selenium for deiodinases to function. This study highlights the importance of consuming alternate dietary sources such as seafood, meat, poultry and nuts on a regular basis if bread is removed from the diet while also emphasizing the risks that dietary trends such as bread avoidance can pose for intake of nutrients such as selenium. It could be suggested that some individuals would benefit from a greater understanding of the nutritional risks associated with bread avoidance, particularly in regard to selenium intake. Public knowledge about alternative selenium sources may not be widely held and therefore individuals may not be equipped with the information needed to make educated choices in regard to their chosen dietary patterns. An attempt to raise awareness of selenium as a nutrient as well as the best alternate dietary sources of selenium in the NZ food supply, to improve both dietary intake and status of selenium amongst at-risk groups such as those who avoid bread is highly recommended.

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Contributions.

Research topic & study design	Dr Louise Brough, Prof. Pamela von Hurst, Jacqui Finlayson
Participant recruitment	Jacqui Finlayson
Participant follow-up Ethical approval • Additional consent • Follow-up emails & phone calls	Jaime Berger
Sample handling • Blood samples	Owen Mugridge PC Tong
Data collation, statistical analysis & interpretation	Jaime Berger
Thesis writing	Jaime Berger
Reviewing & Editing	Prof. Pamela von Hurst, Dr Louise Brough

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List of abbreviations.

AMDR Acceptable macronutrient distribution range
BMI Body Mass Index
EAR Estimated average requirement
FAO Food and Agriculture Organisation
FFQ Food frequency questionnaire
GPX Glutathione peroxidase
MOH Ministry of Health
MUHEC Massey University Human Ethics Committee
NZ New Zealand
NRV Nutrient Reference Values
NZANS New Zealand Adult Nutrition Survey
NZTDS New Zealand Total Diet Study
RDI Recommended dietary intake
SDT Suggested dietary target
Tg Thyroglobulin
TgAb Thyroglobulin antibodies
TPO Thyroid peroxidase
TPOAb Thyroid peroxidase antibodies
TSH Thyroid stimulating hormone
T3 Triiodothyronine
T4 Thyroxine
µg Microgram
UIC Urinary iodine concentration
UIE Urinary iodine excretion
WHO World Health Organization
WOMBI Women, bread and Iodine study
3DDD Three-day diet diary

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Ethics.

Ethics approval for this study (application 20/28) was obtained in 2020 from the Massey Human Ethics Committee, Southern A (MUHEC).

1 Chapter one - introduction

1.1 Background

Selenium is an essential trace mineral which is of fundamental importance for good health (Rayman, 2002). Selenium is important for a number of physiological processes including antioxidant defence, thyroid function, immune function and energy production (University of Otago & Ministry of Health, 2011). Selenium has also been suggested to have the potential to protect against chronic diseases and cancer (Holben & Smith, 1999; Köhrle, Jakob, Contempré, & Dumont, 2005). The diverse physiological and biochemical functions of selenium are carried out by selenoproteins. There are at least 25 known selenoproteins in the human body, these include glutathione peroxidases and thyroid hormone deiodinases (Michalke, 2018).

Selenium deficiency is of concern as it is associated with suboptimal synthesis or function of one or more of the selenium-containing enzymes (selenoproteins). This leads to a reduction in antioxidant protection, redox regulation, immune function and thyroid hormone metabolism. Severe deficiency may result in classical deficiency signs such as Keshan disease and Kashin-Beck disease which are severe cardiomyopathy and osteoarthropathy diseases found historically in low selenium areas in China. However, moderate selenium deficiency and consequential selenoprotein impairment could contribute to chronic diseases and underlying health concerns such as excessive oxidative stress, inflammation and infections (Combs Jr, 2001).

It is widely accepted that selenium deficiency is associated with impaired thyroid function.

This is because the deiodinases which convert inactive thyroxine (T4) to the active hormone triiodothyronine (T3) are selenoproteins. Consequently, there is potential for reduced enzyme activity and conversion from T4 to T3 during selenium deficiency (Michalke, 2018). Selenium is also required for antioxidant defence in the thyroid. Selenoproteins glutathione peroxidases (GPx) protect the thyroid gland against oxidative damage by removing oxygen free radicals which are generated during thyroid hormone production (Thomson, Campbell, Miller, Skeaff, & Livingstone, 2009).

The consequences of poor selenium status for thyroid hormone metabolism are evident from decreases seen in the ratio of T3:T4 when serum selenium concentrations fall. These decreases are indicative of impaired thyroid hormone balance (World Health Organization & Food and Agriculture Organization of the United Nations, 2004). The effects of selenium deficiency on thyroid hormone metabolism may be most profound in tissues such as the brain and pituitary gland which rely on local production of T3 (Arthur, Nicol, & Beckett, 1992).

Dietary intakes of selenium are dependent upon levels of the mineral in the environment, particularly the soil in which foods are grown and stock are grazed upon. Therefore, selenium intakes can vary significantly between countries due to differences in the soil. New Zealand is known to have low levels of selenium in the soil and therefore the population is at risk for deficiency (Thomson, Vannoort, & Haslemore, 2008).

The main dietary sources of selenium in New Zealand are bread, fish, chicken, meat and eggs (Ministry of Primary Industries, 2018). It is found to be uncommon in western populations that brazil nuts and organ meats (which are the richest sources of the nutrient)

are eaten in any great quantity (Rayman, 2002) and therefore seafood, poultry and wheat are often the greatest contributors of selenium in the diet.

Historically low intakes of selenium have been recorded in New Zealand, however since the recent increase in importation of selenium-rich Australian wheat for bread making (particularly in the North Island of New Zealand) intakes have increased (Rayman, 2012) (Thomson, 2004b). A recent study has suggested that intakes are now adequate for the majority of the population (Ministry of Primary Industries, 2018) however, not only is this a single study using old data but it is also important to note that adequate at a population level does not mean adequacy for all. These increases in dietary intake have mostly been seen in the North Island of New Zealand as bread in the South Island continues to be made with mostly locally-grown, selenium-poor wheat.

Globally and within New Zealand there is a growing movement towards reducing carbohydrate intake. The 'high fat, low carb' diets such as the Atkins and Keto diets are becoming increasingly popular leading to a reduction in bread consumption.

Middle-age women in New Zealand have significantly reduced their bread intake over the past 15 years from a median of 3-4 slices per day down to a median of 1.8 slices per day (Mallard, Gray, & Houghton, 2012) (Brough et al., 2017). Brough et al., (2017) also found that a quarter of post-menopausal women in New Zealand ate less than one serve of bread per day.

This dietary pattern of reduced carbohydrate, and therefore bread, consumption illustrates an emergence of an at-risk group of New Zealanders who choose to avoid commercially prepared bread (Thomson et al., 2008) and therefore could be at an increased risk of selenium deficiency if not obtaining the nutrient from other food sources.

Due to the significant contribution that bread (when made with selenium-rich Australian wheat) has made to selenium intake and rising population status, individuals who avoid bread could be at an increased risk of selenium deficiency and therefore associated impairment of thyroid function.

1.2 Significance of the study

Although the most recent Total Diet Survey (2016) suggested adequate intake in the population, selenium deficiency was previously reported and recent studies by Brough et al., (2017) and Jin et al., (2019) both found that women in New Zealand still had inadequate intakes of selenium. These results support the 2008/09 New Zealand Adult Nutrition Survey which estimated that between 44-72% of women aged 19-50 years have inadequate dietary intake of selenium (University of Otago & Ministry of Health, 2011).

If the New Zealand population is already at an increased risk of selenium deficiency due to low soil levels of the mineral, then it is important to investigate the effect that avoidance of bread could have on selenium status especially as high-fat, low-carbohydrate diets become increasingly popular. It is also important to determine what other food sources are contributing most to selenium intake when bread is removed from the dietary pattern.

There is also a lack of research investigating the likely association between inadequate selenium intake and impaired thyroid function in women in New Zealand.

Currently there have been no studies investigating the selenium status of mid-life women who avoid bread in New Zealand and therefore this study aims to investigate this.

1.3 Aims, Objectives and Hypothesis

1.3.1 Aim

The aim of this study is to investigate if avoidance of bread by mid-life women in New Zealand results in low selenium status and subsequent impaired thyroid function.

1.3.2 Objectives

The objectives of this study are as follows:

- To determine if avoidance of bread by mid-life women results in inadequate selenium intake/ status.
- To investigate dietary sources of selenium in mid-life women who avoid bread
- To investigate the association between selenium status and thyroid hormone levels in mid-life women who avoid bread.

1.3.3 Hypothesis

The hypothesis for this study is that women who avoid bread will have inadequate selenium status, resulting in impaired thyroid function.

1.4 Structure of the thesis

Chapter one is an introduction to the study providing a brief background summary stating the significance of the research, as well as providing the aims and objectives. Chapter two of this thesis is a literature review, exploring the current literature relating to selenium, selenium intakes and status both globally and within New Zealand and the role selenium (and its deficiency) play in thyroid hormone function. Following the literature review, chapter three is a manuscript, prepared for publication, outlining the materials and

methods, results, discussion and conclusion of the study. Finally, chapter four provides a discussion as well as recommendations for future research in relation to selenium and thyroid function.

2 Chapter two - literature review

2.1 What is selenium?

Selenium is an essential trace mineral which is of fundamental importance for good health (Rayman, 2002). Dietary intakes of selenium are dependent upon levels of the element in the environment and therefore intakes vary between countries. Countries such as New Zealand have low levels of selenium in the soil and thus are at a greater risk of deficiency (Thomson et al., 2008). Selenium is essential for normal development, growth, metabolism and endocrine function and has the potential to protect against chronic diseases such as cancer (Holben & Smith, 1999; Köhrle et al., 2005).

The diverse biochemical and physiological functions of selenium are carried out by selenium-containing enzymes called selenoproteins. There are at least 25 known selenoproteins found in the human body, all of which have selenocysteine at their centre (Rayman, 2012). These selenoproteins include thyroid hormone deiodinases, glutathione peroxidases, thioredoxin reductases and many more (Michalke, 2018). The functions of these selenoproteins make selenium an essential dietary nutrient as they are involved in a variety of physiological processes including antioxidant defence, thyroid function, immune function and energy production (University of Otago & Ministry of Health, 2011).

Selenium is often described as a “two-edged sword” (Hartikainen, 2005) as both inadequate and excessive intakes can have adverse health effects. It appears that both selenium deficiency and excess have adverse effects and risk is best described as a “U-shaped” relationship as not only is increased mortality associated with both ends of the dietary

intake spectrum but the window between adequate intake and the upper limit is narrow (Rayman, 2019). Low selenium intake is suggested to contribute to morbidity and mortality worldwide due to development of infections and chronic diseases and it is suggested that an increased selenium intake would be expected to reduce rates of cancer across the globe (Combs Jr, 2001).

2.2 Dietary requirement of selenium.

In New Zealand and Australia the Recommended Dietary Intake (RDI) for selenium in adult women is 60 µg/day. The Estimated Average Requirement (EAR) for the same population group is 50 µg/day. The EAR method can be used to assess population intake of nutrient requirements and the percentage below the EAR approximates the percentage of the population that is at risk of inadequate intake (US Institute of Medicine, 2000).

It is suggested that while intakes of 40 - 50ug/day are required to support maximal expression of selenoproteins, perhaps intakes up to 300ug/day can be more beneficial for health (Combs Jr, 2001). These “supranutritional” levels of selenium intake, i.e. intakes greater than the recommended requirements for selenocysteine enzyme expression, may give additional benefits and protection from disease (Combs Jr, 2001; Rayman, 2002) and have even been suggested to reduce cancer risk (Combs Jr, 2001).

The current recommended upper level of intake for both male and female adults is 400ug/day (Ministry of Health, 2014) meaning that supranutritional levels of intake around 300ug/day would be considered safe. However, few populations have intakes approaching this level, and many do not even meet lower, general recommendations for selenium.

This upper level was also recommended as safe by FAO/WHO, the United States National Academy of Sciences and the UK Expert Group on Vitamins and Minerals (WHO, 2001).

It is important to note that selenium status cannot be assessed in isolation as the intake of other nutrients should be considered when determining selenium requirements. For example, if a population is well nourished with good intakes of other antioxidant nutrients such as Vitamin E then it is possible that their requirement for selenium may be lower than the case for selenium deficient populations such as those described in areas of China (Rayman, 2008). It is also suggested that for populations with concurrent iodine deficiency, selenium intake should not be increased before iodine status is optimised, as there may be adverse consequences for brain development (Rayman, 2008).

2.3 Health consequences of deficiency.

The most overt cases of selenium deficiency have resulted in two endemic diseases, both reported in low selenium areas in China and Eastern Siberia. Keshan disease is a cardiomyopathy occurring mostly in children and women of child-bearing age. Kashin-Beck disease is an osteoarthropathy which results in enlarged joints, shortened fingers and toes and in some extreme cases has been associated with dwarfism (Hartikainen, 2005). Both conditions have been found to occur at selenium intakes averaging no more than 11 µg/day (Institute of Medicine, 2000) and are thought to require cofactors such as low iodine status which are most likely more important than selenium status in the development of the conditions (Rayman, 2002). There have been a small number of cases of Keshan disease reported in clinical settings where patients are exclusively nourished long-term using either parenteral or enteral nutrition formulas which are inadequate in selenium (Masanobu, Yasuko, Shinsuke, Yuri, & Shinobu, 2018).

However, excluding clinical settings, there is no record of these diseases outside of China and Eastern Siberia, even in countries such as New Zealand where soils are well understood

to have low levels of selenium (Rayman, 2002). While both Keshan and Kashin-Beck diseases are uncommon in New Zealand, less-overt selenium deficiency is becoming of increasing concern as it is well established that selenium deficiency can have adverse effects on health (Rayman, 2002).

Selenium deficiency is associated with suboptimal synthesis or function of one or more of the selenium-containing enzymes (selenoproteins). This leads to a reduction in antioxidant protection, redox regulation, immune function and thyroid hormone metabolism. These impairments may not result in classical deficiency symptoms however could contribute to chronic diseases and underlying health concerns such as excessive oxidative stress, inflammation and infections (Combs Jr, 2001).

2.4 Selenium's roles in cardiovascular disease, cancer and immunity.

One of selenium's most widely accepted physiological roles is its antioxidant function. Selenium-containing glutathione peroxidases (GPx) protect human tissues from oxidative damage caused by hydrogen peroxide (Michalke, 2018). Diseases such as skeletal muscle myopathies are suggested to be associated with a loss of these antioxidant functions and therefore an increase in free radical-induced cell damage (Arthur et al., 1992).
As demonstrated in Keshan disease, selenium deficiency can significantly impact the cardiovascular system (Michalke, 2018). Potential benefits of selenium to cardiovascular health are due to the roles that selenoproteins play in preventing oxidative modification of lipids, inhibiting platelet aggregation and reducing inflammation (Rayman, 2012). Selenium acts as both an antioxidant and an anti-inflammatory agent thus influencing cardiovascular health (Rayman, 2002).

Some prospective studies have provided evidence that selenium supplementation has a beneficial effect on cancer, in particular cancers of the lungs, bladder, liver, oesophagus, thyroid and prostate (Rayman, 2012). The Nutritional Prevention of Cancer (NPC) trial also found that supplementation of selenium in non-deficient individuals could be effective in reducing cancer risk in a randomized, double-blind, placebo-controlled trial (Combs Jr, 2001). Thus, it is now accepted that supranutritional intakes of selenium (discussed in a later section) can be anti-tumourigenic. This anti-tumourigenic effect is associated with intakes of selenium at least 10 times higher than levels required to prevent clinical signs of deficiency (Combs Jr, 2001).

Adequate selenium intakes are also associated with good immune function. High selenium intakes have been found to have an immunostimulant effect, enhancing T-cell proliferation and increasing cytotoxic lymphocyte-mediated tumour cyto-toxicity which is often compromised in cancer patients (Rayman, 2012). In selenium deficiency cell-mediated immunity is impaired and supplementation with selenium, even in non-deficient individuals, has been shown to have immune-stimulant effects such as increased proliferation of activated T-cells (Rayman, 2002).

2.5 Selenium and thyroid function.

The thyroid gland has the highest concentration of selenium per gram of tissue of all organs in the human body (Rayman, 2012) making it evident that selenium plays a vital role in thyroid function. One of selenium's key roles in thyroid function is in hormone metabolism (Rayman, 2012). The iodothyronine deiodinases which convert inactive thyroxine (T4) to the active hormone triiodothyronine (T3) are selenoproteins and thus selenium plays a vital role in hormone synthesis and activation (Michalke, 2018). Selenium deficiency therefore decreases the synthesis of active thyroid hormones. Selenium is also required for antioxidant defence in the thyroid. Selenoproteins glutathione peroxidases (GPx) protect the thyroid gland against oxidative damage by removing oxygen free radicals which are generated during thyroid hormone production (Thomson et al., 2009).

It was discovered that some of the biochemical changes found in hypothyroidism bear resemblance to those changes found in selenium deficiency, leading to the investigation of the effects of selenium deficiency on thyroid function (Arthur et al., 1992). It is now widely accepted that selenium deficiency is associated with impaired thyroid function.

Selenium deficiency impairs thyroid hormone metabolism through decreased conversion of T4 to T3 due to inhibited iodothyronine deiodinase synthesis and function (Arthur et al., 1992). The consequence of poor selenium levels for thyroid hormone metabolism is evident from decreases in the ratio of T3:T4 when serum selenium levels fall, indicative of impaired thyroid hormone balance (World Health Organization & Food and Agriculture Organization of the United Nations, 2004). The effects of selenium deficiency on thyroid hormone metabolism may be most profound in tissues such as the brain and pituitary gland which rely on local production of T3 (Arthur et al., 1992). Selenium deficiency also leads to

increased oxidative stress on the thyroid gland due to reduced selenium-dependant GPx antioxidant activity (Thomson et al., 2009).

Selenium deficiency can exacerbate the consequences of iodine deficiency (Rayman, 2002) which is of particular concern in New Zealand where there is dietary insufficiency of both elements (Jin, Coad, Weber, Thomson, & Brough, 2019). Selenium dependant glutathione peroxidase antioxidant enzymes are required to degrade harmful hydrogen peroxide which accumulates in the thyroid gland in iodine deficiency (Schomburg & Kohrle, 2008). When hydrogen peroxide is not degraded efficiently then the thyroid gland can become damaged. Thus, if an individual is deficient in selenium then the consequences of even mild iodine deficiency are exacerbated.

2.6 Dietary sources of selenium globally.

As selenium is found in the environment, selenium intake varies significantly between different geographic locations, and both environmental conditions and varying agricultural practices have a significant influence on the selenium content of food sources (World Health Organization & Food and Agriculture Organization of the United Nations, 2004). The selenium content of plants is dependent upon not only the selenium levels in the soil in which they are grown but also factors which determine the availability of the nutrient to the food chain (Rayman, 2012). One such factor is selenium speciation (Navarro-Alarcon & Cabrera-Vique, 2008). Selenite is less soluble and therefore more well absorbed than selenate (Rayman, 2008). Other factors influencing availability include the pH of the soil and the presence of ions which can adsorb or bind with selenium, therefore inhibiting uptake by the plant. These commonly include iron hydroxides and clay minerals (Rayman, 2008). These factors influencing availability are well illustrated by data from areas in China

where Keshan disease is recorded. In the Hebei Province of China there is actually a high soil content of selenium however, the pH of the soil is low and there are high levels of organic matter resulting in low bioavailability, low uptake of the nutrient and therefore resulting in deficiency disease (Rayman, 2008). Some plants are known to absorb more selenium from the soils than others. Brazil nuts and vegetables from the cruciferous/ brassica and allium families (Broccoli, cauliflower, garlic and onion etc.) are known to be selenium accumulators (Ministry of Primary Industries, 2018).

While the selenium content of plants is directly affected by the soil in which they are grown, the selenium content of animal products is determined by the selenium levels in their feed. Selenium supplementation of stock is now common practice in commercial agriculture due to the adverse effects of selenium deficiency on livestock (Combs Jr, 2001).

The richest dietary sources of selenium are brazil nuts, kidney and other organ meats and some seafoods (Rayman, 2008). Brazil nuts in particular, are said to be the richest known food source of selenium (Thomson, Chisholm, McLachlan, & Campbell, 2008). Although there is large variation in the selenium content of all foods, it is estimated that, on average one brazil nut alone contains 48 µg of selenium. In comparison, half a fillet of baked Hoki fish contains approximately 84 µg (NZ Nutrition Foundation, 2018) demonstrating the high selenium content of seafood. The most common dietary sources in Western populations are seafood, meat and wheat (Kodali, 2018). It is said to be unlikely in western populations that brazil nuts and organ meats (which are the richest sources of the nutrient) are eaten in any great quantity (Rayman, 2002) and therefore seafood, poultry and wheat are often the greatest contributors of selenium in the diet.

2.7 Dietary sources of selenium specific to NZ and current selenium intakes in NZ.

In the 1950s it was found that white muscle disease and ill thrift in cattle and sheep throughout New Zealand was due to selenium deficiency in the soils. Since this discovery extensive research has been carried out looking into the selenium status of New Zealanders and the effects of selenium supplementation. Historically, selenium intakes in New Zealand have been low and research carried out in New Zealand from as early as the 1980s found intakes as low as 46µg/day (Ministry of Health, 2000). Most of the research to date indicates that the New Zealand population still has poor selenium intake and status (Thomson, 2004b) with the exception of the latest total diet survey (TDS) (2016) which suggests adequacy of intake (Ministry of Primary Industries, 2018).

The most recent Adult Nutrition Survey (2008/09) found that between 1997 and 2008/09 selenium intakes had increased in New Zealand, however were still considered inadequate for over half of female adults (University of Otago & Ministry of Health, 2011). The survey found that on average dietary selenium intake was inadequate throughout New Zealand, especially for females where the prevalence of inadequate intake was 58% (University of Otago & Ministry of Health, 2011).

Conversely, the more recent NZ Total diet survey (NZTDS) carried out in 2016 found that dietary intake of selenium is adequate and that the New Zealand population is currently consuming sufficient dietary selenium to meet nutritional needs (Ministry of Primary Industries, 2018). It is evident that there is an increasing trend in selenium intake in both male and female adults in NZ. The 2016 Total Diet Survey suggests that this increasing trend is not actually due to an increase in selenium concentrations of the food supply but instead due to an increase in daily consumption of selenium-rich foods such as meat. For example, it

is estimated that between the 2009 NZTDS and the 2016 NZTDS the average New Zealander has increased their consumption of chicken from 47g per day to 58g per day (Ministry of Health, 2011). These changes in dietary patterns seem to have resulted in a subsequent increase of dietary intake of selenium however, the TDS estimates intakes based on standard diets and therefore it could be argued that this is less valid than measuring actual intakes and status.

The 2008/9 NZ Adult Nutrition Survey found that bread and bread-based dishes were together the largest contributor of dietary selenium (15% and 7% respectively) followed by fish & seafood (12%) and poultry (10%) (University of Otago & Ministry of Health, 2011). For non-meat eaters the predominant dietary sources were bread and imported grains such as legumes and pasta.

The 2016, 2009 and 2003/4 New Zealand Total Diet Surveys (NZTDS) all also highlight the significant contribution of bread and grains to selenium intake across all age groups (Ministry of Primary Industries, 2018).

The 2003/4 NZTDS analysed 121 different foods and found that selenium concentration ranged from 0.002 to 0.64mg/kg. The same study found that New Zealand mussels contained the highest concentration of selenium and that overall, seafood in general had higher levels of the nutrient than other food sources (Thomson, Vannoort, & Haslemore, 2008).

The contribution of bread and other cereals to dietary selenium intakes in NZ varies significantly with the source of the crop (Thomson, 2004b). Bread is a rich source of selenium particularly in the North Island of New Zealand due to the use of imported,

selenium-rich wheat from Australia which is used in bread-making in the North Island. In the South Island where bread is predominantly made from locally grown wheat, selenium intake is typically lower (Thomson, 2004b). The variance in selenium content of bread was shown in the 1997-98 New Zealand Total Diet Survey. White bread from Dunedin and Christchurch (South Island) had selenium concentrations of approximately 25 mg/kg while breads from Napier and Auckland (North Island) had concentrations of 78-114 mg/kg (Ministry of Primary Industries, 2018). This variance in selenium content of bread was not accounted for in the latest Adult Nutrition Survey as bread is different between the North and South Islands (University of Otago & Ministry of Health, 2011). For this reason the true contribution of bread to selenium intakes across the country is unknown.

Selenium intakes in New Zealand, which were previously low, have increased significantly over the past ten years, following the increase in importation of high-selenium Australian wheat for bread making (Combs Jr, 2001; Rayman, 2012; Thomson, 2004b). There has also been an increased use of selenium supplements in animal feeds in New Zealand in recent years alongside an increase in selenium fertilisation of NZ pastures, commonly with sodium selenite, (Cox & Bastiaans, 2007) which have also contributed to an increased dietary intake of the population (Thomson, 2004b). Chicken feeds now specifically include selenium which explains the contribution of eggs to total selenium intakes (Thomson, Vannoort, & Haslemore, 2008).

2.8 Association between avoidance of bread and risk of deficiency.

Considering bread is a main contributor of dietary selenium for most New Zealand women there is a probable increased risk of deficiency with reduced bread consumption. In general, bread consumption has decreased over the past 15 years from a median of 3-4 slices per

day to 2 slices per day in middle-age women in New Zealand (Mallard et al., 2012). Further research (Brough et al., 2017) found that median bread intake is low at only 1.8 serves per day and a quarter of the study participants consumed less than 1 serve per day. It is also found that females typically eat less bread than males (Thomson et al., 2008) and therefore are more at risk of deficiency if selenium is not consumed from other food sources.
This dietary pattern of reduced bread consumption illustrates an emergence of an at-risk group of New Zealanders who choose to avoid commercially prepared bread (Thomson et al., 2008) and therefore could be at an increased risk of selenium deficiency if not obtaining the nutrient from other food sources.

Research into consumers knowledge of selenium found that cereal products such as bread are not well-known sources of selenium (Cox & Bastiaans, 2007) and thus the population are not aware of the associated consequences of reduced bread consumption.

Worldwide and within New Zealand there is a growing social movement towards reducing carbohydrate consumption and the 'high fat, low carb' diets are becoming more popular (Gunnarsson & Elam, 2012). Although there is a lack of research investigating bread consumption habits in New Zealand women, anecdotally we know that the avoidance of bread has become more popular in recent years, particularly amongst this demographic.

2.9 Assessment of selenium status.

Biomarkers typically used to assess selenium status include blood (whole, plasma or serum), GPx levels and urine, although hair and nails are sometimes used as well. Approximately half of the body's selenium is contained in the blood system, making it a useful biomarker.

Plasma and serum concentrations have been found to reflect short term selenium intake (several days) while erythrocyte selenium reflects longer-term intake (up to several months) (Thomson, 2004b). Blood selenium concentrations are the most common biomarkers used in research and are considered the most effective biomarker for selenium status at both an individual and population level (Phiri et al., 2020).

The different selenoproteins have various optimal concentrations of selenium and therefore it is difficult to determine an 'optimal' plasma concentration for the nutrient. It is understood that a higher selenium concentration is required to saturate selenoprotein P than to optimize GPx. It is suggested that concentrations greater than 65µg/L optimize activity of iodothyronine 5' deiodinases (Thomson, McLachlan, Grant, Paterson, & Lillico, 2005; Winther, Rayman, Bonnema, & Hegedüs, 2020) while higher concentrations are required for saturation of GPx activity (>95ug/L) (Thomson, Robinson, Butler, & Whanger, 1993) and maximal expressions of Selenoprotein P (>110ug/L) (Hurst et al., 2010). Concentrations below 65µg/L indicate that selenium intake is inadequate (Institute of Medicine, 2000). Toxic effects of selenium are found to occur when selenium concentrations are greater than 1003 µg/L, which corresponds with an intake greater than 850 µg/day (Institute of Medicine, 2000).

Selenium status can also be measured indirectly by measuring activity of individual selenoproteins such as Glutathione Peroxidase (Stefanowicz et al., 2013). GPx measures are said to be the 'gold standard' for assessing selenium status. Glutathione Peroxidases are measured in erythrocytes (GPx1) or plasma (GPx3). Measuring individual selenoproteins such as GPx1 or GPx3 provides more accurate information than selenium intake alone (Thomson, 2004b) as it assesses the antioxidant activity of selenium. Due to the hierarchy of

biological activities, biomarkers such as GPx provide information about functional use of selenium and identify nutritional selenium inadequacy (Combs, Jr., 2015). GPx activity is found to correlate with plasma selenium concentrations up until a threshold level after which the activity of the enzyme is said to plateau at optimum activity (Stefanowicz et al., 2013). For this reason GPx is more beneficial when assessing states of deficiency than when assessing toxicity. Although GPx measures are the gold standard for assessing status they have not been used in this research project due to cost and low stability which would affect the reliability of the test.

Urine is the main route of excretion of selenium, primarily in the form of selenosugar, and it is estimated that 50 to 60% of selenium is excreted this way (Robinson, McKenzie, Thompson, & Van Rij, 1973). Using urinary excretion to assess intake is considered to be more accurate than dietary assessment of selenium and a strong correlation has been established between urinary selenium and dietary intake for a wide variety of populations, (Thomson, Smith, Butler, & Packer, 1996). Dietary intake assessments for selenium are seldom accurate or precise due to differences in geography, food availability and agricultural practices which are difficult to determine for each individual (Combs Jr, 2001). It has been found that for most subjects, doubling the 24 hour urinary excretion of selenium provides a reasonably accurate estimate of intake (Thomson et al., 1996). Due to variation in hydration-driven urinary flow rate, as well as diurnal variation, a 24-hour urine collection is required for accurate measurement of selenium. However a 24-hour urine collection can have logistical challenges for subjects and therefore spot urinary measures are often used. Studies which have used spot urine samples have found challenges determining status due to variations in hydration status and fluid intake and researchers often apply corrections

using creatinine to account for the effect of hydration status (Phiri et al., 2020). There are pronounced differences in urinary excretion between males and females. There also appears to be an influential effect of muscle mass on urinary selenium excretion, most likely due to the fact that nearly 50% of whole body selenium is found in the muscle (Oster & Prellwitz, 1990). For these reasons, the use of the selenium-creatinine ratio is justified for assessment of excretion (Thomson et al., 1996).

Nail clippings are gaining popularity for use as a biomarker of selenium status due to their logistical advantage, particularly for large epidemiological studies. Toenails are an easy and non-invasive measure and represent longer-term exposures compared with other biomarkers. A correlation has been found between selenium content in toenails and selenium status however due to the slow rate at which nails grow, this biomarker provides a time-delayed measure of selenium status. Researchers estimate that the level of selenium measured in toenail clippings is representative of the dietary exposure which occurred 6-12 months earlier (Gutierrez-Gonzalez et al., 2019). This method is useful for assessing past exposure periods which is beneficial for studying the involvement of selenium in the development of chronic disease (Gutierrez-Gonzalez et al., 2019).

Another functional biomarker for selenium status is measuring thyroid hormones, commonly expressed by T4:T3 ratio (Thyroxine: Triiodothyronine) (Combs, Jr. et al., 2009). Blood can be used for assays of serum free T3 and T4 (Thomson et al., 2005). As discussed previously, the deiodinases which convert T4 to T3 are selenoproteins and therefore this conversion relies on good selenium status. Serum concentrations of T3 have been found to be positively correlated to selenium status and deficiency has been found to increase circulating concentrations of T4 and/ or decrease concentrations of T3 (Combs, Jr. et al.,

2009). Therefore, a reduction in selenium status often leads to an elevation in the T4:T3 ratio. This occurs via a build-up of T4 as there is limited conversion to T3 (Olivieri et al., 1996). By measuring levels of both T3 and T4 and determining their ratio the adequacy of selenium for optimal function can be assessed.

Thyroid stimulating H=hormone (TSH) and thyroglobulin are also useful biomarkers for assessing thyroid hormone profile (Ma & Skeaff, 2014). Thyroglobulin (Tg) is a glycoprotein which plays a vital role in the synthesis of both T3 and T4 (Lamas, Anderson, Fox, & Dunn, 1989). During periods of selenium insufficiency, low levels of circulating T4 stimulate the release of thyrotropin-releasing hormone from the pituitary gland, which subsequently increases the production of TSH. TSH increases the synthesis of Tg and therefore selenium deficiency causes an increased amount of Tg to be released into the bloodstream (Ma & Skeaff, 2014).

While there are many methods for assessing status, it appears that varying plasma concentrations of selenium are required for optimal function of various selenoproteins, suggesting a hierarchy of importance and prioritisation of these selenoproteins. (Thomson et al., 2007). This makes the diagnosis of inadequacy difficult as intake may be adequate for optimal function of one selenoprotein but not another.

2.10 Status in New Zealand.

Even though the most recent NZTDS (2016) found that average selenium intakes in New Zealand are adequate to prevent deficiency, there is a lack of research to determine if these adequate intakes are resulting in adequate selenium status across the population. It appears however that researchers are in agreement that selenium status in New Zealand was

previously considered suboptimal (Rayman, 2012; Thomson, 2004b; University of Otago & Ministry of Health, 2011).

In 2008 Thomson et al., reported that despite improvements in intakes, the selenium status of the New Zealand population is marginal and blood concentrations remain lower than those measured in many other western countries. It has also been found that supplementation with selenium results in increased GPx activity and therefore it is argued that selenium intakes are inadequate for optimal functioning of GPx and other selenoproteins (Thomson et al., 2008).

A recent study carried out in the North Island of New Zealand found that mean and median values of urinary selenium excretion were 34 and 31.6µg/ day respectively (Shukri, 2011). These values are consistent with other findings showing that status is higher in the North Island than the South Island, where the mean ranges between 9.4 to 18.7µg/ day (Robinson, Thomson, Jenkinson, Luzhen, & Whanger, 1997; Shukri, 2011).

Another New Zealand study from 2004 found that plasma selenium levels in Otago residents (South Island) range from 0.76-1.65 mmol (60-130mg)/l while mean concentrations from North Island residents (Waikato and Taranaki) were 1.08 mmol (85mg)/l and 1.38mmol (109mg)/l respectively (Thomson, 2004b). This observation of a clear geographical variation demonstrates the inconsistency in status between the North and South Islands.

Due to the large variability in soil content of selenium it is difficult to establish a standard reference range (Thomson, 2004a) and therefore it is difficult to determine whether the population of New Zealand has an adequate selenium status or not.

2.11 Current research relating to selenium in New Zealand women.

Recently both iodine and selenium intakes were investigated in 97 healthy women aged 50-70 years living in the North Island of New Zealand (Brough et al., 2017). Selenium intake was estimated using urinary excretion and it was determined that median intake was 50µg/day which is below the current RDI of 60µg/day. It was found that 49% of participants had intakes below the EAR (50µg/day) demonstrating dietary inadequacy in the population.

Another study investigated selenium intakes of 59 pregnant and 68 lactating women in the North Island of New Zealand using three repeated 24 hour diet recalls, urinary excretion and breastmilk excretion to determine selenium intake of these women. This study found that approximately 60% of pregnant and 68% of lactating women had estimated intakes below the EAR (55µg/day for pregnancy and 65µg/day during lactation) (Jin et al., 2019). These results agree with the suggestion made by Brough et al., (2017) that women in New Zealand are at risk of selenium deficiency.

A further study looking into selenium intake and status of 87 postpartum women in New Zealand found median selenium status was measured at 105.8µg/L. Therefore, all women had adequate selenium status for GPx activities, however 59% of participants did not achieve the suggested plasma selenium concentration for expression of Selenoprotein P (110µg/L) (Jin, Coad, Pond, Kim, & Brough, 2020).

All three of these studies support the most recent New Zealand Adult Nutrition Survey (2008/09) which estimated that between 44-72% of women aged 19-50years have inadequate dietary intake of selenium (University of Otago & Ministry of Health, 2011).

An earlier research paper compared data from five different studies (two observation studies and three interventional) to investigate the relationship between selenium, iodine and thyroid status in the New Zealand population (Thomson et al., 2005). Across the studies it was found that blood selenium concentrations were higher in the North Island than the South Island, due to the differing origins of wheat. There were positive relationships found between selenium intake and selenium status at baseline which were further strengthened after supplementation. There were also positive relationships found between selenium status and GPx activities, again indicating that selenium status in NZ is inadequate for optimal selenoprotein activity (Thomson et al., 2005). Across three of these studies a reduction in T4 levels was measured following selenium supplementation and in all of the studies a negative relationship was found between selenium status and T4 levels. These results indicate a small effect of adequate selenium status on T4 levels, highlighting the requirements for selenium for deiodinase function and therefore conversion of T4 to T3. Other than minor changes in T4 levels, there was a lack of significant associations between plasma selenium status and thyroid status across these studies. This is suggested to indicate that selenium status in New Zealand is sufficient for deiodinase function, however deiodinases rank highly in the hierarchy of selenium supply and are therefore less likely to be affected by deficiency (Thomson et al., 2005).

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3 Manuscript

The methods, results and discussion of this study are presented in the form of a manuscript for publication in a peer-reviewed journal.

3.1 Abstract.

Selenium is an important nutrient for thyroid health, unfortunately the New Zealand food supply is generally low in selenium due to the poor content of the soil. Bread made using selenium-rich Australian wheat is a good dietary source of the nutrient. The avoidance of bread is becoming popular with the rise of lower carbohydrate diets meaning that there is an increased risk of low status and subsequent impaired thyroid function. This study aimed to investigate if avoidance of bread by mid-life women in New Zealand results in low selenium status and subsequent impaired thyroid function

This study is an extension to a previous study which investigated iodine deficiency in women who avoid bread. This study investigated selenium intake (estimated from 3-day diet diaries, urinary excretion and plasma concentration), status (plasma) and thyroid hormone biomarkers (T3, T4, TSH, T3:T4 ratio, Tg, TPOAb) in 37 mid-life women based in Auckland, New Zealand.

Median intake was below the Recommended Daily Intake (60 µg/day) and nearly half of participants had intakes below the Estimated Average Requirement (50 µg/day). Median plasma concentration was 118.9µg/L indicating optimal selenium status, however nearly a third of participants had low status. Impaired thyroid function was only indicated in 2 participants.

Overall, a high prevalence of inadequate intake was observed in women who avoid bread. Subsequently, suboptimal plasma concentrations indicating low status were found in a

number of participants. This study highlighted the importance of consuming alternate dietary sources such as seafood, meat, poultry and nuts on a regular basis if bread is removed from the diet.

3.2 Introduction.

Selenium is an essential trace mineral which is of fundamental importance for good health (Rayman, 2002). Selenium is important for a number of physiological processes including antioxidant defence, thyroid function, immune function and energy production (University of Otago & Ministry of Health, 2011). The diverse physiological and biochemical functions of selenium are carried out by selenoproteins. There are at least 25 known selenoproteins in the human body, these include glutathione peroxidases and thyroid hormone deiodinases (Michalke, 2018).

Selenium deficiency is of concern as it is associated with suboptimal synthesis or function of one or more of the selenium-containing enzymes (selenoproteins). Severe deficiency may result in classical deficiency signs such as Keshan disease and Kashin-Beck disease which are severe cardiomyopathy and osteoarthropathy diseases found historically in low selenium areas in China. However, moderate selenium deficiency and consequential selenoprotein impairment could contribute to chronic diseases and underlying health concerns such as excessive oxidative stress, inflammation and infections (Combs Jr, 2001). It is widely accepted that selenium deficiency is associated with impaired thyroid function. This is because the deiodinases which convert inactive thyroxine (T4) to the active hormone triiodothyronine (T3) are selenoproteins. Consequently, there is potential for reduced enzyme activity and reduced production of T3 during selenium deficiency (Michalke, 2018).

Selenium is also required for antioxidant defence in the thyroid. Selenoproteins glutathione peroxidases protect the thyroid gland against oxidative damage by removing oxygen free radicals which are generated during thyroid hormone production (C Thomson et al., 2009). The consequences of poor selenium status for thyroid hormone metabolism are evident from decreases in the ratio of T3:T4 when serum selenium concentrations fall. These decreases are indicative of impaired thyroid hormone balance (WHO & FAO, 2004). The effects of selenium deficiency on thyroid hormone metabolism may be most profound in tissues such as the brain and pituitary gland which rely on local production of T3 (Arthur et al., 1992).

Dietary intakes of selenium are dependent upon levels of the mineral in the environment, particularly of the soil in which foods are grown and stock are grazed upon. Therefore, selenium intakes can vary significantly between countries due to differences in the soil. New Zealand is known to have low levels of selenium in the soil and therefore the population is at risk for deficiency (Thomson et al., 2008). The main dietary sources of selenium in New Zealand are bread, fish, chicken, meat and eggs (Ministry of Primary Industries, 2018). It is not common for brazil nuts or organ meats (which are the richest sources of the nutrient) to be eaten in any great quantity in western populations (Rayman, 2002) and therefore seafood, poultry and wheat are often the greatest contributors of selenium in the diet. Historically low intakes of selenium have been recorded in New Zealand, however since the recent increase in importation of selenium-rich Australian wheat for bread making (particularly in the North Island of New Zealand) intakes have increased (Rayman, 2012; Thomson, 2004b).

Globally and within New Zealand there is a growing movement towards reducing carbohydrate intake. The 'high fat, low carb' diets such as the Atkins and Keto diets are becoming increasingly popular leading to a reduction in bread consumption.
Middle-age women in New Zealand have significantly reduced their bread intake over the past 15 years from a median of 3-4 slices per day down to a median of 1.8 slices per day (Brough et al., 2017; Mallard et al., 2012). Per slice, bread made with Australian wheat contributes approximately 3.3 – 5.2µg of selenium (Food Standards Australia & New Zealand, 2019) meaning that a reduction of 2 slices per day reduces intake by up to 10.4µg/day. Brough et al., (2017) also found that a quarter of post-menopausal women in New Zealand ate less than one serve of bread per day. This dietary pattern of reduced carbohydrate, and therefore bread, consumption illustrates an emergence of a potentially at-risk group of New Zealanders who choose to avoid commercially prepared bread (Thomson et al., 2008) and therefore could be at an increased risk of selenium deficiency if not obtaining the nutrient from other food sources.

This study aimed to investigate if avoidance of bread by mid-life women in New Zealand resulted in low selenium intake and status and subsequent impaired thyroid function.

3.3 Materials & Methods:

3.3.1 Study design

This cross-sectional study was an extension of the "Women, bread and Iodine" (WOMBI) study which was conducted in Auckland in 2016. The WOMBI study was designed to investigate iodine intake and status in midlife women who consume low amounts of bread.

3.3.2 Recruitment of participants

Participants were recruited for the WOMBI study via poster, email and social media advertising. Potential participants expressed their interest by registration on Massey University's website and this was followed up via an email with an information sheet attached. Eligibility was assessed using a screening questionnaire. At this time the participant requirements were also outlined to each potential participant and recruits were assured that all personal details and responses would be kept private and confidential.

For the extension to the study, participants were contacted again via email with a new information sheet and consent form to request their permission to analyse selenium in their previously collected biological samples (blood and urine). Further emails and follow up phone calls were made to any participants who did not respond to the initial email.

3.3.3 Study sample

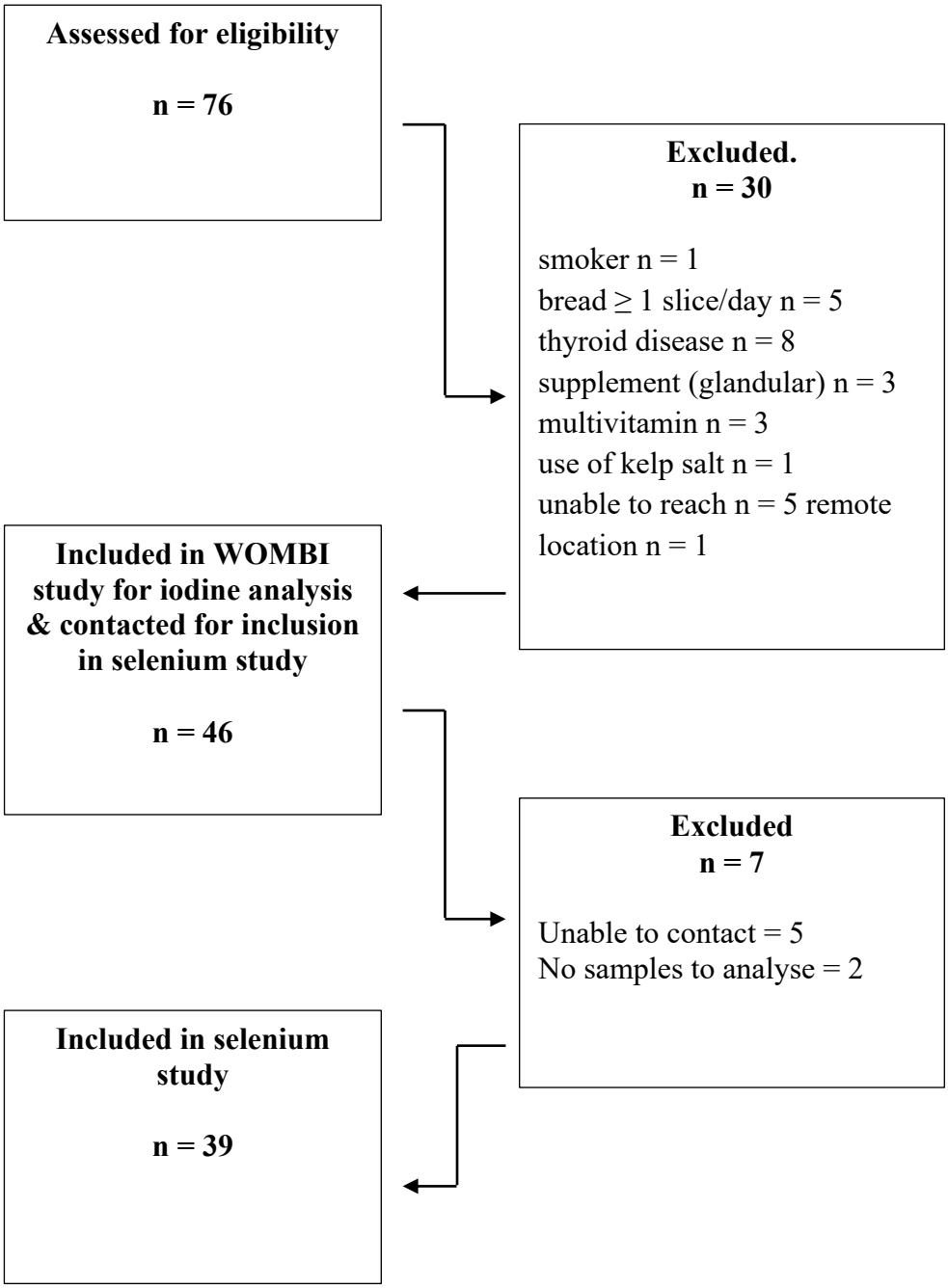
Women living in Auckland, New Zealand aged between 40 to 65 years of age who reported eating less than one slice of bread per day were sought for participation in this study.

A total of 76 women registered for the WOMBI study, 30 of these were excluded (see figure 3.1). The reasons for exclusion were as follows; eating more than one slice of bread per day

(5), smoking (1), taking thyroid medication/thyroid disease (8), remote location (1), unable to contact or could not complete interview (5), the use of porcine derived thyroid extract (3) and other (3). As the original study investigated iodine status, further exclusions were made as follows; taking supplements containing more than 10µg iodine (3) using kelp salt (contains iodine) on a daily basis (1). One potential participant was excluded only after interest in the study prompted a visit to her General Practitioner who subsequently diagnosed thyroid disease.

A total of 46 participants were included in the WOMBI study and 41 of these were included in the study extension investigating selenium (see figure 3.1). Researchers were unable to contact the other 5 participants and therefore, they were excluded from this study.

Figure 3. 1 Flow chart for Participant Inclusion.



3.3.4 Ethics

Ethical approval was obtained from Massey University Human Ethics Committee; Southern A (MUHEC) in 2016 for the original study (application number 16/52). Additional ethics approval was obtained from MUHEC in 2020 (application number 20/28) to contact all participants requesting consent for further analysis. A full explanation of the study was provided, and written consent was obtained from all participants prior to inclusion in this extension to the study. Biological samples from any participants who did not provide consent were not analysed for selenium or thyroid hormones.

3.3.5 Three-day diet diary (3DDD)

Each participant completed a three-day diet diary (3DDD) as an estimated record of food eaten during this timeframe. Participants were asked to estimate portion size and to record as much information as possible regarding method of cooking, brand and type of food. Clear instructions and examples of food records were provided to the participants.

Each 3DDD was entered into the food composition database; Foodworks professional package for windows version 10 (Xyris software (Australia) Pty Ltd, 2019) using the standard database for New Zealand (NZ foodfiles 2016). Each 3DDD was used for analysis of initially iodine and subsequently selenium. Daily selenium intakes and food sources of selenium were determined by Foodworks.

One of the 46 diet diaries was lost in transit during the initial analysis for iodine and therefore, data for only 45 of the 46 participants was available and included in dietary analysis.

3.3.6 Data collection

Eligible participants visited the Massey University Nutrition Research Unit, Auckland, New Zealand for a 30-45 minute session for data collection. At this appointment an explanation was given regarding the 3DDD, the urine collection procedure, and then both the blood draw and anthropometric measurements were obtained.

No information was collected regarding level of education, ethnicity, employment status or living situation as they were not relevant to this study at the time of data collection.

3.3.7 Anthropometric data

Participants' height was determined using a stadiometer (Seca 213). Weight was recorded to the nearest gram and BMI was calculated using weight (kg) / height (m2). Participants were asked to remove bulky clothing and shoes prior to measurements being taken.

3.3.8 Blood samples

A blood draw of 20 ml was taken from each participant's antecubital fossa by a qualified phlebotomist at the Massey University research unit. These non-fasting blood samples were separated into plasma and serum and stored at -80°C.

Blood samples from 38 participants were used for analysis of selenium (plasma Se) and thyroid hormones; free T4, free T3, thyroid stimulating hormone (TSH), thyroglobulin (Tg) and anti-thyroglobulin antibodies (anti-Tg). Out of the 41 participants, 3 had not provided blood samples in the original data collection.

These 38 blood samples (both plasma and serum) were sent to an accredited commercial laboratory; Canterbury Health Laboratory (Christchurch, NZ) for analysis of both selenium and thyroid biomarkers.

Plasma selenium concentration was determined by inductively coupled plasma mass spectrometry at Canterbury Health Laboratories, New Zealand. It has been suggested that plasma selenium concentrations of 65 – 71 μg/L are required for optimal deiodinase function (C. D. Thomson et al., 2005; Winther et al., 2020) and of 95 μg/L to saturate GPx activity (C. Thomson et al., 1993). Hurst et al (2010) even suggest that concentrations greater than 110 μg/L are needed to maximise expression of selenoprotein P and therefore plasma levels above 110μg/L are considered optimal for the expression and function of all three selenoproteins.

Thyroid hormone biomarkers: serum free T3, free T4 and thyroid stimulating hormone (TSH) were measured via chemiluminescent microparticle immunoassay (CMIA). Thyroid peroxidase antibody (TPOAb) concentrations above 10 IU/mL indicates a potential autoimmune disorder. Reference ranges for euthyroid are: TSH, 0.40 – 4.0 mIU/L; free T4, 10 – 24 pmol/L, and free T3 2.5 – 6.0 pmol/L. These reference ranges were used to determine the prevalence of thyroid dysfunction including subclinical hypothyroidism (TSH > 4.00 mlU/L and normal free T4), overt hypothyroidism (TSH > 4 mlU/L and free T4 <10 pmol/L), subclinical hyperthyroidism (TSH < 4 mlU/L and normal T4) and overt hyperthyroidism (TSH < 4 mlU/L and free T4 > 24 pmol/L) (Hunt, 2010).

Serum thyroglobulin (Tg) was assessed by the Beckman Coulter Access method and anti-thyrogloblin antibodies were assessed by the CMIA method at Canterbury Health Laboratories, Christchurch, New Zealand. The Tg value was discounted for any participants

with a TgAb concentration greater than 115 IU/mL as there was a high likelihood of the Tg value being artificially lowered by the Tg antibodies.

3.3.9 Urine collection and processing

Participants were provided with equipment to collect a 24-hour urine sample and were given a thorough explanation. The equipment provided included insulated cooler bags, frozen silica pads, a funnel and four by one L urine collection bottles, previously labelled with the participants' identification number. Clear instruction sheets were provided explaining the procedure for collection of the 24-hour urine sample which were as follows:

- *Collection day 1: participants were asked to void the first morning urine specimen into the toilet (i.e. not retain this specimen) and record the time. This was recorded as the start time of the collection.*
- *All urine voided after this time and for the next 24 hours was retained and poured into the collection bottles provided and kept cool. Participants were also instructed to retain any urine voided during a bowel motion.*
- *Collection day 2: the following day at the same time as the first urine void of the previous day the final and only sample for that day was collected. This was recorded as the time of completion.*

Participants were requested to keep the sample cool at all times using the cooler bags provided. Upon completion and on the same day participants transported the specimens to the Nutrition Research unit where the samples were processed without delay.

On receipt of the samples at the research unit the entire 24 hour urine sample from each participant was poured into large measuring cylinders and the total urine was measured and

recorded to the nearest ml. 60ml was retained from each sample and this was then poured into 2 x 30ml sterile and labelled sample tubes with the date and participant's identification number. The remaining urine sample was discarded. Samples were then transferred to the laboratory freezer, and stored in batches at -80°C until analysis.

Of the 41 participants, 2 had not provided urine samples in the original data collection. The 39 urine samples were sent to an accredited commercial laboratory; Hills Laboratory (Hamilton, NZ) for analysis of both selenium and iodine using inductively coupled mass spectrometry (ICP-MS). Quality control measures included analysis of blanks, analytical repeats and certified reference samples in order to ensure accuracy and precision. On every run calibration standards and checks were undertaken with the limit of detection at 0.002 mg/kg for selenium and 0.001 mg/kg for iodine. Each batch of samples was analysed together with an external reference standard (Seronorm Trace Elements Urine, L-2, Norway) giving a mean (SD) selenium concentration of 74 (5) µg/L (published value and 95 % confidence error: 71.7 +/- 14.4 µg/L) with a coefficient of variance (CV) of 6.5% (n=18) and a mean (SD) iodine concentration of 283 (13) µg/L (published value: 297 µg/L) with a coefficient of variance (CV) of 4.8% (n=12).

Daily selenium intakes were estimated by extrapolation of 24-hour urinary excretion based on the estimation that 55% of dietary selenium is excreted via urine (Thompson, 2004 & Brough, 2017). The mean urine volume collected was 2.18L (SD = 0.81).
A selenium: creatinine ratio can be used when estimating selenium intake from urine samples to allow for diurnal variation which can affect selenium concentration in spot samples of urine. As the samples for this study were 24-hour collections, a selenium: creatinine ratio was not required. No participants reported missing the collection of any of

the urine samples within the 24-hour period and all samples were returned on the final collection day in the cooler bags provided.

3.3.10 Statistical analysis

Statistical analysis was performed using SPSS (Statistics Package for the Social Sciences) Version 24 for windows (ICM Corp, 2016). The Shapiro-Wilk test was used to test for data normality. Nonparametric data was expressed as median (25th, 75th percentile), and parametric data was expressed as mean and standard deviation. Plasma selenium concentrations were divided into two groups (≥110µg/L or <110 µg/L) for comparison with other biomarkers by independent t-test. Correlations were tested using the Pearson's or Spearman's correlation coefficient, depending on the distribution of the data. Multiple linear regression was used to determine predictors of T3;T4 ratio. Predictor variables considered were plasma selenium and urinary iodine concentration (UIC), which were entered into the model simultaneously. Reporting of statistical significance for all data was set at a level of $P < 0.05$.

3.4 Results.

3.4.1 Participants.

A total of 46 women from the Auckland region were recruited for the original study on iodine status. A total of 41 participants gave consent for their biological samples to be re-analysed for selenium. Out of these, only 39 participants provided 24-hour urine samples and 38 provided blood samples. Full data sets were available for 37 participants (diet diary, urine and blood samples).

The women were aged between 40 and 63 years with a mean age of 50.8 (±6.0) years. The mean body mass index (BMI) of the participants was 27.4(±5.4) kg/m^2. BMI ranged between

19.3 and 47.6kg/m^2. The mean height and weight of participants was 163.9cm (±6.2cm) and 73.7kg (±16.7kg) respectively, however one participant declined the measurement of weight.

As outlined in the inclusion criteria, all of the participants consumed an average of less than one slice of bread per day. Mean bread consumption was 1.6 (±1.5) serves of bread per week with a range of 0-5 serves per week.

Median urinary selenium concentration was 14 (12, 21) µg/L. Mean urinary iodine concentration (UIC), as measured in the original study, was 58(±30) µg/L.

3.4.2 Selenium intakes estimated from 3-day diet diaries, urine and plasma.

Daily selenium intakes were estimated from three-day diet diaries, urinary excretion and plasma selenium status. Non-parametric data is reported as median (Q1, Q3), whereas parametric data is reported as mean (SD) and median (Q1, Q3) to allow for comparison. Estimations for intake from 3-day diet diaries and urinary excretion showed median intakes below the recommended daily intake (RDI) of 60µg/day. These estimations also found that 49% of participants had intakes below the estimated average intake (EAR of 50µg/day), which suggests that 49% of the population have inadequate intake. Conversely, estimations from plasma concentration showed mean intake above the RDI with only 3% below the EAR which suggests nutritional adequacy of this group.

Table 3. 1 Selenium intake estimated from 3-day diet diaries, 24-hour urinary excretion & plasma selenium concentration.

Intake	n	Mean (SD)	Median (25, 75)	<60µg/day (RDI) n (%)	<50µg/day (EAR) n (%)
3DDD (µg/day)*	37		52 (36, 84)	22 (59)	18 (49)
Urine (µg/day)*	37		51 (38, 72)	24 (65)	18 (49)
Plasma ** (µg/day)	37	88 (24)	86 (72, 102)	3 (8)	1 (3)

3-day diet diary (3DDD), Recommended Dietary Intake (RDI): 60µg/day; Estimated Average Requirement (EAR): 50µg/day.
**non-parametric data; ** parametric data*

3.4.3 Alternative dietary sources of selenium.

Analysis of the 3-day diet diaries revealed that seafood was the most significant contributor to selenium intake in the women studied at 28%, followed by chicken and meat (17%) and nuts and seeds (13%; Figure 1). The predominant contributor to the nuts and seeds food group was brazil nuts due to their high selenium content. Participants who consumed either seafood and/or brazil nuts on most days had adequate intakes of selenium. It was found that 19 participants (42%) did not consume either seafood or brazil nuts on a daily basis and these participants did not meet the RDI for selenium intake.

The contribution of vegetables and fruit (3%) is mainly through cruciferous vegetables such as broccoli and cauliflower and imported fruit such as bananas.

As expected, bread intake was low and therefore only contributed 5% to selenium intake. However, while participants were eating less than one slice of bread per day, the three-day diet diaries revealed that participants were still consuming other wheat-based products. This included pasta, pizza bases and baked goods made using wheat flour (e.g. homemade muffins etc.) and these non-bread wheat-based products contributed 5% of total selenium intake.

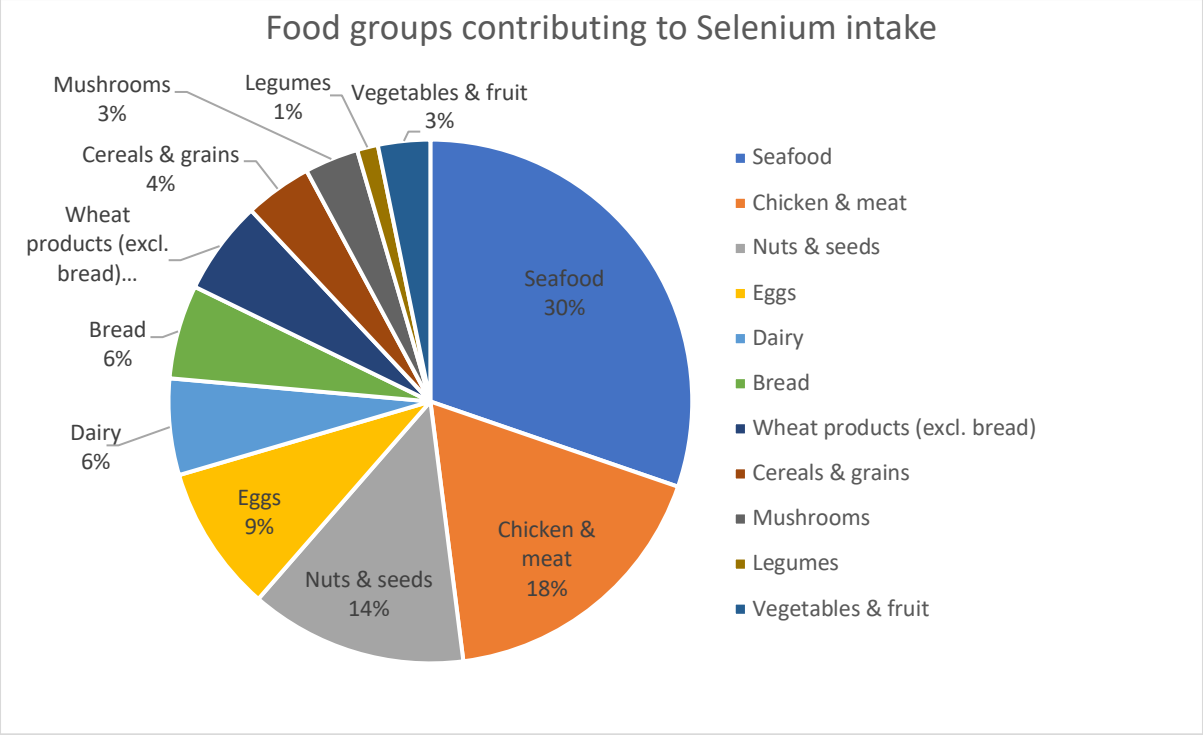


Figure 3. 2 Alternative dietary sources of selenium.

**Wheat products (excl. bread) includes foods made from wheat such as pasta, pizza bases etc. Vegetables and fruits predominantly includes cruciferous vegetables and imported fruit.*

3.4.4 Selenium status, thyroid function markers and prevalence of thyroid dysfunction.

Median plasma selenium concentration for this study was 118.9µg/L which is greater than the amount needed for optimal selenoprotein expression and function. However, this does not mean that all participants have adequate status. Three participants had plasma concentrations lower than 95µg/L and twelve had concentrations lower than 110µg/L, indicating inadequacy for these individuals.

Only two participants had low levels of TSH (with normal free T4) and therefore are considered to have subclinical hyperthyroidism. The majority of participants (n = 34) had thyroid markers indicating euthyroidism.

A thyroid peroxidase antibody (TPOAb) concentration greater than 10 IU/mL indicates a positive result for a potential autoimmune disorder. Four participants (11%) had a TPOAb >10.

Four participants had a TgAb concentration greater than 115 IU/mL and therefore their Tg concentration was disregarded due to the likelihood of it being artificially lowered by the Tg Antibodies.

Table 3. 2 Selenium status, thyroid function markers & prevalence of thyroid dysfunction.

Biomarker	**Median (25th, 75th percentile)**
Plasma Se (µg/L)	118.9 (107.2, 131.3)
TSH (mIU/L)	1.05 (0.8, 1.8)
Free T4 (pmol/L)	12.5 (12, 13)
Free T3 (pmol/L)	4.7 (4.4, 4.9)
Free T3:T4 ratio	0.37 (0.35, 0.4)
Tg (µg/L) (*n=33)*	16.3 (9, 22.7)
	n (%)
TPOAb + (>10 IU/mL)	4 (11)
Subclinical Hypothyroidism	0
Overt Hypothyroidism	0
Subclinical Hyperthyroidism	2 (5)
Overt Hyperthyroidism	0

Plasma selenium > 110µg/L indicates optimal status.
Reference ranges for euthyroidism are TSH 0.4 – 4.0 mIU/L; free T4 10 – 24 pmol/L; free T3 2.5 – 6.0 pmol/L.
Thyroid Peroxidase Antibody (TPOAb) ≥10 IU/mL indicates positive;
Subclinical hypothyroidism: TSH >4.0 mIU/L and free T4 between 10 - 24 pmol/L;
Overt hypothyroidism: TSH >4.0 mIU/L and free T4 < 10 pmol/L;
Subclinical hyperthyroidism: TSH <0.4 mIU/L and free T4 between 10 - 24 pmol/L;
Overt hyperthyroidism: TSH <0.4 mIU/L and free T4 > 24 pmol/L.
N = 37 unless specified otherwise.

Participants were divided into two groups based on selenium status above vs below 110µg/L to determine if there was any difference in thyroid hormone markers between the two groups. A concentration of 110µg/L was chosen for comparison as this is the level at which

maximal expression and activity of iodothyronine 5' deiodinases, GPx and Selenoprotein P occurs. There was no significant difference in T3:T4 ratio, TSH or serum Tg levels between the participants with concentrations above 110µg/L and the participants with plasma concentrations below this level.

3.4.5 Selenium and iodine status and thyroid hormone concentration.

A weak positive correlation was found between selenium status and T3:T4 ratio (r = 0.307, p = 0.065). No other selenium biomarkers were correlated with thyroid hormones. Selenium intake estimated from urine analysis was weakly correlated with selenium intake estimated from 3-day diet diary analysis (r = 0.415, p = 0.011).

Multiple linear regression for the model including both plasma selenium and UIC as predictors of T3:T4 ratio was approaching significance (p=0.072) for predicting 10% of the model (adjusted R2 = 0.099). Plasma selenium significantly predicts T3:T4 ratio, *b* = 0.348, *p* = 0.43*, but UIC does not predict T3:T4, *b* = 0.240, *p* = 0.156.

Table 3. 3 Linear Regression output

	B	SE B	*b*
Step one			
Constant	0.26	0.051	
Iodine (UIC)	0.00	0.00	.24
Plasma Se	0.064	0.03	.348*

*$p = 0.43$

3.4.6 Macronutrient distributions estimated from three-day diet diaries.

Based on the 3DDD, the average percentage of energy provided by macronutrients was 42.5% carbohydrate, 18.8% protein and 38.7% fat (Table 4). According to the Ministry of Health NZ (2006) the acceptable macronutrient distribution ranges (AMDR) for carbohydrate, protein and fat are 45-65%, 15-25% and 20-35% respectively.

Compared to the recommendations, participants were consuming a diet which is lower in carbohydrate and higher in fat. Due to the lower intake of carbohydrates, the participants were only consuming an average of 23.9g of dietary fibre per day (85% of the suggested dietary target (SDT) of 28g/day) and 67% of participants had a fibre intake below the SDT. However, on average participants were consuming more fibre and less sugar compared to the results of the 2008/09 ANS. Mean saturated fat intake as a percentage of total energy was 14.7%.

Table 3. 4 Average macronutrient intakes from 3-day diet diaries, compared to Ministry of Health recommendations and average intakes from the 2008/09 Annual Nutrition Survey.

Macronutrient	**Estimated intake (% of total energy)**	**AMDR (% of total energy)**	**Average intakes from ANS 2008/09 (% total energy)**
Carbohydrate	42.5	45 – 65	47.1
Protein	18.8	15 – 25	16.5
Fat	38.7	20 - 35	33.8
Saturated Fat	14.7	<10	13.1
Monounsaturated	14.6	-	12.3
Polyunsaturated	6.3	-	4.9
	Average intake (g/day)	**Suggested dietary target (g/day)**	**Average intakes from ANS 2008/09 (g/day)**
Fibre	23.9	28	17.5 (*median)*
Sugar	76	<10% total energy	96

3.5 Discussion.

This study of 37 middle-aged women who avoided consuming selenium rich bread found that 49% of participants had inadequate intakes of selenium, 32% had suboptimal selenium status and 5% had thyroid markers indicative of thyroid dysfunction.

Median selenium intake estimated from 3-day diet diaries and urinary excretion was 52 and 51 µg/day respectively, well below the RDI (60 µg/day) with 49% below the EAR (50 µg/day), indicating dietary inadequacy. The US Dietary Reference Values state that for a population to be sufficient it should have a mean intake at or above the RDI and a very low percentage below the EAR (National Research Council Subcommittee on the Tenth Edition of the Recommended Dietary Allowances, 1989). Therefore, due to the large percentage of participants with intakes below the EAR, it cannot be said that intakes overall are adequate. Bread avoidance could result in inadequate intake if alternative sources are not consumed.

Median selenium intake estimated from plasma concentrations was much higher than the other measures at 88µg/day. In contrast to estimations from diet diaries and urinary measures, intakes estimated from plasma concentrations suggested that only one participant had an intake below the EAR. It is difficult to determine which results for estimated intake are most accurate and there is no clear explanation as to why the intakes estimated from plasma are so much higher than those estimated from both urine and diet diaries. It is notable that plasma selenium concentrations did suggest some level of deficiency amongst participants and therefore dietary estimates using plasma selenium are likely an overestimation of intake. Results for median intake from both diet diaries and urinary excretion were very similar to a recent New Zealand based study in 2017 , investigating selenium intakes in 97 healthy women aged 50-70 (Brough et al). Brough et al., found that median intake estimated from urine was 50 µg/day and similar to the current

study, 49% of participants had intakes below the estimated average requirement. These findings also support the most recent Adult Nutrition Survey (2008/09) which found that selenium intakes were inadequate for over half of female adults in New Zealand (University of Otago and Ministry of Health, 2011). Another, more recent study on postpartum women in New Zealand again found that median intake was below the EAR however, similar to the current study, low intake didn't necessarily lead to suboptimal status (Jin et al., 2020). Conversely, the most recent New Zealand total diet study (2016) estimated that the average intake for adult females in New Zealand was 68 μg/day and therefore, adequate. It is worth noting however that this data was generated from simulated diets from dietary data collected over a decade ago, rather than actual current diets. It is also important to note that the average female adult in New Zealand would typically consume more bread than the participants of our study. Participants in the current study consumed an average of 1.6 serves of bread per week. In the study by Brough et al (2017) median bread intake in midlife women was approximately 1.8 serves per day. Therefore, selenium intakes in women who do not avoid bread are likely to be higher than the intakes found in this study, however the other studies discussed did not exclude participants based on bread consumption and still found intakes to be inadequate.

As discussed earlier, the different selenoproteins have differing optimal concentrations required for function; it is suggested that concentrations of 65 – 71 μg/L are required to optimize activity of iodothyronine 5' deiodinases while higher concentrations are required (Thomson et al., 2005; Winther et al., 2020) for saturation of GPx activity (>95 μg/L) (C. Thomson et al., 1993) and maximal expressions of selenoprotein P (>110 μg/L) (Hurst et al., 2010). A large proportion of participants in this study had plasma selenium concentrations greater than 110 μg/L required for optimal health. However, almost a third of participants

(32%, n = 12) had a selenium concentrations below 110 µg/l and therefore could be considered as having suboptimal status. This highlights the risk of deficiency associated with bread avoidance. A small number of participants (n = 3) had concentrations lower than 95 µg/L suggesting inadequate intake for not only maximum expression of deiodinases and selenoprotein P but also for saturation of GPx activity.

Plasma selenium levels in this study were slightly higher than previously found. A 2004 study in New Zealand found mean concentrations of 85 µg/L and 109 µg/L in the Waikato and Taranaki regions respectively (Thomson, 2004b). These regions are also in the North Island of New Zealand where bread is commonly made using selenium-rich Australian wheat and participants of this study were not excluded based on bread consumption. It is surprising that plasma concentrations in this population group were lower than those of the current study where bread was being avoided.

Overall, even though selenium concentrations were optimal for many participants, there is a substantial sub-group of participants (32%) who have sub-optimal concentrations for selenoprotein expression and function. This suggests that bread avoidance may increase the risk of selenium deficiency.

Even though all of the participants within this study were avoiding bread, over 50% still had adequate selenium intake indicating that these women were getting sufficient selenium from alternate food sources. Seafood was the greatest contributor to selenium intake, followed by chicken & meat and nuts & seeds (*figure 1*) with brazil nuts being the predominant contributor in the nuts and seeds food group, due to their high selenium content. A major finding from the analysis of the three-day diet diaries was that the participants with intakes greater than the RDI typically consumed seafood and/or brazil nuts

most days. 19 participants (42%) did not consume either seafood or brazil nuts on a daily basis and these participants did not meet the RDI for selenium intake.

It is important to differentiate between the percentage a food contributes to selenium intake and the percentage it contributes to overall energy intake. For example, seafood contributed more to selenium intake than meat/ poultry however participants were consuming more energy from meat/ poultry than seafood. The 2003/04 New Zealand Total Diet Survey (NZTDS) found that overall seafood had higher levels of selenium than other food sources (Thomson, Vannoort, & Haslemore, 2008) and therefore a smaller quantity of seafood could be eaten in order to consume the same selenium content as a larger quantity of meat or poultry.

Globally the richest sources of selenium are organ meats, brazil nuts and some seafoods (Rayman, 2008) and within New Zealand the food sources which contribute the most significantly to selenium intake are breads and bread-based dishes, seafood and poultry (University of Otago & Ministry of Health, 2011). Therefore, it is not surprising that seafood, poultry, meat and nuts were the greatest contributors of selenium in this group of women who don't eat bread.

An unexpected finding of this study was that even though participants were recruited on the basis that they consume less than one slice of bread per day, on average they were still getting 5% of their total selenium intake from non-bread wheat-based products. These products included pasta, pizza bases and baking which were all made from wheat flour and therefore contain selenium, however they are not technically considered to be 'bread'. It is possible that this finding is significant to the research as the participants are getting a larger proportion of selenium from wheat than originally anticipated. It is possible that deficiency would have been greater if all wheat-based products were omitted from the diet.

Vegetables and fruit contributed approximately 3% of daily selenium intake and this was predominantly through the consumption of cruciferous vegetables such as broccoli and cauliflower and imported fruit such as bananas. Cruciferous vegetables are known to be selenium accumulators (Ministry of Primary Industries, 2018) meaning that they absorb more selenium from the soil than other vegetables grown in New Zealand. Imported fruits such as bananas are grown overseas, in soils richer in selenium and therefore contribute more to selenium intake than fruits grown in New Zealand.

The 3-day diet diaries revealed that it is not necessary to consume bread in order to meet selenium requirements however it is important to ensure alternative sources of selenium are present in the daily diet to maintain adequate intake.

Blood analysis revealed that the majority of participants (92%) in this study had thyroid markers indicative of euthyroidism. Only two participants were considered to have abnormal thyroid function. Both of these individuals had low levels of TSH with normal free T4 indicating subclinical hypothyroidism. Four participants had a TPOAb result > 10 indicating a positive result for a potential autoimmune disorder.

Previous studies have found a negative relationship between selenium status and T4 levels due to the conversion of T4 to T3 requiring selenoprotein deiodinases (Thomson et al., 2005). This means that inadequate status can lead to an increase in free T4. Median free T4 levels in this study were 12.5 pmol/L and all participants had T4 levels within the reference range of 10-24 pmol/L. This could indicate adequate conversion of T4 to T3. However, as mentioned previously plasma concentrations as low as 65-71µg/L are required to optimize activity of iodothyronine 5' deiodinases and the majority of participants in this study had plasma concentrations well above this level. For this reason it would not be expected that

any participants would have limited conversion of T4 to T3 and consequential suboptimal levels of T3.

Participants were divided into two groups based on selenium status above vs below 110µg/L to determine if there was any difference in thyroid hormone markers between the two groups. There was no significant difference in T3:T4 ratio, TSH or serum Tg levels between the participants with concentrations above 110µg/L and the participants with plasma concentrations below this level.

Plasma selenium was weakly positively correlated with T3:T4 ratio, and in a model with UIC was approaching significance. This could corroborate the role of selenium in conversion of T4 to T3 and highlights the effect of increasing selenium on reducing T4 and increasing T3 levels. It could also be suggested that the 65-71µg/L cut off for T4 to T3 conversion may be set too low and that higher selenium concentrations may be required for this conversion to occur.

No other selenium measures (status or intake) were found to correlate with thyroid hormones. This suggests that bread avoidance does not directly result in suboptimal thyroid function if adequate alternate sources of selenium are consumed.

An unexpected finding from this study was made when analysing the 3-day diet diaries. It was found that participants were consuming a larger proportion of their total daily energy from fat sources and a smaller proportion from carbohydrate sources compared to both the recommendations and the findings of the 2008/09 Adult Nutrition survey (Table 3.4), highlighting that this group of participants have different dietary patterns than the general population. This lower carbohydrate, high fat style of eating has risen in popularity recently with the rise of the Atkins and Keto diets. These findings highlight the effects of bread

avoidance and general lower-carbohydrate diets on the other macro-nutrient groups, re-enforcing the idea of an emerging group of at-risk individuals who avoid bread and other carbohydrate-based foods. Interestingly, even with a lower carbohydrate intake participants had a higher intake of fibre than the general population although it was still below recommendations. On average, sugar intake was lower than the general population. This could suggest that the reason these participants have a higher fibre intake with a lower total carbohydrate intake is reduced consumption of high-sugar foods combined with an increased consumption of fruit and vegetables. The 3-day diet diaries also revealed that saturated fat intake for participants of this study was higher than both the recommendations and the ANS 08/09, however intakes from both mono- and poly-unsaturated fats were also higher than the general population. When eating a diet higher in fat it is important to identify the types of fat consumed due to their differing effects on overall health. A high intake of saturated fat in individuals who avoid bread may lead to other health complications outside of selenium deficiency (Ministry of Health, 2015) and further research into this area would be recommended.

3.6 Strengths and Limitations.

To our knowledge this was the first study to investigate the effect of bread avoidance on selenium status and thyroid function in New Zealand women. One of the strengths of this study is the use of dietary and biological markers to estimate selenium intake and status, together with a wide range of hormone biomarkers to assess thyroid hormone function. One of the limitations of this study is the method used to assess iodine status. Urinary iodine concentration was not found to significantly correlate with thyroid hormones however, a single measure for UIC is not valid for individual status and at least 10 measures

should be taken to determine an individual's iodine status (Andersen, Karmisholt, Pedersen, & Laurberg, 2008). This limitation in the method for measuring iodine status could be a possible explanation for the lack of correlation between UIC and thyroid hormones. Other limitations of this study include the small sample size and the close geographical proximity of the participants. All participants were located in the Auckland region and therefore this sample may not be representative of the wider New Zealand population.

Selenium status was measured using plasma concentrations as this has been found to be the most useful biomarker for assessing selenium status at both an individual and population level (Phiri et al., 2020). However, plasma and serum concentrations have been found to reflect only short term selenium intake (several days) and therefore using an additional biomarker such as erythrocyte selenium which reflects longer-term intake (up to several months) may have been of benefit (Thomson, 2004b).

3.7 Conclusion.

Overall, a high prevalence of inadequate intake was observed in this group of middle-aged New Zealand women who avoid bread. Subsequently, almost a third of participants had suboptimal plasma concentrations. Plasma selenium was suggested to predict T3:T4 highlighting the role of selenium in thyroid function, however bread avoidance did not directly result in impaired thyroid function for the majority of participants. Even though bread in the North Island of New Zealand is a rich source of selenium due to the Australian-grown wheat with which it is made, it appears that bread avoidance does not directly result in inadequate intake and suboptimal status as long as individuals are consuming a diet rich in alternative selenium sources such as seafood, meat, poultry and nuts. Further larger studies are required to investigate the effect of suboptimal selenium status on thyroid

hormone function as well as investigating the accuracy of estimations for intake based on diet diaries, urine excretion and plasma concentrations.

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4 Conclusion – Recommendations chapter.

The aim of this study was to investigate if avoidance of bread by mid-life women in New Zealand results in low selenium status and subsequent impaired thyroid function. The findings conclude that bread avoidance can lead to inadequate selenium intake in relation to the estimated average requirements, and subsequent low selenium status however it does not directly result in impaired thyroid function.

This study addressed a gap in the literature by investigating the effect of removing bread from the diet on selenium intake and status. The hypothesis for this study was that women who avoid bread will have inadequate selenium status, resulting in impaired thyroid function. The findings of this study suggest that this hypothesis is partially correct as avoidance of bread did result in inadequate plasma concentration for nearly a third of mid-life women in this group, however this did not result in subsequent impairment of thyroid function as only 2 participants (8%) were found to have impaired thyroid function.

Median intake was found to be below the Recommended Daily Intake (RDI) and nearly half of participants had intakes below the Estimated Average Requirement (EAR). The US Dietary Reference Values state that for a population to have sufficient intake it should have a mean intake at or above the RDI and a very low percentage below the EAR (National Research Council, 1989). For this reason, it cannot be said that intakes were adequate in this population group. Estimations of intake based on 3-day diet diaries and urinary excretion found that 49% of participants had intakes below the EAR however, intakes estimated from plasma selenium found that only one participant (3%) had a dietary intake of selenium

below the EAR. Considering over a third of participants were found to have low plasma selenium concentrations it is likely that intake estimated from plasma is an overestimation. The findings of this study support findings from previous studies carried out in New Zealand which also found that women in New Zealand have inadequate intakes (Brough et al., 2017) (Jin et al., 2019). Plasma concentrations were sufficient for expression and function of deiodinases, GPx and selenoprotein P, suggesting that a large proportion of participants in this study had adequate selenium status for health. However 32% (n=12) of participants had a selenium concentration below 110µg/l and therefore could be considered as having suboptimal status.

Alongside selenium intake and status, this study also investigated the primary alternative food sources of selenium for mid-life women who don't eat bread. It was found that seafood, meat, poultry and nuts were the main contributors to dietary selenium intake. Participants who had intakes above the RDI typically consumed either seafood and/ or brazil nuts most days showing that these foods are key sources of selenium in the New Zealand food supply. Participants (42%) who did not consume seafood and/or brazil nuts on a daily basis had intakes below the RDI. The findings of this research highlight an at-risk group of individuals who don't eat bread and who also do not eat seafood or nuts regularly. Further research with a larger population group is recommended to investigate the selenium intakes and status of these women. It is also important to note that this participant group came from a high socio-economic area and so results cannot be generalized to the wider population as both seafood and brazil nuts are expensive foods.

Participants of this study were found to be consuming a larger proportion of their total daily energy from fat sources, and a smaller proportion from carbohydrates, compared to both

the national recommendations and the findings of the 2008/09 Adult Nutrition survey. This finding highlights the implications of bread avoidance on macro-nutrient distribution and it is recommended that further research is carried out investigating the effects of this higher fat and lower carbohydrate intake on other aspects of health.

In terms of thyroid hormones, the majority of participants (92%) had thyroid biomarkers indicative of euthyroidism. Only two participants were considered to have abnormal thyroid function. Both of these individuals had low levels of TSH with normal free T4 indicating subclinical hypothyroidism. This shows that the majority of participants in this study had adequate selenium intakes and status for thyroid function.

There was a weak positive correlation found between plasma selenium and T3:T4 ratio, highlighting the role that selenium plays in the conversion of thyroid hormones. Plasma selenium was suggested to predict T3:T4 ratio, emphasizing the importance of ensuring adequate selenium in the diet for optimal thyroid function.

To our knowledge this was the first study to investigate the effect of bread avoidance on selenium status and thyroid function in New Zealand women. One of the strengths of this study is the wide range of biomarkers used to assess thyroid hormone function as well as the variety of biomarkers used to estimate intake.

One of the limitations of this study is the method used to assess iodine status. It is known that iodine plays a major role in thyroid function and could be expected to correlate with thyroid hormones regardless of selenium status. Iodine status determined by UIC was not found to significantly correlate with thyroid hormones, nor was UIC found to significantly predict T3:T4 ratio however, a single measure for UIC is not valid for individual status and at

least 10 measures should be taken to determine an individual's iodine status (Andersen et al., 2008). This limitation in the method for measuring iodine status could be a possible explanation for the lack of correlation between UIC and thyroid hormones. Iodine was not the focus of this thesis and therefore iodine status and the role of iodine in thyroid function was not fully investigated in the extension to this study. Other limitations of this study include the small sample size and the close geographical proximity of the participants. Participants were all located in the Auckland region and therefore this sample is unlikely to be representative of the wider New Zealand population, particularly not the South Island population where bread is typically made using local wheat.

Selenium status was measured using plasma concentrations as this has been found to be the most useful biomarker for assessing selenium status at both an individual and population level (Phiri et al., 2020). However, it may have been useful to incorporate other biomarkers as well such as erythrocyte selenium to assess status over a longer time frame or specific selenoproteins such as plasma GPx or Selenoprotein P to measure optimal functionality (Gutierrez-Gonzalez et al., 2019).

Bread avoidance and, in general, lower carbohydrate diets are becoming more commonplace and this study highlights the risks that bread avoidance can pose on selenium intake if alternative food sources of selenium are not consumed as part of a daily diet. It could be suggested that some individuals do not fully understand the nutritional risks of bread avoidance in regard to selenium intake and that knowledge about alternative selenium sources is not widely held. However, these are only suggestions and further research relating to public perception and knowledge about selenium and available food sources of selenium is recommended. It is important that the general population are aware

of the nutritional risks of bread avoidance and have an understanding of alternative dietary sources of nutrients such as selenium so that they can make educated choices about their dietary patterns. Public health recommendations from this study are to increase awareness of selenium as a nutrient and to educate the New Zealand population on alternate food sources of the nutrient.

Overall, this study demonstrates that avoidance of bread does not directly result in impaired thyroid function, however it can become difficult to meet nutrient recommendations for selenium and maintain optimal status when bread is removed from the diet. This highlights the importance of promoting alternate food sources of selenium for women who choose to avoid bread as avoidance can lead to inadequate intake and poor status if alternate food sources of the nutrient are not consumed.

Appendices 1 – 9.

Appendix 1: Original WOMBI participant information sheet.

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

The WOMBI (Women bread and iodine) Study

Does the exclusion of iodine fortified bread in the diet of mid-life women result in low iodine status?

Information for Participants

You are invited to take part in a post graduate research study investigating the effect of avoiding iodine fortified bread products on iodine status of mid-life women living in the northern suburbs of Auckland. The principal investigators are as follows:

Principal Investigator: Jacqui Finlayson – Postgraduate student School of Food and Nutrition Massey University, Albany Tel: 09 4241020 Mob: 027 2927152 Email: jacqui.finlayson.1@uni.massey.ac.nz	Supervisor: Dr Pamela von Hurst School of Food and Nutrition Massey University, Albany Tel: 414 0800 ext. 43657 Email: P.R.vonHurst@massey.ac.nz	Supervisor: Dr Louise Brough, School of Food and Nutrition Massey University, Palmerston North Tel: (06) 356 9099 ext. 84575 Email: L.Brough@massey.ac.nz

We are looking for 50 mid-life women to participate in this study. To be eligible for our study you should:

- Be female, between 40 and 65 years of age.
- Live in the northern suburbs of Auckland
- Have no have thyroid, kidney, or heart disease.
- Not be taking thyroid medication, supplements containing more than 10 mcg of iodine, lithium medication, or hormone replacement therapy
- Not smoke more than 5 cigarettes per day
- Not consume more than one slice of bread daily

Following your expression of interest for this study, you will be contacted by the Research team from Massey University to assess your eligibility. Once accepted into the study you will be required to visit the Human Nutrition Research Unit at Massey University on one occasion. At this visit you will be asked to provide personal and medical details. Your height, weight and percentage of body fat will be measured and you will be asked to provide a 16ml blood sample (approximately one tablespoon of blood). A container and instructions for collecting a 24hour urine sample will be given to take home and complete. Lastly you will be asked to complete a food frequency questionnaire and a 3- day food diary.

About the Study

We will make an appointment for you to visit Massey University, Albany campus.

Involvement in this study will include:

A visit to the research unit:

You will be requested to complete a food frequency questionnaire and provide your date of birth. The questionnaire may be completed while you are at the research unit. You will spend approximately one hour with the researcher who will also measure your height, weight and body fat percentage. All measurements will be made in a private area by female researchers over light clothing so you do not need to get undressed.

At this visit you will also be provided with containers to take home to collect a sample of urine collected over 24 hours. You are requested to collect all your urine over a consecutive 24-hour period and keep it in the cool bags provided until collected by the one of the research team. The urine will be subsequently analysed for iodine concentration.

Also, you will be asked to complete a 3-day food diary which can be taken home and returned using the stamped, addressed envelope provided and sent to the Nutrition Research Unit at Massey University.

Lastly you will be asked to provide a blood sample of 16ml. The blood sample will be analysed for thyroglobulin, thyroglobulin antibodies and thyroid hormones (TSH, fT3, fT4).

Risks and Benefits

There will be no charges for any of the tests that you undertake. The main benefit of taking part in this study is that you contribute to a greater understanding if avoiding bread that is fortified with iodine results in a lower iodine status. New Zealand (NZ) has low soil levels of iodine which adversely affects the amount of iodine in the food that is grown and produced here. As a result, the NZ people are vulnerable to iodine deficiency. To improve the iodine status of the population in 2009 the NZ government introduced the mandatory fortification of all non-organic and commercially produced bread with iodised salt. However, as some people choose not to eat bread or choose to eat only organic bread the contribution to dietary iodine intake from bread sources is limited.

You will also receive information about your dietary intake and body composition.

There are no personal risks to your health, but the blood tests could potentially identify thyroglobulin, thyroglobulin antibody and/or thyroid hormone levels outside the normal range. If we identify any possible abnormalities, we will advise you to consult your General Practitioner for further investigation.

Participation

You are under no obligation to accept this invitation to take part in this study. If you do decide to participate, you have the right to:

- Decline to answer any question;
- Withdraw from this study (at any time without having to give a reason);
- Ask any questions about this study at any time during participation;
- Provide information on the understanding that your name will not be used unless you give

permission to the researcher;

- Be given access to a summary of the project findings when it is concluded.

General

If you want to discuss any aspect of this study you should contact the Principal investigator, Jacqui Finlayson (027 2927152; email; jacqui.finlayson.1@uni.massey.ac.nz)

If you have any queries or concerns regarding your rights as a participant in this study you may wish to contact the Health and Disability Advocacy; telephone 0800 555 050.

At the conclusion of the study we will provide a report of the outcome to those involved in this study and we will send the results by mail.

Confidentiality

No material which could personally identify you would be used in any reports on this study. Information collected from you in this study will be stored securely in the Department of Nutrition and will only be available to study personnel. When this study is completed, all material will be destroyed.

Compensation for Injury

If physical injury results from your participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic and your claim will be assessed by ACC in accordance with the Accident Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to, treatment costs, travel costs for rehabilitation, loss of earnings, and/or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred as a result of physical injury. If your ACC claim is not accepted, you should immediately contact the researcher. The researcher will initiate processes to ensure you receive compensation equivalent to that to which you would have been entitled had ACC accepted your claim.

Please feel free to contact the researcher if you have any questions about this study.

This project has been reviewed and approved by Massey University Human Ethics Committee: Southern A, application no 16/52. If you have any concerns about the conduct of this research, please contact Mr Jeremy Hubbard, Chair, Massey University Human Ethics committee: Southern A, telephone 04 801 5799 x 63487, email humanethicssoutha@massey.ac.nz

Appendix 2: Original participant consent form.

School of Food and Nutrition
Massey University
Private Bag 102-904
North Shore Mail Centre
Albany, Auckland
New Zealand

T 09 414 0800

The Women, Bread and Iodine Study (WOMBI) Study

Does the exclusion of iodine fortified bread in the diet of mid-life women result in low iodine status?

PARTICIPANT CONSENT FORM

This consent form will be held for a period of five (5) years

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time. I agree to participate in this study under the conditions set out in the Information Sheet. I am aware that I can:

- Decline to answer any particular question;
- Withdraw from this study (at any time without having to give a reason);
- Ask any questions about this study at any time during participation;
- Provide information on the understanding that your name will not be used unless you give permission to the researcher;
- Be given access to a summary of the project findings when it is concluded.

Signature: ..... **Date:** .....

Full Name - printed .....

Appendix 3: Study extension information sheet.

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA
Institute of Food, Nutrition and Human Health
School of Sport and Exercise Science
College of Health

Women, bread and iodine

INFORMATION SHEET

Dear Wombi Participant,

We are writing you to share important news about the Women, bread and Iodine study. As a valued study participant, we would like to ask you for your permission to analyse the samples (blood and urine) that we have already collected from you for selenium.

As you know from participating in this study, we believe that women who avoid bread may be at a higher risk of iodine deficiency. We also believe that avoidance of bread could increase risk of a selenium deficiency due to wheat being a rich source of selenium. Selenium deficiency could result in impaired thyroid function, a weakened immune system and contribute to chronic health concerns which occur as a result of excessive inflammation and infection. The selenium status of women in the Auckland region has not previously been investigated directly. This study aims to investigate the selenium status of women who avoid bread and any effect of selenium status on thyroid hormone levels.

We thank you for your dedication and commitment to this study. You should already have received a summary of results from the Iodine study. Once complete, we will send another summary of these new results out to you.

If you have any questions about the project or any of the tests planned, please contact the lead researchers named below.

Name; Associate Professor Pamela Von Hurst
Number; +64 (09) 414 0800 ext. 43657
Email; P.R.vonHurst@massey.ac.nz
School of Sport, Exercise and Nutrition
Massey University, Albany

Name; Jaime Berger
Email; jberger@massey.ac.nz
School of Sport, Exercise and Nutrition
Massey University, Albany

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 20/28. If you have any concerns about the conduct of this research, please contact Dr Negar Partow, Chair, Massey University Human Ethics Committee: Southern A, telephone 04 801 5799 x 63363, email humanethicsoutha@massey.ac.nz.

Kind Regards,

Jaime Berger from the School of Sport, Exercise and Nutrition,
Associate Professor Pamela Von Hurst from the School of Sport, Exercise and Nutrition,
Dr Louise Brough from the School of Food and Advanced Technology.

Appendix 4: Study extension consent form.

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Institute of Food, Nutrition and Human Health
School of Sport and Exercise Science
College of Health

Women, bread and Iodine

PARTICIPANT CONSENT FORM - INDIVIDUAL

I have read the Information Sheet and have had the details of the additional analysis for selenium explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

I agree to the additional analysis of selenium in the samples I provided for this study under the conditions set out in the Information Sheet.

Full Name: ..... **Date:** .....

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Code: _____

Appendix 5: Diet diary instructions.

The WOMBI Study – The Women, Bread & Iodine Study

Date of visit:
Day_____ _____

3-day Food Dietary Diary

Month_____ Year

PLEASE READ THROUGH THESE PAGES BEFORE STARTING YOUR DIARY

We would like you to record in this diary everything you eat and drink over **3 DAYS**, including food consumed at home and outside the home. It is very important that you continue to eat and drink what you normally eat and drink during the period of recording. Please describe all the food you eat in as much detail as possible. Be as specific as you can.

When to fill in the diary

Please record the food you eat as you go, **do not list from memory** at the end of the day. Use written notes on a notepad if you forget to take your diary with you. Each diary day covers a 24-hour period, so please include any food or drinks that you may have had through the night. Remember to include foods and drinks between meals (snacks) including water.

Home-made dishes

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COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Code: _____

Please record the name of the recipe, ingredients with amounts (including water and other fluids) for the whole recipe, the number of people the recipe serves, and the cooking method; record how much of the whole recipe you personally have eaten.

Take-away and eating out

Please record as much detail about the amount and ingredients as you can, e.g. Vegetable curry containing chickpeas, eggplant, onion and tomato.

Brand name

Please note the brand name (if known). Most packed foods will list a brand name, e.g. Bird's eye, Watties, or Supermarket own brands

Portion Size

Examples for how to describe the quantity or portion size you had of a particular food or drink are shown on pages 17-21 of this diary.

For foods, quantity can be described using:

- household measures, e.g. two thick slices of bread, 4 tablespoons (tbsp.) of peas.
- weights from labels, e.g. 500g steak, 420g tin of baked beans, 125g pot of yoghurt
- number of items, e.g. 4 fish fingers, 2 pieces of chicken nuggets,

For drinks, quantity can be described using (see page 21 for a real size glass):

- the size of glass, cup or the volume (e.g. 300ml).
- volumes from labels (e.g. 330ml can of fizzy drink).

We would like to know the amount that was actually eaten which means taking any leftovers into account. You can do this in two ways:

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Code: _____

- Record what was served and make notes of what was not eaten e.g. 3 tbsp of peas, 1 tbsp not eaten; 1 large sausage roll, ½ not eaten
- Only record the amount actually eaten e.g. 2 tbsp of peas, ½ a large sausage roll

At the end of each recording day, you will be prompted to tell us

Was it a typical day?

After each day of recording you will be prompted to tell us whether this was a typical day or whether there were any reasons why you ate or drank more or less than usual.

Did you take any supplements?

At the end of each recording day there is a section for providing information about any supplements you took. Brand name, full name of supplement, strength and the amount taken should be recorded.

Overleaf (page 4-8) you can see an example day that has been filled in to show you how we would like you to record your food and drink.

It only takes a few minutes for each eating occasion!

Thank you for your time- we really appreciate it!

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Code: _____

EXAMPLE

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Code: _____

DAY 1		**Date: _____Day _____Month_____Year**		
Time	**Where**	**Food/drink description & preparation**	**Brand name**	**Portion size or quantity eaten**
		6am to 9am		
6.30am	Kitchen	Filter coffee, decaffeinated Milk (fresh, blue top) Sugar white Toast, multigrain bread Marmalade	Robert Harris Anchor Pams Pams Pams	Mug A dash 1 level teaspoon 1 slice 1 heaped teaspoon
		9am to 12noon		
		Did not eat or drink anything		

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Code: _____

Time	Where	Food/drink description & preparation	Brand name	Portion size or quantity eaten
		12noon to 2pm		
12.30pm	Work tea room	Ham salad sandwich from home: Bread wholemeal thick sliced Margarine light Smoked ham thin sliced Lettuce, iceberg Cucumber with skin	 Pams Sunlight Supermarket	 2 slices 1 tablespoon 2 slices 1 leaf 4 thin slices
		2pm to 5pm		
3pm	Meeting room	Herbal tea Louise slice	Healtheries bakery	1 cup 1 regular slice

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Code: _____

Time	Where	Food/drink description & preparation	Brand name	Portion size or quantity eaten
		5pm to 8pm		
6.30pm	At table with husband and children	Spaghetti, wholemeal Bolognese sauce (see recipe) Courgettes Orange juice	Pams Homemade Fresh Just Juice	100g 1 serve 50g 200mls
		8pm to 10pm		
9pm	Sitting room alone	Milk Chocolates	Canterbury	25g
		10pm to 6am		
10pm	bedroom	water	tap	200mls

Please record the details of any recipes or (if not already described) ingredients of made up dishes or take-away dishes.

Write in recipes or ingredients of made-up dishes or take-away dishes			
Name of Dish: Bolognese sauce		Serves: 4	
Ingredients	Amount	Ingredients	Amount
Low fat beef mince	500g		
garlic	3 cloves		
Brown onion	100g		
Sweet red pepper (capsicum)	50g		
Watties chopped tomatoes	400g		
Tesco tomato puree	1 tablespoon		
Pams canola oil	2 tablespoon		
Greggs mixed herbs	2 tablespoon		
Pams Worcester sauce	1 teaspoon		
Brief description of cooking method: Fry onion and garlic in oil, add mince and fry till brown. Add pepper, tomatoes, puree, Worcester sauce and herbs. Simmer for 30 minutes.			

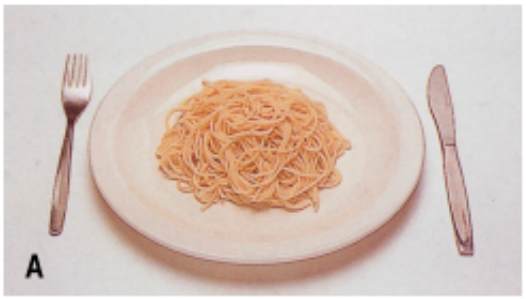
Use the pictures to help you indicate the size of the portion you have eaten.
Write on the food record the <u>picture number and size A, B or C</u> nearest to your own helping.

Remember that the pictures are much smaller than life size.
The actual size of the dinner plate is 10 inches (25cm), the side plate, 7 inches (18cm), and the bowl, 6.3 inches (16cm).

The tables on pages 16-21 also give examples of foods that you might eat and how much information is required about them.

Breakfast cereal

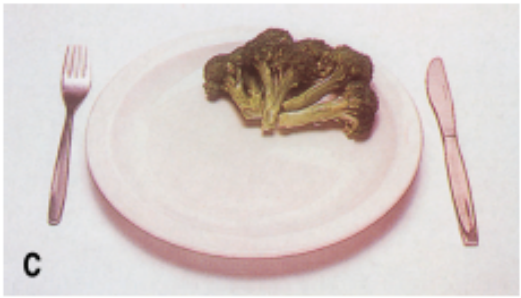
Spaghetti/noodles



Rice

Chips

Broccoli or cauliflower

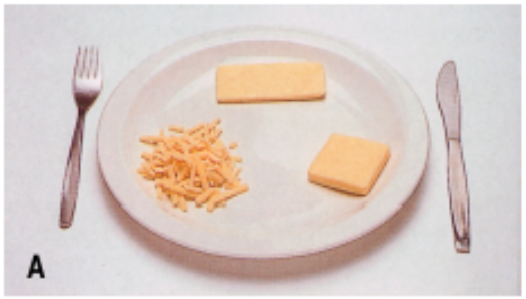


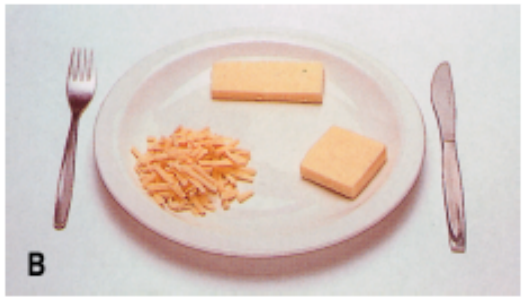
Stew or curry

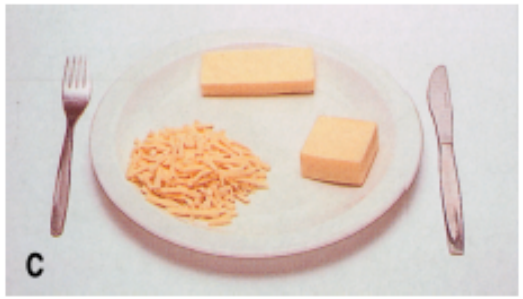
Battered fish

Quiche or pie

Cheese







Spongy cake

Appendix 6: Diet diary forms.

Day

DAY 1		Date: _____Day _____Month_____Year		
Time	**Where**	**Food/drink description & preparation**	**Brand name**	**Portion size or quantity eaten**
6am to 9am				
9am to 12noon				
Time	**Where**	**Food/drink description & preparation**	**Brand name**	**Portion size or quantity eaten**
12noon to 2pm				

2pm to 5pm				
Time	**Where**	**Food/drink description & preparation**	**Brand name**	**Portion size or quantity eaten**
5pm to 8pm				

8pm to 10pm				
10pm to 6am				

1. Was the amount of **food** that you had today about what you usually have, less than usual, or more than usual?

   ☐ Yes, usual

   ☐ No, **less** than usual.

   ☐ No, **more** than usual

Please tell us why you had less than usual	Please tell us why you had more than usual

2. Was the amount you had to **drink** today, including water, tea, coffee and soft drinks (and alcohol), about what you usually have, less than usual, or more than usual?

☐ Yes, usual

☐ No, **less** than usual ☐ No, **more** than usual

Please tell us why you had less than usual	Please tell us why you had more than usual

3. Did you finish all the food and drink that you recorded in the diary today?

☐ Yes ☐ No

If no, please **go back to the diary and make a note of any leftovers**

4. Did you take any **vitamins, minerals or other food supplements** today?

☐ Yes ☐ No

If yes, **please describe the supplements you took below**

Brand	Name (in full) including strength	Number of pills, capsules, teaspoons
Example Thomson's	Calcium (1000mg) with vitamin D	1 tablet

Please record the details of any recipes or (if not already described) ingredients of made up dishes or take-away dishes

Write in recipes or ingredients of made-up dishes or take-away dishes			
Name of Dish:		Serves:	
Ingredients	Amount	Ingredients	Amount

Brief description of cooking method:			

Write in recipes or ingredients of made-up dishes or take-away dishes			
Name of Dish:		Serves:	
Ingredients	Amount	Ingredients	Amount

Brief description of cooking method:			

Appendix 7: Urine collection instructions.

School of Food and Nutrition **Study ID:_____**
Massey University **Date of collection:_____**
Albany

COLLECTION OF URINE

HUMAN NUTRITION RESEARCH UNIT LABORATORY PROCEDURE

You have been provided with:

- Two urine collection bottles.
- Cooler bags containing frozen ice packs
- Measuring jug for urine collection.

Collection
Start collection first thing in the morning & store bottle in the refrigerator or cool bag between collecting.

1. Pass urine into the toilet. This urine is **not wanted,** but this is the "time Commenced", enter this here: Time: am/pm (please circle)

2. From now on collect **all** urine you pass during the rest of the day and night. Use measuring jug for collection and pour carefully into sample bottle. ***When you empty your bowels,*** please collect urine so that you do not lose the urine.

3. **Collect the last** specimen the next morning (i.e. 24 hours after starting). This is the time finished. Note even if you do not feel the need to pass this urine, you must empty your bladder completely. Time finished:- am/pm (circle one)

Store urine bottles in cooler bags containing frozen ice packs and contact:-

Jacqui Finlayson: ph. 027 2927152 (call/text) to arrange collection of sample.

OR if dropping off to the Research unit;

Owen Mudgridge: ph. 09 213 6650 O.Mugridge@massey.ac.nz

Appendix 8: Ethics approval letter.

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Date: 06 August 2020

Dear Jaime Berger
Re: Ethics Notification - SOA 20/28 - The consequences of avoiding bread: The effect of bread

avoidance on selenium status and thyroid function.

Thank you for the above application that was considered by the Massey University Human Ethics Committee: Human Ethics Southern A Committee at their meeting held on Thursday, 6 August,

Approval is for three years. If this project has not been completed within three years from the date of this letter, reapproval must be requested.

If the nature, content, location, procedures or personnel of your approved application change, please advise the Secretary of the Committee.

Yours sincerely

Professor Craig Johnson
Chair, Human Ethics Chairs' Committee and Director (Research Ethics)

Research Ethics Office, Research and Enterprise
Massey University, Private Bag 11 222, Palmerston North, 4442, New Zealand T 06 350 5573; 06 350 5575 F 06 355 7973 E humanethics@massey.ac.nz W http://humanethics.massey.ac.nz

Appendix 9: Pearson's correlation matrix among biomarkers for selenium intake, status and thyroid hormones.

Correlations

		Iodine UIE	Iodine UIC	Plasma Se mcmol/L	Log-transformed Intake from 3DDD	Log-transformed Intake form Urinary excretion	T3	T3:T4 ratio	Log-transformed TSH	Log-transformed Tg
IodineUIE	Pearson Correlation	1	.985**	-.147	-.063	.652**	.231	.235	.268	-.214
	Sig. (2-tailed)		.000	.384	.712	.000	.169	.161	.109	.203
	N	37	35	37	37	35	37	37	37	37
Iodine UIC	Pearson Correlation			-.159	-.042	.658**	.167	.184	.314	-.206
	Sig. (2-tailed)			.362	.812	.000	.338	.289	.067	.235
	N			35	35	33	35	35	35	35
Plasma Se mcmol/L	Pearson Correlation				.025	.013	.213	.307	.151	-.005
	Sig. (2-tailed)				.885	.941	.206	.065	.372	.975
	N				37	35	37	37	37	37
LogIntake3DDD	Pearson Correlation					.237	-.315	-.024	.126	.152
	Sig. (2-tailed)					.170	.058	.889	.458	.370
	N					35	37	37	37	37
LogIntakeUrine	Pearson Correlation						.047	.132	.257	.137
	Sig. (2-tailed)						.787	.451	.136	.433
	N						35	35	35	35
T3	Pearson Correlation							.655**	.169	.033
	Sig. (2-tailed)							.000	.317	.845
	N							37	37	37
T3:T4 ratio	Pearson Correlation								.257	-.058
	Sig. (2-tailed)								.125	.732
	N								37	37
Log TSH	Pearson Correlation									.085
	Sig. (2-tailed)									.616
	N									37

**. Correlation is significant at the 0.01 level (2-tailed).