Validation and Reproducibility of an Iodine and Selenium Specific Food Frequency Questionnaire in Breastfeeding Women

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Abstract

Background: New Zealand has poor levels of iodine and selenium in its food sources. During lactation, women have increased selenium and iodine requirements, as their breastfeeding infant relies on their intake, putting them at increased risk of deficiency. Thyroid function is impacted by iodine and selenium status, and if these nutrients are low can cause consequences for the mother and breastfed infant. Dietary assessment methods, such as a Food Frequency Questionnaire (FFQ), can be utilised to assess nutrient intake, and validating an FFQ shows that the questionnaire can be used on the intended population to predict nutrient intake. To the best of our knowledge, New Zealand currently does not have a valid iodine and selenium specific FFQ for breastfeeding women. Due to this population risk with iodine and selenium, it is justified to test the validity and reproducibility of this FFQ on breastfeeding women in New Zealand.

Aim: The aim of this study is to assess the validity and reproducibility of an iodine and selenium specific Food Frequency Questionnaire for breastfeeding women living in New Zealand.

Methods: As part of the Mother and Infant Nutrition Investigation study (MINI), data was collected from breastfeeding mothers from three months to 12 months postpartum (PP). Participants (n = 87) were administered an iodine and selenium specific FFQ at three months and a four-day diet diary (4DDD) to assess iodine and selenium intake. To assess reproducibility the FFQ was readministered at 12 months PP (FFQ2). FFQ1 was validated via 4DDD and selected biomarkers (urinary and breastmilk iodine concentrations and plasma selenium); statistical analysis was used, including Wilcoxon signed ranked test, Spearman's correlation, cross-classifications, weighted kappa statistics, Bland Altman plots, the same statistical analysis carried out to assess reproducibility between FFQ1 and FFQ2.

Results: For the validation, the correlation observed ranged from 0.317 (selenium) to 0.532 (total iodine) between the FFQ and 4DDD and for FFQ to EIB (Estimated Intake from Biomarkers), 0.146 (selenium) and 0.155 (total iodine). Cross-classifications for majority of the nutrient groups were >50% correctly classified (32.9% (selenium) to 71.6% (iodine food only)) when comparing the FFQ to 4DDD. Most of the groups were <10% grossly misclassified (1.37% (iodine and salt) to 11.0% (selenium)). For the FFQ to EIB, the correctly classified participants were 50% (iodine) and 73.1% (selenium), and the grossly misclassified participants were 16.35% (iodine) and 3.4% (selenium). For FFQ to 4DDD, the weighted kappa values showed poor agreement (k<0.21) for two groups and fair agreement (k 0.21-0.41) for three groups. For EIB, the weighted kappa showed poor agreement (k<0.21) for four groups and fair agreement (k 0.21-0.41) for one. The Bland-Altman plots showed fair agreement for the difference between FFQ1 to 4DDD or EIB. For reproducibility, the correlation between FFQ1 and FFQ2 was 0.625 (iodine) and 0.429 (selenium).

Cross-classification for correctly classified participants was >50% for iodine; for selenium and iodine, <10% were grossly misclassified. The weight kappa value showed poor agreement (k>0.21) for both iodine and selenium.

Conclusion: The FFQ showed reasonable validity when assessing iodine and selenium intake using the FFQ for breastfeeding women in New Zealand and showed good reproducibility for iodine and selenium. This FFQ could be used in future research on this population and could be used in primary care as a convenient way to assess iodine and selenium intake for breastfeeding women in New Zealand.

Keywords: iodine; selenium; FFQ; breastfeeding; lactation; validation; reproducibility; dietary assessment; questionnaire.

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Abbreviation list

4DDD	Four-day diet diary
BMIC	Breast Milk Iodine Concentration
DIO	I 5'-iodothyronine deiodinase
EAR	Estimated Average Requirements
EIB	Estimated Intake from Biomarkers
FFQ	Food Frequency Questionnaire
FFQ1	Food Frequency Questionnaire administered at three months
FFQ2	Food Frequency Questionnaire administered at 12 months
GPx	Glutathione peroxidases
I-Se-FFQ	Iodine and Selenium Specific Food Frequency Questionnaire
I-Sup	Iodine intake with supplements added without iodised salt
I-Sal	Iodine intake with iodised salt without supplements
LOA	Limits of Agreement
MINI	Mother and Infant Nutrition Investigation
MOH	Ministry of Health
MUIC	Median Urinary Iodine Concentration
NIS	Sodium-iodine symporter
NZ	New Zealand
PP	Postpartum
SD	Standard deviation
T3	Triiodothyronine
T4	Thyroxine
Tg	Thyroglobulin
TH	Thyroid Hormone
TSH	Thyroid-stimulating hormone
WHO	World Health Organization
RDI	Recommended Dietary Intake
UIC	Urinary Iodine Concentration
Q1, Q3	Quartile 1, Quartile 3

Chapter One: Introduction

1.1 Background

Iodine and selenium requirements are highest during lactation, as they are essential for thyroid function for the mother and infant. Breastfeeding mothers' iodine and selenium status can predict breastfed infants' iodine and selenium status (Stravik et al., 2021). Mothers are recommended to exclusively breastfeed their infants for six months; during this time, the infant relies on the mother's breastmilk for iodine and selenium (Dorea, 2002a, 2002b). The consequences of an iodine or selenium deficiency can cause life-long development problems in infants (Dorea, 2002a). Iodine and selenium have a co-dependent relationship in maintaining thyroid function. They have a role in regulating and synthesising thyroid hormones. These thyroid hormones regulate metabolism, growth, and development (Andersson & Braegger, 2022). The National Academy of Medicine states the Estimated Average Requirements (EAR) of iodine for breastfeeding women is 209 µg/d; this increased from 95 µg/d for non-pregnant, non-lactating women (Institute of Medicine, 2002). For selenium, the EAR for pregnancy is 49 µg/d, which increases to 59 µg/d for lactation (Institute of Medicine, 2000). Compared to New Zealand's EAR for iodine for lactating women is 190 µg daily and selenium intake for lactating women is 65 µg daily (NHMRC, 2006). Due to the increased demands associated with breastfeeding, these women's nutrient requirements are increased to keep up with these demands.

The iodine and selenium status of populations is concerning in some countries due to poor soil nutrient content and limited food sources containing high amounts of these nutrients (Thomson, 2004b). New Zealand's poor iodine and selenium levels in food have posed a population risk for decades. New Zealand soil has poor levels of iodine, leading to poor nutrient levels in crops and animals, while crops poorly absorb selenium (McNally, 2011; Zimmermann, 2011), putting New Zealanders at risk of nutrient deficiencies (Thomson et al., 2008). Iodine deficiency is linked to thyroid dysfunction, with its role as a cofactor in making thyroid hormones; thyroxine (T4) and triiodothyronine (T3). Selenium is integrated into selenoproteins, which are antioxidants with anti-inflammatory properties that regulate the thyroid (Rayman, 2012).

In the 1940s, iodine deficiency was endemic in New Zealand. To combat this endemic, the government initiated iodine fortification of table salt (Thomson et al., 2008). Population intake was then adequate until the 1990s when iodine deficiency reemerged due to the dairy industry

discontinuing the use of iodophors, increased use of plant-alternative milk, decreased bread consumption and public health measures promoting reduced salt usage (Brough, 2022). Thus, in 2009, further iodine fortification was initiated, including mandated fortification of commercial bread with iodised salt (Food Standards Australia and New Zealand, 2008). However, recent studies show that iodine deficiencies are still prevalent among lactating women (Brough et al., 2015; Jin et al., 2021). During breastfeeding, iodine becomes concentrated in breast milk and transferred to the breastfeeding infant. Furthermore, iodine requirements are increased due to maternal thyroid requirements. Due to these increased requirements and the adverse consequences of iodine deficiency, a subsidised iodine supplement (150mcg/day) is recommended for pregnant and breastfeeding mothers (Manatu Hauora: Ministry of Health, 2023).

Postpartum depression affects 13% of women in New Zealand after childbirth (Plunket, 2014). Selenium is a powerful antioxidant that is incorporated into selenoproteins. The selenoproteins, glutathione peroxidases (GPxs) and thioredoxin reductases (TrxR) both have a role in the redox system in the brain and thyroid (Rayman, 2012). These have a role in reducing oxidative stress on the brain. A selenium deficiency increases the risk of depression (Jin, Coad, Pond, et al., 2020) (Pasco et al., 2012). The increased selenium requirements during pregnancy and lactation predispose mothers to postpartum depression (Pedersen et al., 2007).

A maternal iodine or selenium deficiency has significant consequences on the fetus. Thyroid hormones play a role in brain development by impairing myelination, cell migration, differentiation, and maturation (Zimmermann, 2011). Cretinism is a severe consequence of iodine deficiency; it is a condition that negatively affects an infant's mental and physical development due to thyroid hormone deficiencies. Low maternal selenium concentrations also increase the risk of pre-eclampsia (Mistry et al., 2008), pregnancy-induced hypertension (Rayman et al., 2015), and preterm birth (Rayman et al., 2011). Low selenium intake can cause dysregulation in thyroid hormones, as it acts as an antioxidant to protect thyroid cells from oxidative damage (Zimmermann & Köhrle, 2002).

Recent research suggests that breastfeeding mothers in New Zealand still do not meet their iodine and selenium requirements. A study by Brough et al. (2015) showed that only 36% of lactating women were taking a daily 150 µg iodine supplement, with only 74% of participants meeting the EAR. Jin et al. (2022) assessed the iodine status of postpartum mothers and their breastfed infants and found that the breastfed infants had suboptimal iodine status. Another study by Jin et

al. (2019) observed that 68% of lactating women had intakes below the EAR for selenium. These findings are concerning as they show that a large proportion of this population are still at risk of an iodine or selenium deficiency.

In addition to assessing dietary intake of iodine and selenium, biomarkers can be used to determine an individual's iodine and selenium status. Various biomarkers can be used; however, some biomarkers provide greater reliability of the long-term status of these nutrients. Iodine is partitioned between breast milk and urine, meaning to gather an accurate measure, urine iodine concentration (UIC) and breast milk iodine concentration (BMIC) should be used in unison (Dold et al., 2017). Selenium can be measured through plasma/ serum, tissue, breastmilk and urine. Plasma or serum selenium provides a more accurate measure of selenium status (Thomson, 2004a).

The ability to assess dietary intake helps to provide an understanding of the population's nutrition status. There are multiple ways to assess an individual's dietary intake; different methods have various advantages and disadvantages (Gibson, 2005). A Food Frequency Questionnaire (FFQ) is a dietary assessment method that can be used on large populations in clinical practice or research settings (Gibson, 2005). An FFQ comes in the form of a checklist; participants fill in the checklist retrospectively. FFQs can be developed or adapted to be used for a specific population. They can be developed to identify a particular food, food group or nutrient (Cade et al., 2004).

The main advantage an FFQ holds over other dietary assessment methods is that it captures information over a broader time frame. It provides greater insight into the habitual intake of foods. There is no set time frame for a FFQ to assess; the researcher can set the time frame based on the study objectives (Gibson, 2005). The other advantage of FFQ is its relatively low participant burden. It is also self-administered, making it inexpensive compared to other dietary assessment methods (Gibson, 2005). Compared to other methods, that only measure food intake, a FFQ can also assess dietary patterns, which gives a more detailed understanding of a participant's dietary intake and habits.

Validating an FFQ is an important step to ensure the accuracy of this dietary assessment method. If an FFQ is not validated, it can lead to incorrect data and bias in the results, leading to false conclusions on the dietary assessment data (Cade et al., 2004). A ‘one-size-fits-all’ FFQ does not exist; every population is different and has different dietary requirements. Different ethnicities,

ages, groups, genders, and health statuses must be considered when developing an FFQ (Cade et al., 2002). Validating an FFQ can assure the accuracy of that FFQ to be used on the intended population. Two main methods for validating an FFQ are comparing it against another dietary assessment method or relevant biomarkers (Cade et al., 2004). These methods can also be combined to form an analysis called triangulation (Willett, 2001). This analysis involves comparing a triad of measures: 1) a food frequency questionnaire, (2) an alternative dietary reference method, and (3) a biomarker (Willett, 2001). These are compared using varying statistical analysis methods; there is no gold standard statistical assessment for validating dietary assessment methods (Gibson, 2005).

There is limited research on the nutrient status of breastfeeding women in New Zealand. While many studies focus on pregnancy, it is important that there is differentiation in research between pregnant and lactating women. A validated FFQ that can accurately assess iodine and selenium intake in breastfeeding women would help identify women at risk of or with a low iodine or selenium status. Currently, there is no validated FFQ for assessing iodine and selenium in breastfeeding women in New Zealand. This FFQ must be validated using data collected from the population of its intended use (Cade et al., 2004). The validated FFQ could then be used in clinical or research settings as a quick tool to assess the iodine and selenium status of breastfeeding women.

1.2 Aim

The aim of this study is to assess the validity and reproducibility of an Iodine and Selenium-Specific Food Frequency Questionnaire for breastfeeding women living in New Zealand.

1.2.1 Objectives

- To validate the Iodine and Selenium Specific Food Frequency Questionnaire using a Four-Day Diet Diary assessing the iodine and selenium intake of breastfeeding women living in New Zealand.
- To evaluate the reproducibility of the Iodine and Selenium Specific Food Frequency Questionnaire using two FFQs administered nine months apart to assess selenium and iodine intake of breastfeeding women living in New Zealand.

- To validate the Iodine and Selenium Specific Food Frequency Questionnaire using Estimated Intake from biomarkers to assess the iodine and selenium intake of breastfeeding women living in New Zealand.

1.2.2 Hypotheses

The I-Se-FFQ is a valid dietary assessment tool for assessing the iodine and selenium intake of breastfeeding mothers living in New Zealand.

The I-Se-FFQ is a reproducible dietary assessment tool for assessing the iodine and selenium intake of breastfeeding mothers living in New Zealand.

1.3 Thesis structure

Chapter one introduces the research topic. It aims to provide background, the purpose of why the study is being carried out, and the significance of the results from the study. Chapter two includes a review of the current literature involving the research topic, including a detailed analysis of the current research on selenium and iodine intake of breastfeeding women, consequences, importance, and current studies. It also describes the importance of validating FFQ and why a validated FFQ is needed for the study population. Chapter three will be written as a manuscript and contain a brief introduction, methods followed by the research findings and a discussion of these. Chapter four concludes the study and includes the limitations of the study and recommendations for future research.

1.4 Researcher contributions

Table 1. 1 Contribution of researchers to study

Researcher	**Role and Contribution to Thesis**
Charlotte Bertasius	**MSc Nutrition and Dietetics student** Main researcher; analysis of 4DDD, FFQ and biomarkers, statistical analysis, interpretation of results, and writing of thesis and manuscript.
Associate Professor Louise Brough	**Main Academic Supervisor**

	Research topic and MINI study design, ethical approval, reviewing the thesis, editing the thesis.
Dr Ying Jin	**Academic Co-Supervisor** Ethics approval, MINI study design, methodology, recruitment & screening, data collection, handling of samples, Assisted in result dissemination. Revised and approved the thesis chapters and manuscript.

Chapter Two: Literature Review

2.0 Introduction

This literature review explores the aspects related to the assessment of iodine and selenium dietary intake in breastfeeding women, various dietary assessment methods and challenges in breastfeeding women, iodine and selenium requirements for breastfeeding women, and the importance of iodine and selenium for breastfeeding women. The literature particularly focuses on the validation of food frequency questionnaires (FFQ) within the breastfeeding population living in New Zealand.

Relevant literature was gathered from the following databases: Massey University Library database, Google Scholar, Scopus, Web of Science and PubMed. The publications of reviews ranged from 1968 to 2023. The key search words were food frequency questionnaires, dietary assessment, selenium and iodine intake, thyroid, validity, reproducibility, New Zealand, lactation, breastfeeding and postpartum. When searching, these key terms were also used in combination with the functions ‘AND’ and ‘OR’.

2.1 Selenium and iodine for lactating women

2.1.1 Selenium, Iodine and Thyroid Function

Iodine and selenium are essential nutrients for thyroid function. During lactation, the demand for iodine and selenium increases significantly because breastfed infants rely on breast milk to meet their requirements. Thyroid hormones are crucial for infant growth and development. Iodine and selenium are critical for optimal thyroid hormone production and regulation. Additionally, thyroid hormones regulate basal metabolic rate. Both hyperthyroidism (overactive thyroid gland) and hypothyroidism (underactive thyroid gland) can impact psychiatric disorders such as depression and anxiety. An association has also been found between thyroid dysfunction and postpartum depressive disorder (Sylven et al., 2013). Poor iodine and selenium intake can impact the mother's and infant's thyroid function. Lactation increases the demand for maternal thyroid hormone production (Soldin, 2011). Further, a dysfunctional thyroid can negatively impact milk production (Andersson & Braegger, 2022).

The thyroid gland, functioning as an essential endocrine gland, is responsible for the formation and secretion of thyroid hormones, triiodothyronine (T3) and thyroxine (T4). Playing a pivotal role

in metabolism and regulating metabolic rate. These thyroid hormones consist of proteins and iodide; T3 comprises three iodide atoms, and T4 comprises four iodide atoms (Andersson & Braegger, 2022; Chung, 2014). T3 is responsible for the regulation of the development of the brain and cognition, with main roles in neuronal proliferation and migration, glial differentiation, and myelination of the central nervous system (Andersson & Braegger, 2022). T4 role is not as pronounced as T3 but acts as the precursor in converting T4 to T3. Nonetheless, T4 helps to regulate normal metabolism in the body. During periods of accelerated growth and development, it is essential that thyroid hormones are functioning optimally. These thyroid hormones help with physical growth and maturation and the development of the central nervous system.

Iodine requirements are increased for lactating women as the infant relies on the maternal iodine intake and stores (Semba & Delange, 2001). Mammary glands can hold concentrated iodine levels (Semba & Delange, 2001). Iodine is a rate-limiting element for synthesising thyroid hormones (Chung, 2014). During lactation, there is an increase in maternal thyroid hormones and the sodium-iodine symporter (NIS) responsible for transporting iodine to the mammary gland and into the breast milk (Cho et al., 2000), resulting in a high concentration of iodine in the mammary gland (Semba & Delange, 2001). Iodide is transported into the thyroid gland via the NIS, thyroid peroxidase oxidases I^- becoming bound to thyroglobulin (Tg) to help form T4, which can be converted to T3 (Andersson & Braegger, 2022). In iodine-deficient states, the thyroid stimulating hormone (TSH) will increase to maximise iodine absorption. This increases the thyroid gland's workload, resulting in a condition called goitre (Zimmermann et al., 2008). Iodine deficiency causes thyroid dysfunction in the mother and can put the infant at risk of poor cognitive development and intellectual disabilities (Zimmermann et al., 2008).

The thyroid contains the highest amount of selenium in the body. Selenoproteins are antioxidants containing selenium; these reduce oxidative stress through redox reactions with different micronutrients (Rayman, 2012). Thus, selenium can be protective against the buildup of oxidative stress and may be associated with depression (Pasco et al., 2012). Secondly, selenium has a role in thyroid regulation. Selenium is a component of the enzyme I 5'-iodothyronine deiodinase (DIO) required for the conversion of thyroxine (T4) to triiodothyronine (T3) (Thomson, 2004a). Low selenium status can reduce the conversion of T4 to T3, subsequently causing an increase in TSH production. Selenium stays concentrated in the thyroid even during times of low selenium status. TSH stimulates the conversion of T4 to T3 via DIO; this reaction's byproduct is hydrogen peroxide (Ventura et al., 2017). Selenium protects the thyroid gland from these free radicals

(Zimmermann et al., 2008). A selenoprotein called glutathione peroxidase is an enzyme that reduces hydrogen peroxide to water and oxygen, preventing oxidative damage to thyroid tissue (Zimmermann & Köhrle, 2002).

While infants are born with some selenium stores, they rely on breastmilk to provide them with selenium to maintain and build these stores (Dorea, 2002b). Maternal selenium intake is directly associated with breastfed infants' selenium intake through breast milk (Alaejos & Romero, 1995). Low selenium status adversely affects both immunity and cognitive function for mothers and infants (Varsi et al., 2017). While iodine and selenium have roles that are independent of each other, they also have codependent roles in thyroid function. In individuals who are iodine deficient, a selenium deficiency can exacerbate thyroid dysfunction (Thomson, 2004a). If the body is selenium deficient, it could seriously damage thyroid tissue and increase the risk of thyroid disease.

2.1.2 Iodine and Selenium Requirements

Three of the most significant conditions caused by iodine and selenium deficiencies are myxedematous cretinism (iodine), Keshan disease and Kashin-Beck disease (selenium). Keshan disease is a heart condition (endemic cardiomyopathy). While Kashin-Beck disease is a chronic condition that affects bones. Overall, impaired thyroid function during pregnancy can impair growth in children and cause neurodevelopmental delays (Stravik et al., 2021). Severe iodine deficiency can result in cretinism, characterised by severe mental and physical impairment. New evidence suggests that iodine deficiencies may play a role in infant allergy risk (Stravik et al., 2021). While there are no reported cases of cretinism in New Zealand due to the risk of iodine and selenium deficiencies within our population, measuring these nutrients is essential.

Based on the Australia and New Zealand Nutrient References Values (NHMRC, 2006), the Estimated Average Requirement (EAR) for iodine for lactating women is 190 μg daily and selenium intake for lactating women is 65 μg daily. During lactation, the body has a process to help protect the infant when the mother has low iodine status. Iodine is partitioned between urine and breastmilk; a higher proportion of iodine is excreted in breastmilk than urine, helping the infant maintain iodine intake (Dold et al., 2017). In recent years, many trends within the New Zealand diet could negatively impact iodine and selenium status.

Selenium status can be measured using plasma selenium; it is the most widely used biomarker as it best reflects selenium intake (Ashton et al., 2009). Plasma selenium can be converted from μg/L to μg/d, which is better to use when estimating selenium intake from biomarkers (Han et al., 2018). A study by Thomson et al. (2001) recruited 35 pregnant women in Dunedin, New Zealand. Eighteen women were asked to take a 50μg selenium supplement daily from pregnancy until 12 months postpartum. Monthly visits were required during pregnancy, and then postpartum visits at 3, 6, 9 and 12 months were used to collect blood tests and urine samples. The results showed that renal clearance of selenium was much higher during pregnancy than during postpartum. Also, non-supplemented plasma selenium and urine selenium excretion were significantly lower in postpartum compared to pregnancy. This shows that during postpartum, there is either a decrease in selenium intake or an increase in selenium requirements. Inadequate iodine status in a population of lactating women is indicated by a median urinary iodine concentration (MUIC) of less than 100 μg/L, and adequate iodine status is indicated by greater than 100 μg/L; this is lower for lactating women than pregnant women, as a proportion of iodine is exerted through breast milk (World Health Organization, 2007). Another study in Dunedin by Mulrine et al. (2005) assessed the iodine status of breastfeeding mothers. 109 lactating mothers were recruited and supplemented with either 75μg/d, 150 μg/d iodine or a placebo for six months. From preliminary testing of the participants' iodine status prior to giving birth, the study population's average MUIC was 43 μg/L, showing an iodine deficiency. With these results, if lactating mothers are not advised to take iodine supplements throughout breastfeeding, it will put them and their infants at risk of iodine deficiency.

Breastfed infants rely solely on their mother's iodine stores and intake until solids are introduced. A study by Skeaff et al. (2005) investigated a group of New Zealand infants and toddlers who were breastfed or formula-fed. In New Zealand, formulas are permitted to have an iodine content of 30 to 315 μg/L. The study was conducted on children aged 6 to 24 months in the South Island. The study found that MUIC was lower in breastfed than formula-fed infants (44 μg/L vs 99 μg/L). This study identified that breastfed infants in 2003 were at a more significant risk of developing an iodine deficiency due to the iodine level in breast milk.

2.1.3 Sources of Iodine and Selenium

Largely, iodine and selenium concentrations of foods vary depending on the environment in which they are grown. In recent years, there has been an increase in selenium intake due to importing

wheat and cereal products from Australia into New Zealand, which has a higher selenium content (Rayman, 2012). Brazil nuts, organ meats and seafood are food sources with the highest selenium content (Rayman, 2012; Thomson, 2004b). Yeast, cereals, and grains enriched with selenium are the most bioavailable sources of selenium. Dairy products, fruit, and vegetables are the lowest sources of selenium. For iodine, seafood, iodised salt, milk, and eggs are the best sources of iodine. Seafood of saltwater origin is higher in iodine than food gathered from freshwater environments. Other sources of iodine are meat and cereals; however, these are secondary sources of iodine (Thomson, 2004b).

In the New Zealand diet, the main sources of selenium are seafood, poultry, and eggs (NHMRC, 2006). Grain-based foods also contribute largely to selenium intake. Across the New Zealand population, female adults had the lowest selenium intake (68μg/day), and of the paediatric cohort, infants had the lowest selenium intake (24μg/day) (Pearson et al., 2016). Previously, dairy products were the main sources of iodine intake before bread fortification. Now, the main food sources of iodine part of the New Zealand diet are cereal-based foods and bread, followed by fish, meat and eggs (Pearson et al., 2016).

Whilst some salt is fortified with iodine, other types of salt are not. The salt market has significantly expanded in recent years with the addition of Himalayan salt, sea salt, rock salt and herbal salts. There has also been a shift in the New Zealand diet where some individuals actively try to decrease salt usage to restrict sodium intake; while this can be positive for heart health, it can negatively affect iodine intake (Skeaff & Lonsdale-Cooper, 2013). Additionally to this diet, trends such as keto, low carb or gluten-free diets have become more common, resulting in a decrease in commercial bread consumption. With bread being one of the main sources of iodine, having a diet low in bread could increase the risk of low iodine status (Mallard et al., 2012). There has also been an increase in plant milk usage. These products tend to have lower levels of iodine compared to dairy milk (Dineva et al., 2021).

Goitrogens are compounds that interfere with the metabolism of iodine. They block the uptake of iodine, resulting in thyroid dysfunction. Goitrogens are found in cruciferous vegetable sources such as cabbage, broccoli, cauliflower and cassava (NHMRC, 2006). A diet high in these food sources can increase the risk of developing an iodine deficiency, increasing the risk of thyroid dysfunction (Pearson et al., 2016).

2.1.4. Government Iodine-Specific Initiatives

In New Zealand, the government has implemented initiatives to help improve iodine intake: 1) iodisation of salt, 2) fortification of bread with iodised salt, and 3) subsided iodine supplements for pregnant and breastfeeding women. Studies have examined how these initiatives have impacted the iodine status of pregnant and breastfeeding mothers. Brough et al. (2015) conducted a study looking at 57 participants in 2009 and 70 participants in 2011, including pregnant and breastfeeding women. The results showed an increase in iodine status, with MUIC for the pregnant population for 2011 and 2009 being 85 and 47 μg/L, respectively, and for the breastfeeding population, 74 and 34 μg/L. While overall iodine status has increased since the initiatives began, only 74% of pregnant women achieved the EAR for iodine. A study by Reynolds and Skeaff (2018) found that only 52% of pregnant and breastfeeding women adhered to the recommendation of at least a 150 μg/day iodine supplement.

Australia has the same requirement for mandatory iodine fortification of bread. A study by Huynh et al. (2017) measured the iodine status of lactating women's pre-fortification and post-fortification of bread to see the effect this initiative had on this population. The study analysed breastmilk samples for a population of 1660 (enrolled in the study) from 2006 and 2007 (pre-fortification) and then from 2012 and 2013 (post-fortification). An 84 μg/L increase in median breast milk iodine concentration (BMIC) was identified between pre-fortification and post-fortification ($P<0.05$). In the post-fortification group, fewer participants were identified as having inadequate iodine status (BMIC <100μg/L) compared to the pre-fortification group. These results showed that the mandatory iodine fortification of bread has improved overall iodine status. However, 49% of women had BMIC <100 μg/L in the pre-fortification group. Showing that the recommendations of taking an iodine supplement during lactation are still essential to help meet the increased iodine requirements of lactating women.

2.1.5 Biomarkers for Selenium and Iodine

Selenium

Selenium intake can be determined using biochemical markers such as urine samples or serum plasma concentrations. Biomarkers such as urinary selenium excretion can be a good indicator of recent selenium intake as it is estimated that 50-60% of selenium intake is excreted into the urine (Thomson, 2004a). Urinary markers of selenium are more accurate than tissue markers

(toenail and hair clippings) due to tissue markers varying depending on the type of selenium that is ingested (Thomson, 2004a). Tissue markers that can assess long-term selenium intake include toenails and hair clippings. The form of selenium impacts the status of selenium, as different forms can affect other parts of the body (Levander et al., 1987).

Plasma or serum selenium can be used to assess short-term selenium status. In comparison, erythrocyte selenium is used to assess long-term selenium status (Thomson, 2004a). Many factors can affect plasma selenium concentrations. Pregnancy and lactation have one of the most profound effects on plasma selenium concentration. Smoking negatively affects plasma selenium concentration; it is unclear whether this is due to the increased antioxidant requirements caused by tobacco or inadequate dietary intake (Gibson, 2005). There are also age-related factors, as plasma selenium status has a decreasing pattern in individuals over 60.

Breastmilk selenium concentration is positively associated with dietary intake, making it an appropriate biomarker to determine short-term selenium status in lactating women. However, this partitioning of iodine between breastmilk and urine should be taken into consideration when using breastmilk independently. Other factors that can affect the selenium concentration of breast milk include parity and the stage of lactation (Benemariyal et al., 1995). Selenium concentrations are higher in the hindmilk compared to the foremilk (Mannan & Picciano, 1987).

Iodine

Iodine is predominantly concentrated in the thyroid gland, with 70-80% of iodine found in the thyroid gland (Gibson, 2005). There are multiple different methods to assess iodine intake and status. Urinary iodine is correlated with dietary iodine intake as over 90% of iodine intake is excreted in urine, and a small amount is excreted in feces and via the skin (Gibson, 2005; Skeaff et al., 2005; Thomson, 2004b). Iodine can be measured in 24-hour or morning spot urine samples (Thomson, 2004b). Median urinary iodine concentration (MUIC) is the recommended biomarker for assessing iodine status in populations (World Health Organization, 2007). However, while it is a good indicator, it has some limitations. Iodine intake is highly variable, making it not suitable for individuals. However, these variables tend to even out when averaged in a population study (World Health Organization, 2007).

Thyroid hormones can also be used as an indicator of iodine status. While this method is less recommended than urinary iodine, it still provides an indication of iodine status. Hormone monitoring methods are less practical than iodine blood or urine tests, which are more expensive and require greater laboratory analysis (World Health Organization, 2007). Thyroglobulin is a protein found in the thyroid and a precursor in synthesising thyroid hormones. Tg markers can be used with urinary iodine to assess iodine status (World Health Organization, 2007).

Iodine concentration can also be measured in breast milk and directly correlates with maternal iodine intake. During lactation, iodine becomes concentrated in the mammary gland and is directly excreted into breast milk. Thus, breast milk iodine concentration is a good biomarker for assessing iodine status in breastfeeding mothers (Dold et al., 2017). In lactating mothers, dietary iodine intake is mainly absorbed by three main organs: the mammary gland, thyroid and kidneys. The thyroid is responsible for absorbing a fraction of dietary iodine, with the mammary gland and kidneys absorbing the most to be excreted in breast milk and urine (Dold et al., 2017). A study by Andersen et al. (2014) found that breast milk iodine concentration alone was more appropriate for a lactating population as fluid intake directly affected urinary iodine concentration along with diurnal variation. Moreover, combining the two measures, BMIC and UIC, will overcome any effect of partitioning and will give a more accurate estimate of iodine status in lactating women (Dold et al., 2017).

2.1.6 Iodine and Selenium problem in New Zealand

These nutrients are trace elements found in soils that are taken up into crops and are subsequently ingested by animals and humans. New Zealand soil has poor levels of iodine, and crops poorly absorb selenium (McNally, 2011), contributing to widespread iodine and selenium deficiencies (Thomson, 2004b). Pregnant or lactating women are at the most significant risk for developing iodine and selenium deficiencies due to their increased requirements (Soldin, 2011). If the mother is unable to meet these increased requirements, the fetus/infant becomes at risk of developing a deficiency. A deficiency in these nutrients can lead to thyroid dysfunction and disruption of thyroid hormones (Zimmermann et al., 2008).

From the 2008/09 Adult Nutrition Survey, it was found that women 31-51 years old, on average, had a selenium intake of 58.1 μg, with an estimated prevalence of 58% of these women not meeting the EAR for selenium intake (University of Otago & Ministry of Health, 2011). A study by

Kendall (2016) assessed breastfeeding women's selenium and zinc status in New Zealand. It showed a large proportion of lactating women are not consuming enough selenium to meet their increased selenium requirements. Therefore, increasing the risk of them or their breastfed infant developing a selenium deficiency. Another study by McLachlan et al. (2004) assessed the selenium intake of 6 to 24-month-old infants and their postpartum mothers in the South Island, New Zealand. The findings showed that the infants and mothers were at risk of suboptimal selenium status.

New Zealand implemented iodine fortification in 1924 when table salt was iodised (Thomson et al., 1997). Before this goitre, a disease caused by low iodine status was common in the late 1800s and early 1900s (Thomson, 2004b). Prior to colonisation, there were reports of goitre (Brough & Skeaff, 2020). Initially, a low concentration of iodine was added to salt until further evidence of widespread goitre was found in New Zealand. In 1938, the iodine concentration of salt was increased to help alleviate the goitre epidemic (Mann & Aitken, 2003). In 1962, the dairy industry started using iodophors as cleaning agents, accidentally increasing the iodine content of dairy products in New Zealand. However, this process became less common in the 1980s as other cleaning agents were used, causing a decrease in the iodine content of dairy products (Thomson, 2004b). This resulted in iodine deficiency emerging in New Zealand. More recently, in 2009, further fortification occurred when it became mandatory for bread to be fortified with iodised salt (Ministry for Primary Industries & Ministry of Health, 2016; Skeaff & Lonsdale-Cooper, 2013). With other products, manufacturers and consumers can add iodised salt to food products (Thomson, 2004b).

Even with mandatory fortification of bread with iodised salt, iodine intakes are insufficient to ensure pregnant and lactating women meet their increased requirements. In 2010, New Zealand further initiated subsided iodine supplements for all pregnant and breastfeeding women. In a study by Brough et al. (2015) in 2011, only 28% of lactating women were aware of the iodine supplement recommendations. However, some supplements did not meet the recommendation of 150 mg/d. In New Zealand, women are entitled to free antenatal healthcare up to 6 weeks after delivery. After this time, women would need to pay to renew their iodine supplement prescription, making it less accessible for low socioeconomic communities (Brough et al., 2015).

2.2 Dietary Assessment Methods

There are multiple methods to assess an individual's dietary intake. These methods can be classified into quantitative daily consumption methods, including diet recalls and diet records. The second group is retrospective information, including food frequency questionnaires and diet history (Gibson, 2005).

2.2.1 Food Frequency Questionnaire

A food frequency questionnaire assesses how frequently food items are consumed (Gibson, 2005). The questionnaire entails a list of foods or beverages in which the participant states how often they have consumed those products/foods in the specified amount of time. The questionnaire is self-administered. The questionnaire can be computerised or on paper; most recently, they are computerised. FFQs can be designed to assess a specific nutrient or group of foods (Willett, 2012). There is no standard timeframe for an FFQ; the period assessed can vary from a week to a year. This means a wider range of an individual's diet is captured (Gibson, 2005).

FFQs are frequently seen in research due to their self-administration ability and having less participant burden than other dietary assessment methods (Willett, 2012). This method is also economical as it does not require an interviewer. However, FFQs are limited, as this method is retrospective and relies on the participant's memory to maintain accuracy. Participants completing an FFQ need a certain level of literacy and numeracy to interpret and answer questions correctly.

2.2.2 Diet History

A diet history aims to gain more in-depth information about the participant’s diet. This gathers information about usual food intake and meal patterns; this information can be taken over a long time. This is the most common dietary assessment method used in clinical practice as it provides diet intake and food consumption behaviours. The interviewer will typically take a diet history, starting with questions about meal patterns and mealtimes.

They will also ask about frequently consumed meals or foods and the portion sizes of these meals and foods. Secondly, the interviewer may cross-check the information with an FFQ to check that the information given is accurate (Gibson, 2005). The accuracy of the diet history largely depends on the skill and ability of the interviewer (Thompson & Subar, 2017). Due to the complexity of diet

history requiring a trained interviewer and being rather time-consuming, they are less likely to be used in research.

2.2.3 24-Diet Recall

The 24-hour recall is an assessment method used to gather a single day of data the participant consumed over the last 24 hours. While a diet recall is relatively easy to carry out, there is an interview technique to gather an appropriate level of information. This technique is made up of 4 stages. The first stage involves taking a general diet overview with limited food details. Stage two involves reviewing the listed food/meals and gathering information such as cooking methods and brands of foods. The third stage involves repeating the recall, but this time gathering information such as serving size and amounts of meals and foods. The final stage is the interviewer recalling the information to the participant to ensure a mutual understanding (Gibson, 2005). A diet recall is generally used with another tool, as it only shows one day of data. It is important not to assume it is an individual standard diet (Willett, 2012). This method can be used to assess dietary intake at a population level. The 24-hour diet recall is economical and has little participant burden. However, the interviewer does require training to be carried out properly, and the information is more likely to be inaccurate compared to other dietary assessment methods (Thompson & Subar, 2017).

2.2.4 Food record

A food record is considered one of the most accurate dietary assessment methods. It involves the participant recording what they are eating prospectively. The participant records a detailed description, including the food, brand, method of preparation and cooking and the amount/measurement of the food (Gibson, 2005). There are two types of food records: an estimated or weighed food record. An estimated food record is when the participant estimates the amount of food and then records it.

In contrast, a weighed food record is when the participant weighs each component of a recipe or food consumed and records it. Food records are usually carried out over multiple days, usually 3 or 4 days. One of these days is usually a weekend day. This gives a more accurate representation of an individual's weekly diet. As food records are self-administered by the participants, there is a high participant burden as they must write down every food or beverage

consumed. For the researchers, there is a high workload that comes along with this, as all the data needs to be inputted into a food analysis system (Willett, 2001).

Table 2. 1 Challenges of Different Dietary Assessment Methods

Dietary Assessment Methods	Challenges
FFQ	- Relies on participant memory. - Participants need a certain level of numeracy and literacy level to interpret and answer questions. - Food lists can be restricted to certain foods and some composite foods can be excluded from the list. - Portion size interpretation may differ between participants.
Diet history	- Requires a high level of interviewer training to be carried out correctly. - Experience and time consuming.
24-hour recall	- Only gives one day of data. - Dependent on participant memory. - Requires interviewer training to be carried out correctly. - Probing questions can cause bias and inaccuracy in answers.
Food Records	- High participant burden. - Time consuming for the researcher to analyse.

2.3 Dietary Assessment Challenges

2.3.1 Under- and over-reporting

When administering a dietary assessment, there will inevitably be under or over-reporting of dietary intake from participants leading to inaccurate results in data collection. Macdiarmid and Blundell (1998) found that the prevalence of underreporting in numerous nutritional studies is 18 to 54% but can also differ in different demographic subgroups. For instance, women are more likely to underreport their diet than men, as are participants with a higher Body Mass Index (BMI). Individuals who partake in dieting or diet restricting are more likely to underreport their dietary intake (Gibson, 2005). The environment in which the dietary intake is recorded can also lead to underreporting, possibly due to social embarrassment, inconvenience, or guilt. Dietary assessment tools used in a group environment or involving an interviewer can lead to these effects (Gibson, 2005).

2.3.2 Dietary variations

Many factors influence dietary variation within a population, including ethnicity, age, economic status, lifestyle, and many other factors. Day-to-day variation within an individual's diet is to be expected, meaning one day of eating can look different compared to the following day (Willett, 2012). Mothers tend to have fluctuating schedules throughout the week. Some days, they might have help, or their child could be at daycare. There are also factors, such as some mums continuing to work while some may be on maternity leave. These factors influence dietary variation as different lifestyles influence an individual's meal patterns.

When comparing dietary intakes, sex and age can influence the dietary variation within that population. Females' and males' diets should always be presented separately due to the differing natures of their diets. Traditionally, males tend to eat larger portion sizes compared to females (Gibson, 2005). Along with this, the age of the individuals within a population can increase the dietary variation. At different ages, individuals will have differing lifestyles and dietary needs. Not only will portion sizes be different, but also meal patterns. In populations with a significant range of ages, these should be split into smaller categories for the results to be presented to prevent inaccurate results (Gibson, 2005).

2.3.3 Assessing iodine and selenium intake

Challenges can arise when assessing iodine and selenium intake. Current advice from the World Health Organisation is for the universal iodination of salt to help combat widespread iodine deficiency (Secretariat et al., 2007). While salt iodisation is not mandatory in New Zealand, adding iodised salt to commercial bread is (Food Standards Australia New Zealand, 2009). It is still common in households to use iodised table salt in cooking and adding to meals at the dining table. This can pose a challenge in dietary assessment as quantifying this amount is difficult as measuring tools are often not used. Edmonds et al. (2016) have suggested estimating the amount of iodine consumed through iodised salt by adding 48 μg of iodine (from 1g of salt) to the dietary intake for each participant who indicates they use iodised salt in cooking or at the dining table.

Selenium content can vary across New Zealand, which results in food sources having differing amounts. The South Island food supply is reported to have lower levels of selenium compared to the North Island. Furthermore, more commonly, wheat from Australia has been seen being imported into New Zealand, therefore increasing the selenium content of these wheat-based

products (Thomson, 2004b). Australian wheat is typically used more frequently in the North Island, whereas the South Island use their own. Thus, assessing dietary intake can make it hard to measure selenium and iodine intake accurately. Food databases used for dietary analysis, such as FOODWORKS, are not frequently updated; therefore, nutrient information can be out of date, leading to inaccurate dietary analysis.

2.3.4 Supplement usage in breastfeeding women

In New Zealand, the government provides a subsidised iodine supplement (150 mcg/day) for all pregnant and breastfeeding women (Manatu Hauora: Ministry of Health, 2023; Shukri et al., 2014). Selenium supplementation is less extensively studied compared to iodine. The New Zealand government does not provide selenium supplements to help with the increased selenium requirements during breastfeeding. With the recommendation to take an iodine supplement during breastfeeding, it is important to consider this factor when assessing a breastfeeding population. Specifically, the amount of nutrients in the supplement can be accounted for in the dietary intake. Some women use the government-subsided supplement, others may use there another supplement with a different amount of iodine.

2.3.6 Measurement errors

Both systematic and random errors are likely to occur during dietary assessment. While it may be unavoidable in some cases, there are ways to minimise the chance of these errors occurring and creating bias within the results. Systematic errors can be harder to control, including under and overreporting, misreporting, and participants wrongly estimating portion sizes. Random errors can affect the reproducibility of a dietary assessment method and can be minimised by the frequency of the dietary assessment used in the same group (Gibson, 2005). For some mothers, the postpartum lifestyle can be sporadic, and some mothers have irregular meal patterns. Irregular meal patterns can be hard to quantify; hence, gathering accurate dietary intake can take time and effort. Additionally, participants can inaccurately record dietary intake, leading to bias within the results. Control procedures can be used to minimise these errors, for instance, providing thorough instructions, training interviewers, and using the most appropriate dietary assessment method for the study population (Willett, 2012).

2.3.7 Analysis of Dietary Intake

The technique used to analyse dietary assessments can cause significant bias in the results if the wrong food composition tables are used. These errors caused by food composition tables can be random or systematic (Gibson, 2005). Food composition databases are sources of nutrient values for different food items, including national or international databases. Using food composition databases from the same country or demographic as a study population is important to reflect the population's true dietary patterns, such as gaining accurate nutrient information for selenium and iodine. New Zealand crops have lower selenium and iodine nutrient profiles (Brough & Skeaff, 2020; Thomson, 2004b). Accurately analysing iodine intake can be difficult; with the mandatory fortification of bread with iodised salt, it is important to use food databases that reflect the current iodine level in bread (Skeaff & Lonsdale-Cooper, 2013).

Additionally, the ability to quantify salt intake can be challenging. Most individuals do not measure salt intake when adding to cooking or meals. Therefore, estimating the iodine intake from the salt consumed can be challenging if the salt being used is iodised. In New Zealand, the food composition database, FOODfiles, is jointly owned by the Ministry of Health and Plant and Food Research (Sivakumaran et al., 2018). However, even when using food composition databases from the relevant country, there are limitations. The food items are limited to what is contained in the database, and if certain food items are missing, then the most appropriate alternative food item needs to be selected instead. These limitations must be considered when analysing the dietary intakes of different populations.

2.4 Considerations when Assessing the Validity and Reproducibility of Food Frequency Questionnaires

Validation of an FFQ is the best way to ensure its accuracy for its intended population. Willett (2012) defines validation as “the degree to which the questionnaires actually measure for the aspect of dietary intake it was designed to measure” (Willett, 2012). There are different methods to assess an FFQ's validity; however, comparing a ‘gold standard’ or far superior dietary assessment is considered the best method of validation (Cade et al., 2002; Willett, 2012). Validation is imperfect as the comparison is not done with an absolutely accurate dietary assessment, only a more superior or accurate assessment method. These two measures must be independent of each other to ensure there is no correlation to give an inaccurate validation

result. Weighed food records are the recommended choice of dietary assessment method to be used for comparison or 24-hour recalls. However, with 24-hour recalls, there is a higher chance of correlation due to similar characteristics compared to FFQ, such as relying on memory and participant perception of portion size (Cade et al., 2002).

Understanding the reproducibility of an FFQ is just as important as validation. Reproducibility tests the precision of the FFQ by taking the FFQ data from two different time points and comparing them to see if the same result can be produced at separate time points from each other (Willett, 2012).

2.4.1 Sample/population selection

Factors such as population selection can affect the validation of an FFQ. The study population used to validate an FFQ, must be validated using a sample of the intended population (Cade et al., 2002). This includes matching characteristics such as age, ethnicity, gender, and health study. Suppose there are significant differences between the study population and the intended population. In that case, it can cause inaccurate results in the validation, leading to inaccurate data when utilising the FFQ in the intended population (Cade et al., 2002). For example, mothers of child-bearing age ranges will have different dietary assessment answers compared to young or older adult males.

2.4.2 Sample size

The sample size of the study population largely depends on the choice of statistical analysis. Different statistical analysis methods can be used to determine validity; different methods have different sample size requirements. When using the Bland-Altman method, there needs to be a sample size of at least 50 participants to estimate the Limits of Agreement (LOA). This is based on a review of validation studies undertaken by Cade et al. (2002). The study investigated sample sizes ranging from 6 to 3750 participants, with the median found to be 110 participants.

2.4.3 Reference method and recording day required

To validate an FFQ, an appropriate reference method needs to be selected. The reference method refers to the dietary assessment method used compared to the FFQ (Cade et al., 2002). The reference method selected must use different assessment techniques than an FFQ, e.g.

memory or method of estimating portion size (Gibson, 2005). This limits the degree of errors in the validation. While no reference method will accurately measure dietary intake, the point of validation is to analyse the degree of agreement between the two methods to indicate validity (Gibson, 2005).

Additionally, studies have found it essential to administer the reference method following the FFQ. This is due to the reference method possibly influencing the participants' answers if they complete the FFQ second. This could cause an inaccurate or false result in the validation (Cade et al., 2002).

Commonly, food records are used to validate FFQs due to the independent errors they both possess. Food records are widely considered a superior dietary assessment method as they can be completed as weighed or estimated. This method can assess dietary intake over several days and allows the habitual dietary intake to be considered (Cade et al., 2002).

The number of recorded days must be decided using reference methods such as a food record. There is no gold standard number of days for carrying out a food record. There are benefits to using a more extended period, such as gathering data on habitual intake, which allows better recognition of ‘usual intake’ and meal patterns. However, it is important to consider the participant burden; while a long food record would be ideal, it may not provide the best accuracy. Longer diet records carry a higher participant burden and could lead to inaccurate results as participants become complacent. Additionally, in research, it can cause a decrease in the rate of respondents (Cade et al., 2002). A study by (Stram et al., 1995) found that an ideal number of days to do a food record for a validation study is four to five days.

2.5 Food Frequency Questionnaires Available for Breastfeeding Mothers in New Zealand and Internationally

Overall, there needs to be more research on breastfeeding mothers' dietary intake. With most research being directed during pregnancy rather than postpartum. To the best of our knowledge, there are no validated selenium specific FFQs. Selenium has only been investigated in multiple nutrient FFQs. While there are iodine-specific FFQs, these have not been developed and validated for a breastfeeding population. There is no current literature to suggest that there is a selenium and iodine-specific FFQ that has been developed or validated.

In Australia, Condo et al. (2015) developed an iodine-specific FFQ to assess pregnant women's iodine status. This iodine-specific FFQ was a forty-four-item questionnaire. The questionnaire was validated through the correlation between the iodine intake of the I-FFQ and a 4DDD. Additionally, the I-FFQ was validated by correlating the iodine intake from FFQ and biomarkers, including 24-hour urinary iodine excretion, 24-hour urinary iodine concentration, spot UIC and thyroid function. These were all measured at 28 weeks' gestation. When comparing the I-FFQ and 4DDD, a moderate correlation of 0.349 ($P<0.001$) was observed; this correlation increased to 0.876 ($P<0.001$) with the addition of iodine supplements. From cross-classifications, there was a fair agreement between the I-FFQ and 4DDD, classified into inadequate (<160μg/L) and adequate (>160μg/L) groups; 66% were classified into the same category, followed by 90% with the addition of supplements. An association between iodine intake and 24 UIE was identified. It was found that thyroid function had limited ability to be predict from the iodine-specific FFQ. These results showed that Condo et al. (2015) a valid screening tool was developed to identify pregnant Australian women with inadequate iodine intake.

In Norway, Naess et al. (2019) the validity and reproducibility of a 60-food item iodine-specific FFQ was assessed. Norway does not have a mandatory fortification policy, as is seen in Australia and New Zealand. This study was conducted on 137 pregnant women; the iodine-specific FFQ and 6DDD were administered at 16-18 weeks gestational age. Along with using the 6DDD as the reference model, biomarkers were also used, including urinary iodine concentration and thyroid function tests. When comparing the I-FFQ and 6DDD, a strong correlation was obtained at 0.62 ($P<0.001$). Between I-FFQ and UIC, an acceptable correlation of 0.21 ($P=0.018$) was seen; however, no significant association between I-FFQ and thyroid function tests were observed. The limits of agreement from the Bland Altman plot were large. The reproducibility of the I-FFQ observed a strong correlation, 0.63 ($P<0.001$), between the iodine intake at 16-18-week gestion and 35-36 gestion. The results from the validation and reproducibility demonstrate that this I-FFQ can be used to assess the iodine status of pregnant Norwegian women.

Prpic et al. (2021) conducted a study in Croatia with 133 lactating women and their breastfed infants. Both the urinary iodine concentration (UIC) and thyroid markers were measured in both the mother and the infant. Specifically, iodine concentrations (BMIC) were measured in the mother's breast milk. These biomarkers were compared to the iodine and salt-rich food-specific FFQ analysis. Croatia has initiatives in place for salt iodisation. Of the participants, 99.2% used

iodised salt, and only 20.4% used iodine-containing supplements. The analysis revealed a correlation between the mother's BMIC and the infant's UIC. Additionally, there was a positive correlation between the mothers' and the infants' thyroid function. This study demonstrated that BMIC was a reliable biomarker for lactating mothers. The study found that iodised salt and dairy products were the highest sources of iodine in the participant's diet; the analysis showed a positive correlation between dairy intake and BMIC.

There are limited studies worldwide that have validated a selenium-specific food frequency questionnaire. A study by Pestitschek et al. (2013) used a selenium-specific food frequency questionnaire to examine the correlation between selenium intake and thyroid function. The FFQ included five food groups and 15 selenium-rich foods to assess selenium intake. The study was conducted in a thyroid outpatient clinic in Austria with 212 participants (176 women and 36 men); 21 participants were used as a control with no thyroid abnormalities. The FFQ consisted of 5 food groups with 15 selenium-specific foods outlined. The results revealed that 86% of the participants were not meeting the US EAR for selenium. The participants with hypothyroid function had a lower selenium intake than those with normal thyroid function. The study's key finding was a positive correlation between selenium intake and thyroid function.

Table 2. 2 Comparison of Food Frequency Questionnaire studies carried out on Lactating women and Comparison of Iodine or Selenium Specific Food Frequency Questionnaires in New Zealand and Internationally

Food Frequency Questionnaire Studies Carried Out on Lactating Women							
Reference	***Country***	***Sample size***	***FFQ type, consumption period,***	***Validation method***	***Nutrients assessed***	***Asked about iodine supplement use***	***Findings***
Aakre et al. (2021)	Norway	1,004 participants enrolled during pregnancy	Web-based, semi quantitative FFQ Previous 3 months	FFQ previously validated by (Dahl et al., 2011), and adapted to be used for this population.	Iodine	Yes	Iodine intake is insufficient during pregnancy and up to 18 months postpartum.
Bzikowska-Jura et al. (2023)	Poland	30 mothers at 6-8 weeks postpartum	Semi-structured in accordance with WHO guidelines Previous 3 months	Validated against 3-day diet record using Spearman's/ Pearson's correlation coefficients	Calcium Phosphorus	N/A	Maternal calcium and phosphorus were directly related to the content of both minerals in human milk.
Lovell et al. (2016)	New Zealand	63 term, healthy, singleton exclusively breastfed infants aged 2-3 months and their mothers	Semi quantitative FFQ	FFQ was not validated due to limited vitamin D containing foods in NZ	Vitamin D	N/A	Exclusively breastfed infants vitamin D status is dependent on maternal vitamin D intake and sunlight exposure.
Ding et al. (2021)	China	112 lactating women	Semi quantitative FFQ	Validated against a 3-day diet record, Spearman's rank correlation coefficient was used to compare. Reproducibility was also tested	Food groups Diet patterns Macro and micronutrients	N/A	This FFQ was a valid and reasonably reproducible tool to use to assess the dietary intake for lactating Chinese women.

				between two FFQ 4 weeks apart			
Prpic et al. (2021)	Croatia	133 lactating women and their infants	Iodine and salt rich FFQ	The FFQ was carried out along with biomarkers on both mothers and infants including UIC, thyroid function tests and BMIC (only in the mothers).	Iodine	Yes	A positive correlation between thyroid function in lactating women and their infants. Only 20.4% participants used iodine containing supplements while 99.2% used iodised salt. A regression analysis identified that BMIC was a predictor of infant UIC (P<0.001).
Huang et al. (2021)	Taiwan	198 women who were lactating	Quantitative FFQ	Not stated	Iodine	Yes	The iodine status of lactating women in Taiwan is adequate however this may vary in certain subgroups.

Iodine or Selenium Specific Food Frequency Questionnaire Studies							
Reference	***Country***	***Sample size,***	***FFQ type, consumption period,***	***Validation method***	***Country has a mandatory salt fortification policy?***	***Asked about iodine/selenium supplement use***	***Findings***
Naess et al. (2019)	Norway	137 pregnant women 18-19 weeks' gestation	Semi quantitative iodine specific FFQ	Validated against a 6-day diet record, Spearman's rank correlation coefficient was used to compare. Reproducibility was also tested in a subgroup by comparing FFQ1 with a FFQ administered at 35-36 weeks' gestation.	No	Yes	Study found the I-FFQ was a valid tool to be used on Norwegian pregnant women. Results suggest this FFQ could be used in similar populations where salt is not iodised.
Tan et al. (2013)	Australia	84 men and women aged 60-95 years old	Semi quantitative iodine specific FFQ	Validated against 3 repeated 24-hour recalls and urinary spot iodine concentrations. Reproducibility was assessed by repeating the FFQ after 9 months comparing the results.	Yes	Yes	The FFQ was a valid tool to be used to assess the iodine intake of older Australians. The reproducibility is yet to be demonstrated.
Condo et al. (2015)	Australia	122 pregnant women at 28 weeks' gestation	Semi quantitative iodine specific FFQ	Validated against a 4DDD and biomarkers (UIE, UIC, spot UIC, and	Yes	Yes	Study identified the I-FFQ to be a valid tool to screen pregnant

				thyroid function tests)			Australian women at risk of inadequate iodine status.
Combet et al. (2015)	United Kingdom	43 women of child-bearing age	Iodine-specific FFQ	Validated using triangular (triad) method – against 4-day semi quantitative food dairies and biomarker (iodine in duplicate 24-hour urine collection)	No	No	The FFQ and food diary showed good agreement with cross-classification and moderate correlation with food diary and UIC. The iodine specific FFQ was found to be a valid tool for estimating dietary iodine exporusure.
Pestitschek et al. (2013)	Austria	212 patients from a thyroid outpatient clinic (21 control patients)	Selenium specific FFQ	Unvalidated but compared to selenium and thyroid biomarkers	Yes	No	A positive correlation between selenium intake and blood selenium levels was obtained. No significant difference in blood selenium levels between patients that are non- and autoimmune thyroid diseases.

Karita et al. (2003)	Japan	215 adults	Self-administered selenium specific FFQ	Validated against a four seasonal 7-day food records and selenium biomarkers	No	No	No positive or significant correlation between the FFQ and serum biomarkers. The FFQ was found to be valid between energy adjusted intake and erythrocyte level of Se.
Duffield and Thomson (1999)	New Zealand	110-free living adults from Otago	Selenium specific FFQ	Comparison of FFQ with chemical analysis of duplicate diet and diet records to assess selenium intake.	Yes	Yes	Diet record was not adequate at predicting Se intake. Significant correlation between duplicate diet and plasma Se, diet record and plasma Se and FFQ and whole-blood Se.

2.6 Methods of Validation

Validation of FFQ is important to ensure the information collected is accurate and can be used to draw associations between dietary intake and dietary factors and disease (Cade et al., 2002). The standard method of validating a FFQ is to compare a FFQ to a dietary reference method such as diet diary. However, some studies additionally use biomarkers which increase the strength of the validation.

Using Estimated Intake from Biomarkers (EIB) and a diet record to validate an FFQ is advantageous, as any errors should be independent of the questionnaire. This provides a more significant result of the validity of the questionnaire (Willett, 2012). Using biomarkers in conjunction with dietary reference methods can help to strengthen the validity of an FFQ, as the measurements are generally independent of the ones obtained from the dietary assessment. A method of validation is using a diet diary and Estimated Intake from Biomarkers to assess the validity of an FFQ. A study by Combet et al. (2015) used the triad method to validate an iodine-specific FFQ. To understand the agreement between these methods, coefficients are used to assess the correlation between these three methods. To establish the validity of the variables used in calculations, they must be linearly related to actual intake, and errors must be independent of each other (Ocke & Kaaks, 1997).

While biochemical markers provide more substantial validity, there are some limitations. Biomarkers are influenced by the diet and other external and internal factors affecting an individual, such as metabolism and absorption (Willett, 2012). A significant limitation of using biomarkers is that they cannot provide an exact quantitative measure of dietary intake of a specific nutrient; they can only provide a qualitative indicator of the information of a particular nutrient. Another limitation to using biomarkers is the time of day or period the marker takes. Different features can show an additional value of a nutrient, which could be based on short-term or long-term dietary intake. Biomarkers can be used to estimate nutrient intakes; the biomarkers can be converted from μg/L to μg/d. Thus allowing them to be better compared with nutrient intakes measured through dietary assessment methods. Estimated Intake from Biomarkers (EIB) decrease the likelihood of measurement error in dietary data collection (McNamara & Brennan, 2020). In a validation study, using these to compare the nutrient intakes gathered from the questionnaire or dietary reference method would further strengthen the association between the two measurements.

2.7 Statistical Analysis of Validity of Food Frequency Questionnaires

Different statistical analyses are used to assess the validity of an FFQ. Different methods of statistical analysis can be used. There needs to be more consensus regarding choosing an appropriate statistical method to validate an FFQ (Gibson, 2005). Multiple statistical methods should be used in this field to get the best result of validity (Cade et al., 2002). When using triangulation to validate an FFQ, the appropriateness of the statistical methods differs depending on the comparison to a reference dietary assessment or biomarker.

2.7.1 Correlation coefficients

To assess the strength of the relationship between an FFQ and a diet diary, Spearman's or Pearson's correlation can be used for normal or non-normally distributed data, respectively. Correlation coefficients are the most common statistical method used in validation studies, with 83% of validation studies using it as a statistical method (Cade et al., 2002). Masson et al. (2003) a correlation greater than 0.5 shows a good association between two dietary assessment methods. Cade et al. (2004) found that correlations above 0.45 showed good associations and correlations below 0.3 or 0.4 would be difficult to detect an association (Cade et al., 2002). Correlations above 0.8 or 0.9 are statistically unlikely (Willett, 2012).

Bland and Altman (1999) argued that coefficient correlations should not be used alone but with another statistical method, such as linear regression or a Bland-Altman plot. The correlation coefficient should not be used to show actual agreement between the two methods, instead as a measure to show the association between the two methods.

2.7.2 Comparison of Means

A comparison of means is a technique used to assess the relative validity at a group level and to see if two means are statistically different (Gibson, 2005). Both data groups need to have a test of normality (Shapiro-Wilk or Kolmogorov Smirnov) carried out to test for normal distribution. Data that shows a normal distribution will be tested using a paired t-test, while non-normally distributed data will use Wilcoxon's signed rank test.

2.7.3 Cross-classification and weighted kappa statistic

Cross-classification is a statistical test that is used to classify subjects based on thirds (tertiles), fourths (quartiles) or fifths (quintiles) of the test (FFQ) and reference (diet diary) methods. This classification calculates percentages based on the number of subjects classified into the same category for both the test and reference methods. Along with calculating the number of subjects grossly misclassified into categories, the complete opposite

category. In validation studies using tertiles to classify data, at least 50% should be correctly classified into the same tertile. Less than 10% of the data should be grossly classified into the opposite tertile (Masson et al., 2003). However, cross-classification has limitations, as the variables can be classified into the same category by chance. This can be avoided by cross-classification with the weighted kappa statistic. The weighted kappa statistic is a summary measure of cross-classification; this considers the agreement expected by chance and the degree of misclassification (Masson et al., 2003).

2.7.4 Bland-Altman analysis

Bland and Altman (1999) argued that correlation coefficients are inappropriate for assessing the agreement between two dietary methods. Instead, they proposed Limits of Agreements (LOA) to compare two dietary assessment methods. The Bland-Altman analysis is a visual analysis that uses a scatter plot with the mean difference between two methods (Y-axis) plotted against the mean intake of the two dietary methods (X-axis). The graph displays outliers and trends when nutrient intake is increased (Bland & Altman, 1999). Three lines are plotted on the graph; the middle indicates the total mean average between the two dietary methods. The closer this line is to 0 on the y-axis, the greater the limits of agreement between the two dietary methods. The additional two lines with side of the mean difference are two 95% confidence intervals.

2.8 Summary

From the literature, to the best of our knowledge, there are no validated iodine and selenium specific FFQs in New Zealand or internationally. There were studies that validated iodine specific FFQs, these were mostly validated on a pregnant population or non-pregnant, non-lactating adult population (Combet & Lean, 2014; Condo et al., 2015; Naess et al., 2019; Prpic et al., 2021; Tan et al., 2013). The literature around the use of FFQs shows that FFQs need to be validated against the intended population (Willett, 2012). Thus there is currently no appropriate validated iodine and selenium specific FFQ that can be used to assess iodine and selenium intake of a breastfeeding population.

Iodine and selenium have an important relationship as they both have a joint role in thyroid regulation. During lactation iodine and selenium requirements increase to reflect the increase demands as the breastfed infant also relies on the mother's iodine and selenium intake. Studies have shown that a proportion of New Zealand breastfeeding women are not meeting

their iodine or selenium requirements (Brough et al., 2015; Jin et al., 2022; Jin et al., 2021; Mulrine et al., 2005; Skeaff et al., 2005; University of Otago & Ministry of Health, 2011). Additionally even with government initiatives such as iodisation of salt, bread fortification with iodised salt, and subsided iodine supplements for lactating women there is still issues with adherence.

When validating a FFQ it is recommended to not only use a dietary assessment method but also a biomarker to increase the strength of the validation (Cade et al., 2002). When selecting a suitable biomarker it's important to consider the relationship and association between the biomarker and estimating nutrient status. Furthermore, for the validation process, it is advantageous to use multiple statistical methods to measure the association between the FFQ, dietary reference method and biomarkers (Cade et al., 2002).

The literature shows that there is justification for a valid and reproducible iodine and selenium specific FFQ for lactating women specifically for breastfeeding women in New Zealand. Having this tool would be valuable for future research or in primary care for assessing the iodine and selenium intake of breastfeeding women in New Zealand.

Chapter Three: Research Manuscript: Validation and Reproducibility of an Iodine and Selenium Specific Food Frequency Questionnaire in Breastfeeding Women

3.1 Abstract

Background: During lactation, women have increased iodine and selenium requirements. Iodine and selenium status can predict thyroid function; if breastfeeding women have poor iodine and selenium intake, it can cause thyroid dysfunction in both the mother and breastfed infant. Currently, there is no valid iodine and selenium-specific Food Frequency Questionnaire (FFQ) available to assess the iodine and selenium intake of breastfeeding women in New Zealand.

Aim: The aim of this study is to assess the validity and reproducibility of an Iodine and Selenium Specific Food Frequency Questionnaire for breastfeeding women living in New Zealand.

Methods: 87 participants in the Mother and Infant Nutrition Investigation (MINI) study completed a 69-item self-administered semiquantitative iodine and selenium specific food frequency questionnaire (FFQ). The first FFQ (FFQ1) was compared to a four-day diet diary (4DDD), and intakes from biomarkers (urinary and breastmilk iodine concentration, and plasma selenium) were estimated to determine the validity through comparison to a diet diary and estimated intake from biomarkers (EIB). FFQ1 was repeated nine months later (FFQ2) to assess reproducibility. Agreement between the FFQ1, 4DDD and estimated intakes from biomarkers (EIB) was statistically assessed via the Wilcoxon signed ranked test, Spearman's correlation coefficients, cross-classifications, weighted kappa statistics, and Bland Altman plots.

Results: For the validation, Spearman's correlation observed a range from 0.317 (selenium) to 0.532 (total iodine) between the FFQ and 4DDD ($p<0.05$). Cross-classifications for correctly classified participants ranged from 32.9% (selenium) to 71.6% (iodine food only) when comparing the FFQ to 4DDD, the grossly misclassified participants ranged from 1.4% (iodine and salt) to 11.0% (selenium). The weighted Kappa statistics ranged from 0.007 (selenium) to 0.421 (iodine and supplements) for the FFQ compared to 4DDD. The Bland-Altman plots showed fair agreement between FFQ and 4DDD/EIB and the mean difference between the two groups. For reproducibility, the correlation coefficients between FFQ1 and FFQ2 were 0.625 (iodine) and 0.429 (selenium) ($p<0.05$). Cross-classification for correctly classified participants was 65.0% (iodine) and 37.3% (selenium), and grossly misclassified participants were 1.7% (iodine) and 3.4% (selenium). The weighted kappa statistics were 0.155 (iodine) and -0.006 (selenium). For FFQ1 to EIB, 0.146 (selenium) and 0.155 (iodine) (p-value <0.05).

Cross-classifications between the FFQ1 to EIB showed correctly classified participants were 50.0% (iodine) and 73.1% (selenium), and the grossly misclassified participants were 16.4% (iodine) and 3.4% (selenium). The Kappa statistics were 0.115 (iodine) and 0.320 (selenium) between FFQ1 and EIB.

Conclusion: The studied Iodine Selenium Specific FFQ demonstrated reasonable validity and good reproducibility for assessing iodine and selenium intake in breastfeeding women in New Zealand. This FFQ could be used in clinical practice as an easy and cost-effective screening tool or a way to assess habitual iodine and selenium intake of breastfeeding women.

3.2 Introduction

During lactation, the mother's iodine and selenium requirements increase, as breastfed infants are dependent on maternal intake, with the Estimated Average Requirements (EAR) for iodine in lactating women being 190 µg/d for iodine and 65 µg/d for selenium. In recent years, there have been improvements in iodine and selenium status globally; however, vulnerable populations are still at risk of deficiencies. Around the world, countries have implemented public health policies such as mandatory salt fortification with iodine, subsidised iodine supplements and fortified commercial bread with iodised salt. A study (Greenwald et al., 2022) analysed the legislation around salt fortification for 196 countries; 110 (63%) of these countries have mandatory salt iodination legislation. The New Zealand government has introduced initiatives to help minimise the risk of iodine deficiencies: 1) fortification of bread with iodised salt and 2) subsidised iodine supplements for pregnant and breastfeeding women. In New Zealand, Brough et al. (2015) showed that after the implementation of these initiatives, only 36% of breastfeeding women were taking an iodine supplement throughout breastfeeding. Another study by Reynolds and Skeaff (2018) observed that 54% of pregnant and lactating women were taking a supplement with 150µg/day iodine as per recommendations. Selenium is found in various levels in soil, with factors such as soil type, texture, organic matter content and rainfall impacting the selenium level (Mehdi et al., 2013) with plant content corresponding to the level of the soil. While the government does not subsidise selenium supplementation, the literature suggests taking a selenium supplement with an iodine supplement can support thyroid regulation and prevent deficiencies (Contempre et al., 1991).

The thyroid gland plays a vital part in growth and development, with specific functions in cognitive development in infants (Andersson & Braegger, 2022). Both selenium and iodine are required to synthesise thyroid hormones, and deficiency can cause thyroid dysfunction. Iodine is a component of thyroid hormones (Chung, 2014), and selenium is a component of the conversion enzyme I 5'-iodothyronine deiodinase (DIO) required for the conversion of

thyroxine (T4) to triiodothyronine (T3) (Thomson, 2004a). Thyroid dysfunction can lead to significant consequences for the mother and her infant, impaired thyroid hormone production can affect metabolism, growth and development (Andersson & Braegger, 2022). During breastfeeding, exclusively breastfed infants solely rely on the mother's iodine and selenium stores and intake for the first six months until complementary feeding begins. While iodine becomes concentrated in the mammary gland during this time, partitioning occurs to ensure the infant's needs are met (Brough, 2023). If the mother has low iodine intake, it can lead to thyroid dysfunction, poor immunity, and cognitive impairment (Varsi et al., 2017).

Multiple dietary assessment methods can be used to assess an individual dietary intake, each with its advantages and disadvantages. An FFQ is a dietary assessment method self-administered by the participant; it assesses the frequency at which certain foods are consumed. The questionnaire lists foods and beverages; the participant indicates how often these are consumed within a specific period (Willett, 2012). The questionnaire can be developed to assess a specific nutrient, making it useful for assessing nutrient intake at an individual or population level (Willett, 2012). Furthermore, FFQs can be developed to be used on a specific population. The benefits of an FFQ are that they are inexpensive, require little to no training to administer, and are less time-consuming than other methods. The additional advantage of FFQs is that they assess dietary intake over a longer period, giving a better idea of habitual dietary intake (Gibson, 2005). However, prior to using an FFQ, it should be validated to ensure its accuracy in determining dietary intakes (Cade et al., 2002). An FFQ is validated by comparing the outcomes of the FFQ and a superior dietary assessment method, such as a diet record (Willett, 2012). Additionally, an FFQ can be validated against biomarkers to strengthen the validity. Moreover, along with validating FFQ, the reproducibility of the FFQ should be assessed. This assesses whether the FFQ can produce a similar outcome at a different time point (Cade et al., 2002).

The aim of this study is to validate and assess the reproducibility of an Iodine and Selenium Specific Food Frequency Questionnaire in a breastfeeding population in New Zealand. To our knowledge, this is the first study to develop and validate an iodine and selenium-specific FFQ to be used for breastfeeding women, with limited studies looking purely at a breastfeeding population, even though they also have increased nutrient requirements. Similar studies that have validated either an iodine or selenium-specific questionnaire on different population groups (Combet & Lean, 2014; Condo et al., 2015; Fu et al., 2023; Glabska et al., 2017; Kelliher et al., 2023; Naess et al., 2019; Prpic et al., 2021; Tan et al., 2013), however these FFQs have not been validated on New Zealand population, and many of them were validated against a pregnant population and they only assess iodine or other nutrients individually.

3.3 Materials and Methods

Study overview

The FFQ validation and reproducibility study was undertaken as part of the Mother and Infant Nutrition Investigation (MINI) study at Massey University (Jin, Coad, Zhou, et al., 2020). The MINI study was conducted in Palmerston North, New Zealand between June 2016, and December 2017. The study's overarching aim was to investigate maternal thyroid function, postnatal depression and iodine, selenium and iron intake and status in mothers and their infants during the first postpartum year. The MINI study was an observational longitudinal cohort study of breastfeeding mothers and their infants in Manawatu, New Zealand. The study had three visits at three months, six months, and 12 months postpartum for both mother and infant.

Study population

The eligibility criteria for this study were breastfeeding mothers over sixteen years of age who had given birth to a healthy singleton infant three months prior. Mothers and their infants were excluded if they had pre-existing or developed significant health problems (such as metabolic disorders or cancer) or if they had been diagnosed or treated for hyper - or - hypothyroidism. Potential participants were recruited via posters displayed at health professionals' workplaces that often work with prenatal and postnatal women. The potential participants could then express their interest online or via telephone/email, where they were then provided with a study information sheet. Following this, the potential participants were asked to complete an eligibility questionnaire. All mothers were asked to provide written consent for themselves and their infants.

Ethics

The Mother and Infant Nutrition Investigation study was approved by the Health and Disability Ethics Committee (reference: 15/ NTA/ 172) in December 2015. The study's ethical approval was registered with the Royal New Zealand Plunket Ethics Committee in June 2016. The MidCentral District Health Board approved the MINI study and registered it with the Australian New Zealand Clinical Trials registry [ACTRN1261500102854]. The research was conducted at the Human Nutrition Research Unit at Massey University Palmerston North.

Development of the FFQ

The FFQ used for this study was a 69-item self-administered semiquantitative iodine and selenium-specific food frequency questionnaire. This FFQ was adapted from an iodine-specific FFQ used in an Australian study of pregnant women (Condo et al., 2015). The FFQ aimed to estimate habitual maternal iodine and selenium intake. Condo et al. (2015) had an FFQ made up of 44 food items; this was altered to assess both iodine and selenium by adding food items that were appropriate for assessing selenium intake in New Zealand. The food items were added to the FFQ if they had a selenium content greater than 5% of the Recommended Dietary Intake (RDI). Of the 69 food items, these were categorised into nine categories: dairy products, eggs, fish, seafood, meat, cereal products, vegetables (fresh and frozen), snacks and sweets and ready-made food. A serving size was given for each food item, either volume or weight, along with cups or spoons. There were four frequency types for assessing frequency: per day, per week, per month, and rarely (<1 month)/never.

In addition to the FFQ, further questions were asked about iodised salt and iodine supplement usage. The questions were designed to elicit 'yes' or 'no' responses and inquired whether the respondents utilised iodised salt either while cooking or at the dinner table. For participants who reported using iodised salt regularly, 48 µg of iodine was added to their estimated iodine intake (Edmonds et al., 2016). For iodine supplement usage, the participants were asked to identify the supplement's name, along with whether it was prescribed and the start and stop (if applicable) date of the supplement. The frequency of consumption throughout the week was asked, as well as the supplement dosage.

Dietary data collection

For the 4-day diet diary, the four days were consecutive and included one weekend day. Each mother was given a diary template and was asked to record food items, brands, amounts consumed, and the nutritional information panel if applicable. Each participant was given kitchen scales (Digitech) and household measuring cups and spoons to measure all food and beverages consumed. To ensure the accuracy of the measurements, every participant was given an oral and written demonstration on how to use the scales correctly. The women were also asked to record all food and beverages together with supplement usage.

Average iodine and selenium intake were calculated from the responses from the FFQ. The frequency of consumption of food items was converted to daily amounts; this was done by dividing the response by the indicated frequency; yearly was divided by 365; monthly was divided by 30; weekly was divided by seven; and daily remained the same. The dietary intake

from the FFQ was calculated using Excel, and the concentration for the corresponding food item was determined using FoodWorks.

The 4DDD was entered into and analysed using FoodWorks 9 Professional (Xyris Pty Ltd) and New Zealand FOODfiles (2016) to estimate nutrient intake. If certain food products/items were not included in FOODfiles 2016, new food items were created and added based on the food item/product information provided by the participant or appropriate databases from Australia and the United States. Participants who indicated the use of iodised salt were calculated using Food Works, the iodine from the iodised salt was added to the total iodine for the 4DDD. The Foodworks database does not contain accurate and up-to-date information on the current iodine concentrations of bread due to the now mandatory fortification of bread with iodised salt. The iodine concentration of the different types of bread was based on the Ministry of Primary Industries data (Ministry for Primary Industries & Ministry of Health, 2016). Also, if a participant used a supplement, this was entered into Food Works as a new food item.

Estimated dietary intake from iodine and selenium biomarkers

Multiple biomarkers were taken to measure iodine and selenium status; these included urinary, blood and breastmilk markers.

At three months postpartum non-fasting maternal spot urine samples were collected to measure iodine concentrations. A breast milk sample was also collected (if able to collect breast milk). The samples were approximately 30-50ml, and the participants were provided with an Allegro electric breast pump (Unimom NZ). The samples were collected before noon on the study visit days; however, the breast milk collection was not standardised. Urinary and breast milk iodine concentrations were determined by an accredited commercial laboratory (Hill Laboratories, Hamilton, New Zealand) using inductively coupled plasma mass spectrometry (ICP-MS). To ensure quality control, procedures such as analysis of blanks, analytical repeats, and certified reference material to ensure accuracy and precision. These were collected in the morning, before noon, frozen, and stored at -20°C prior to analysis.

Non-fasting blood samples were all taken by experienced phlebotomists for analysis of plasma selenium. This was collected at six months postpartum. Samples were then centrifuged and aliquoted into microcentrifuge tubes with each participant's unique identification number. These samples were stored at -80°C. Plasma selenium was measured using inductively coupled mass spectroscopy (ICP-MS) at Canterbury Health Laboratories, New Zealand (Jin, Coad, Zhou, et al., 2020). Plasma selenium was converted to an Estimated Selenium Intake

Biomarker. This was done using the calculation Y = 1.623 log X + 3.433, with X = Plasma Selenium (μg/L) and Y = selenium daily intake (μg/d) (Han et al., 2018).

Due to the partitioning of iodine in breastfeeding women instead of using urinary (Watanabe et al., 1997) or breast milk (BMIC) iodine concentration individually, these values were proportionately added to estimate total iodine excretion. Both UIC and BMIC were converted from μg/L to μg/day. This was done by multiplying UIC by 1.5L/day (assumed total urine volume) and multiplying BMIC by 0.78L/day (assumed daily breastmilk in lactating women); these were added together and finally divided by the assumed excretion rate in urine and breastmilk (0.92) to calculate the total iodine (Jin et al., 2022).

Statistical analysis

Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) software version 25 (IBM SPSS, Inc., Chicago, IL, USA). The data was first checked for normality of distribution using Kolmogorov-Smirnovs and Shapiro-Wilks test. Normally distributed data were reported as means and standard deviation (mean±SD), and non-normally distributed data were reported as median with 25th and 75th percentiles (Q1, Q3).

The validity of iodine and selenium intake from the FFQ was determined by comparison with 4DDD and EIB. Iodine was categorised into four groups: food-only, food with supplements added but without iodised salt (I-Sup), food with iodised salt but without supplements (I-Sal), and total iodine (food, iodised salt and supplements). For selenium, there was only one category of intake. The FFQ did not include selenium from supplements, however for 4DDD selenium from supplements was included along with a food-only group. To compare the strength of the relationship, Spearman's correlation was used if $p<0.05$, then coefficients were determined (with the range of between 0 to ± 1). The correlation coefficient indicates the strength of the relationship where 0.9-1 perfect, 0.7-0.9 very high, 0.5-0.7 high, 0.3-0.5 moderate, 0.1-0.3 low, and 0.1-0 insubstantial (Lombard et al., 2015). Based on the normality of the data, Wilcoxon signed ranks were used to compare the mean differences between the FFQ and 4DDD; here, the effect size was calculated if a p-value > 0.05 was obtained. Cross-classification was used, categorising iodine and selenium into tertiles for the FFQ and 4DDD. The recommendation is that >50% of participants should be correctly classified into the same tertiles, and only <10% should be grossly misclassified into opposite tertiles (Masson et al., 2003). The weighted kappa statistic can be used to investigate the level of cross-classification agreement further. The weighted kappa statistic calculates the agreement based on the observed percentages compared to the expected percentage from the cross-classification,

with K=1 meaning complete agreement and K=0 meaning no agreement. The levels of agreement for the kappa statistic are >0.8 very good agreement, 0.61-0.8 good agreement, 0.41-0.6 moderate agreement, 0.21-0.4 fair agreement, and <0.2 poor agreement (Masson et al., 2003).

The level of agreement was further investigated using Bland-Altman scatterplots. These scatterplots show the level of agreement by plotting the difference between the two measurements for iodine or selenium on the vertical axis and then plotting the average on the horizontal axis. The limits of agreement are also plotted; these were calculated by difference ± 2 SD (Altman & Bland, 1983).

To determine the reproducibility, the FFQ1 administered three months postpartum was compared to the FFQ2 administered at 12 months postpartum. This was determined using the same statistical analyses as for validation.

3.4 Results

3.4.1 Participants demographic

A total of 87 participants took part in the MINI study. The majority of the participants were New Zealand Europeans (76%). The mean age±SD of the participants was 31.5±4.2 years. The mean±SD gestational age was 39.4±1.5 weeks. Of the participants, 22% gave birth via cesarean, and 44% reported it was their first time giving birth. The majority of the participants (77%) received a tertiary level of education or higher, with 62% having an annual household income above the New Zealand median (Statistics New Zealand, 2018).

Table 3. 1 Characteristics and demographics of the study participants; adapted from Jin et al. (2021).

Maternal Characteristics (n=87)	
Age, years (Mean ± SD)	31.5±4.2
Ethnicity (n (%))	
Māori Asian NZ European Other	9 (10) 9 (10) 66 (76) 3 (4)
Household income	
Above median (n (%))	54 (62)
Tertiary education	67 (77)
Cesarean delivery (n (%))	19 (22)
Primiparity (n (%))	38 (44)
Gestational age at birth, weeks (Mean±SD)	39.4± 1.5

* Median annual household income based on Statistics New Zealand is 75,995 New Zealand dollars for the year ended June 2016 (Statistics New Zealand, 2018).

3.4.2 Validity of FFQ1 to 4DDD

Median comparison and correlations

The median intake of iodine (food only) was 90.0 µg/d (74.8, 121.6) from FFQ1 and 96.7 µg/d (81.0, 116.2) for the 4DDD, with a difference of 6.7 µg/d. The median for total iodine was 214.1 µg/d (133.8, 322.8) and 150.6 µg/d (100.5, 284.8), with a difference of 63.5 µg/d. The median for selenium was 72.11 µg/d (55.9, 94.5; FFQ1) and 61.7 µg/d (50.7, 84.6; 4DDD), with a difference of 10.4 µg/d. Spearman's correlation comparing FFQ1 to 4DDD ranged from 0.317 (selenium), 0.413 (iodine food only) to 0.532 (total iodine). All correlations were significant ($p<0.05$). The Wilcoxon signed-rank test returned a statistically non-significant difference between the FFQ1 and 4DDD for total iodine, I-Sal and selenium. The effect size for total iodine and selenium was found to be moderate effect size (0.313) whereas the effect size for I-Sal medium (0.477).

Table 3. 2 Comparison of median iodine and selenium intake from FFQ1 and 4DDD and Spearman coefficients.

Nutrients	Median FFQ1 (25th,75th percentile)	Median 4DDD (25th,75th percentile)	Wilcoxon (p-value)	Wilcoxon Effect size	Spearman's coefficient	Spearman's Correlation (p-value)
Iodine (food only)	90.03 (74.8, 121.6)	96.7 (81.0, 116.2)	0.994	-	0.413	<0.001
Total iodine (with food, supplements and salt)	214.1 (133.8, 322.8)	150.6 (100.5, 284.8)	0.007	0.313	0.532	<0.001
Iodine (food and supplements)	149.7 (84.6, 238.1)	122.96 (88.4, 244.0)	0.899	-	0.524	<0.001
Iodine (food and salt)	150.71 (102.1, 199.6)	123.2 (85.5, 150.6)	<0.001	0.477	0.486	<0.001
Selenium (food only)	72.11 (55.9, 94.5)	61.7 (50.7, 84.6)	0.003	0.342	0.317	0.02

Cross-classification and weighted kappa statistic

When comparing the participants classified into the same tertiles for FFQ1 and 4DDD, the correctly classified percentage ranged from 32.9% (selenium) to 71.6% (iodine food only). All the iodine nutrient variables had greater than 50% of participants correctly classified into the same tertile. Selenium only had 32.9% of participants classified into the same tertile. Selenium has 56.1% of participants classified into adjacent tertile. Most of the nutrients had less than 10% of participants grossly misclassified into opposite tertiles, although for selenium, it was 11.0%. The grossly misclassified tertiles for iodine measures ranged from 1.4% to 6.8%. When comparing the agreement between FFQ1 and 4DDD, the weighted Kappa ranged from 0.007 (selenium) to 0.421 (I-Sup). Selenium and I-Sal showed poor agreement (K<0.20), fair agreement was observed for iodine (food only) and total iodine (K=0.21-0.40), moderate agreement was observed for iodine with supplements (K=0.41-0.60).

Table 3. 3 Cross-classifications and weighted Kappa for iodine and selenium intake comparison between FFQ1 and 4DDD.

Nutrients	Correctly classified into same tertiles (%)	Classified into adjacent tertile (%)	Grossly misclassified into opposite tertiles (%)	Weighted Kappa statistics (K)
Iodine (food only)	71.6	25.7	2.7	0.218
Iodine (with salt and supplements)	62.2	31.1	6.8	0.360
Iodine (food and supplements)	68.5	28.8	2.7	0.421
Iodine (food and salt)	54.8	43.8	1.37	0.174
Selenium (food only)	32.9	56.2	10.96	0.007

Bland Altman plots for comparison of FFQ1 to 4DDD

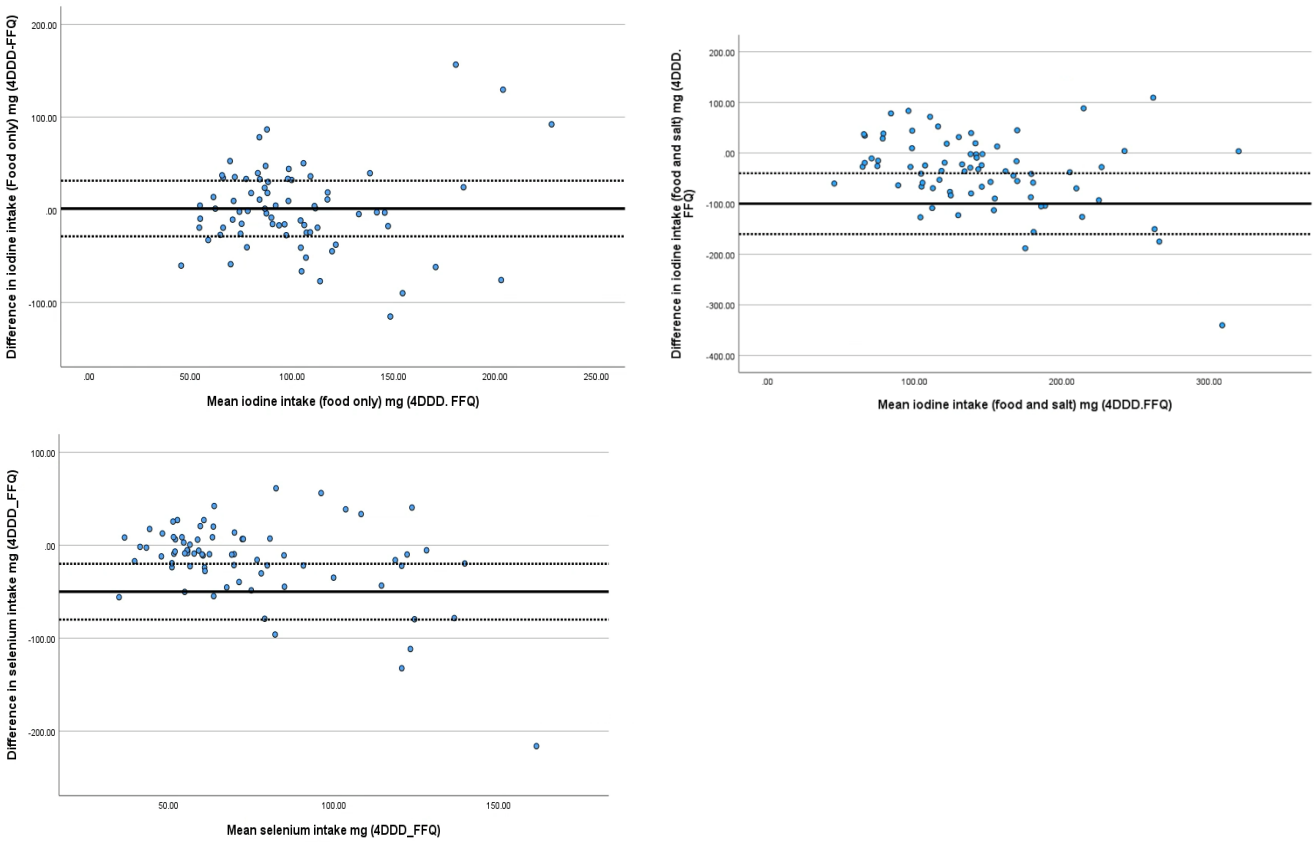

Figure 3. 1 Bland-Altman plot of the agreement for iodine intake (food only), iodine intake (food, supplements, and salt), iodine intake (food and salt), iodine intake (food and supplements) and selenium intake (food only) The middle line represents the mean difference between the FFQ1 and 4DDD; the dotted lines represent the limits of agreement.

Bland-Altman plots were constructed to assess the strength of agreement between iodine and selenium intake from the FFQ1 and 4DDD (Bland & Altman, 1999). Bland-Altman plots are also used to identify outliers in the results. For most nutrient comparisons (excluding total iodine (FFQ-4DDD)), most of the measures fell between the LOA.

3.4.3 Reproducibility of the FFQ

Median comparison and correlations

The median intake of iodine from food only was 90.0 µg/d (74.8, 121.6) for FFQ1 and 80.7 µg/d (58.3, 115.9) for FFQ2. When comparing FFQ1 collected at three months postpartum and FFQ2 collected at 12 months postpartum, there was a 10.3% difference in medians for iodine. The median for selenium (food only) was 72.11 µg/d and 56.68 µg/d, with a 21.4% difference in the median between FFQ1 and FFQ2. FFQ1 had a higher nutrient intake for iodine and selenium. From Spearman's coefficients, iodine (food only) was 0.625, and selenium was 0.429; both coefficients were statistically significant. The Wilcoxon signed-rank test returned a statistically significant difference between FFQ1 and FFQ2 for iodine and selenium; effect sizes were of medium value.

Table 3. 4 Comparison of mean iodine and selenium intake from FFQ1 and FFQ2 and Spearman coefficients.

Nutrient	Median FFQ1 (25th,75th percentile)	Median FFQ2 (25th,75th percentile)	Wilcoxon (p-value)	Wilcoxon Effect size	Spearman's coefficient	Correlation significance
Iodine (food only)	90.03 (74.82, 121.6)	80.73 (58.3, 115.9)	<0.001	0.4340	0.625	<0.001
Selenium (food only)	72.11 (55.88, 94.51)	56.68 (44.27, 78.76)	<0.001	0.5514	0.429	<0.001

Cross-classification and weighted kappa statistic

When comparing the participants classified into the same tertiles when comparing the FFQ1 and FFQ2, 65% for iodine and 37.3% for selenium were classified into the same tertile. In comparison, 33.3% of iodine and 59.3% of selenium were classified into one tertile difference. Less than 5% of participants were grossly misclassified into opposite tertile, 1.7% of iodine and 3.4% of selenium. The agreement between FFQ1 and FFQ2 was shown through the weighted Kappa statistic; both iodine and selenium showed poor agreement (K<0.20).

Table 3. 5 Cross-classifications and weighted kappa for iodine and selenium intake comparison between FFQ1 and FFQ2.

Nutrients	Correctly classified into same tertiles (%)	Classified into adjacent tertile (%)	Grossly misclassified into opposite tertiles (%)	Weighted Kappa statistics (K)
Iodine (food only)	65.0	33.3	1.67	0.155
Selenium (food only)	37.3	59.3	3.39	-0.006

Bland Altman plot analysis

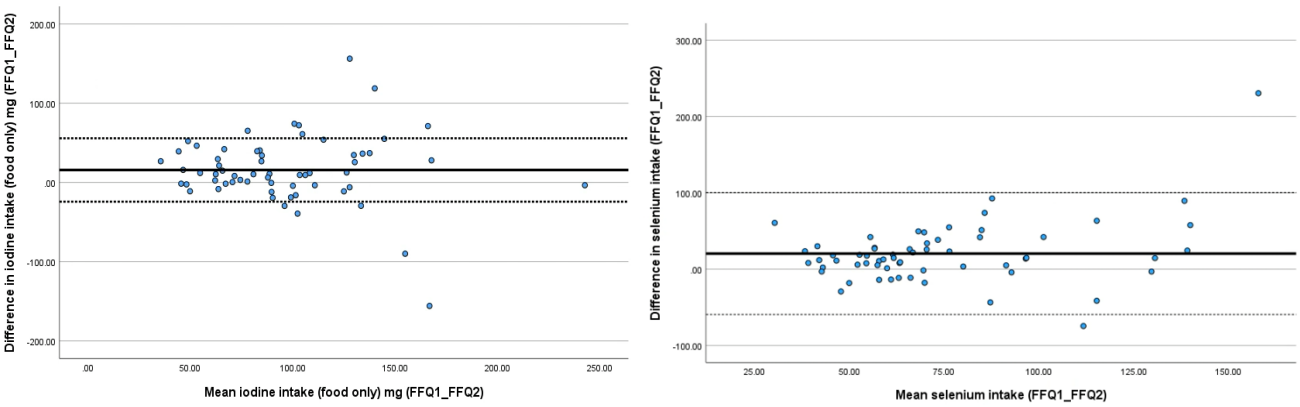

Figure 3. 2 Bland-Altman plot of the agreement for iodine intake (food only) and selenium intake (food only). The middle line represents the mean difference between the FFQ1 and FFQ2; the dotted lines represent the limits of agreement (LOA = mean difference ± 1.96 standard deviation).

The Bland-Altman plots were constructed to show trends and bias in the data for reproducibility. In both plots, outliers that are further outside the LOA can be seen. The selenium plot demonstrates a larger degree of variance as the LOA range is greater than the iodine plot.

3.4.5 Validity of FFQ1 to Estimated Intake from Biomarkers (EIBs)

Median comparison and correlations

The median for the EIB iodine excretion (urine and breast milk) was 219.5 µg/d (142, 330), and for EIB selenium, it was 11 µg/d (10.8, 11.1). The Wilcoxon signed-rank test showed a statistically significant result for selenium with a medium effect size of 0.58. The Wilcoxon for total iodine has a p-value > 0.05. Both iodine and selenium showed a non-significant Spearman's coefficient between FFQ1 and EIB. Comparing the EIB to the 4DDD, Spearman's coefficient for total iodine was 0.118 and for selenium, 0.051 (p-value > 0.05). The Wilcoxon signed-rank test showed a statistically significant result for total iodine and selenium. The effect size for total iodine was small (0.32), while the effect size for selenium was large (0.79).

Table 3. 6 Comparison of median iodine and selenium intake from FFQ1, 4DDD and EIB and Spearman coefficients.

Estimated Intake from Biomarker (µg/d)	Median EIBs (Q1, Q3)		Wilcoxon Effect size	Spearman's coefficient	Spearman's Correlation (p-value)
Comparison between EIBs and FFQ (food only)					
Iodine (based on urine and breast milk)	219.46 (142, 330)	0.464	-	0.155	0.163
Selenium (Based on plasma)	11 (10.83, 11.14)	<0.001	0.582	0.146	0.228
Comparison between EIBs and 4DDD (food + supplements)					
Iodine (based on urine and breast milk)	219.46 (142, 330)	0.007	0.320	0.118	0.313
Selenium (Based on plasma)	11 (10.83, 11.14)	<0.001	0.868	0.051	0.201

Cross-classification and weighted kappa statistic

When comparing the classifications between biomarkers and the FFQ1, for total iodine, 50% were classified into the same tertile, while for selenium, 73.1% were classified into the same tertile. For the classification as adjacent tertile, 33.8% of total iodine was classified into this category and 23.9% for selenium. For the classification into opposite tertiles, 16.3% were grossly misclassified for total iodine and 3.0% for selenium. The agreement between the FFQ1 and EIB was shown through the weighted Kappa statistic; total iodine showed poor agreement (K<0.20), while selenium had fair agreement (K=0.21-0.40). When comparing the classification between EIBs and 4DDD, 60.3% of participants were correctly classified, while for selenium, 38.8% were correctly classified. For the classification into one tertile difference, total iodine was 35.6% and 55.2% for selenium. When comparing grossly misclassified participants into opposite tertile, total iodine was 5.5% and for selenium, 6.0%. The agreement between FFQ1 and EIBs with the weighted Kappa statistic; total iodine and selenium showed poor agreement (K<0.20).

Table 3. 7 Cross-classifications and weighted Kappa for iodine and selenium intake comparison between EIBs, FFQ1 and 4DDD.

Estimated Intake from Biomarker (µg/d)	Correctly classified into same tertiles (%)	Classified into adjacent tertile (%)	Grossly misclassified into opposite tertiles (%)	Weighted Kappa statistics (K)
Comparison between EIBs and FFQ (food only)				
Iodine (based on urine and breast milk)	50.0	33.8	16.3	0.115
Selenium (Based on plasma)	64.2	34.3	1.5	0.204
Comparison between EIBs and 4DDD (food + supplements)				
Iodine (based on urine and breast milk)	60.3	35.6	5.5	0.150
Selenium (Based on plasma)	38.8	55.2	6.0	0.070

Bland Altman plot analysis

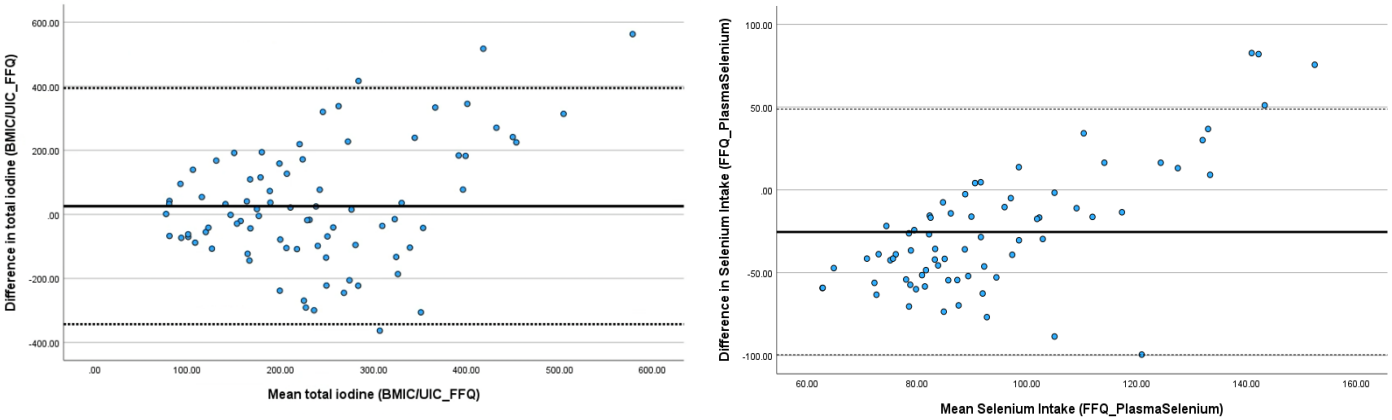

Figure 3. 3 Bland-Altman plot of the agreement for iodine intake and excretion (urine and breastmilk) and selenium intake and plasma selenium. The middle line represents the mean difference between the FFQ1 and EIBs; the dotted lines represent the limits of the agreement.

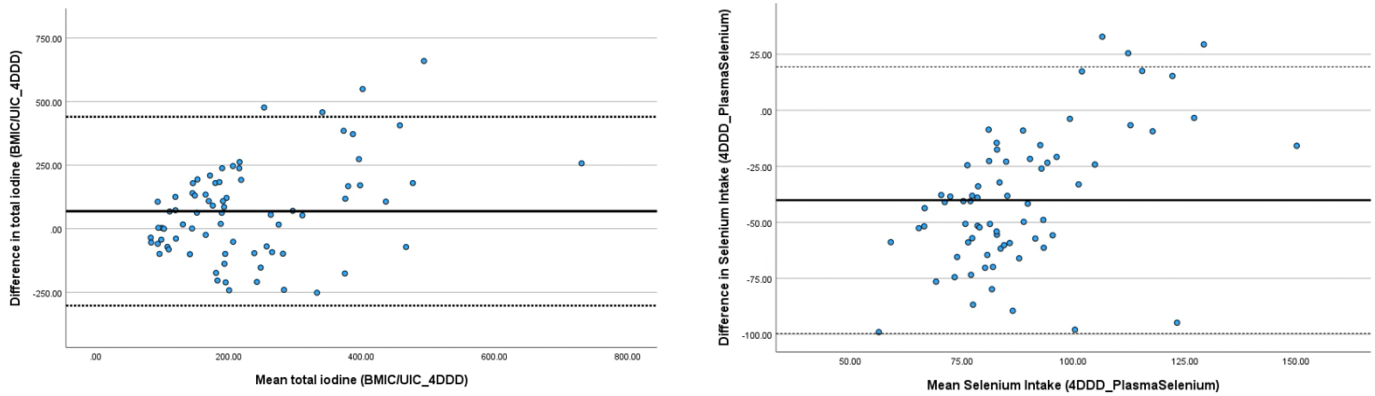

Figure 3. 4 Bland-Altman plot of the agreement for iodine intake and excretion (urine and breastmilk) and selenium intake and plasma selenium. The middle line represents the mean difference between the 4DDD and EIBs; the dotted lines represent the limits of agreement.

Bland-Altman plots were constructed to assess the strength of agreement between iodine and selenium intake from the EIB to FFQ1 and 4DDD (Bland & Altman, 1999). A wider range of variance can be observed in Figures 3.2 and 3.3. Outside the LOA outliers can be seen.

3.5 Discussion

This study is the first to use a dietary reference method and estimated intake from biomarkers to investigate the validity and reproducibility of a selenium and iodine-specific food frequency questionnaire for breastfeeding women in New Zealand. The results showed moderate relative validity for selenium and iodine intake. Moreover, the FFQ showed moderate reproducibility of the assessment tool when compared to FFQ2 re-administered nine months later.

3.5.1 Validity of the FFQ

When assessing the validity, the FFQ and the 4DDD had low to moderate correlations (0.317 (selenium) to 0.532 (total iodine)). Studies with a similar approach that examined iodine or selenium intake found a correlation between an FFQ and a dietary reference method (such as diet diary or 24-hour recall) with the ranges 0.349 to 0.876 (Condo et al., 2015), 0.52 to 0.69 (Combet & Lean, 2014), 0.52 (Rasmussen et al., 2001), 0.377 (Tan et al., 2013), 0.76 (Fu et al., 2023), 0.123 to 0.571 (Kelliher et al., 2023), and for selenium 0.32 to 0.36 (Karita et al., 2003), 0.30-0.32 (Brantsæter et al., 2008). Producing similar results further validates the findings, as they were expected. When looking at the correlations between the FFQ1 and 4DDD for iodine, the food-only group has the smallest correlation; when supplements or iodised salt are added, the strength of the association increases. Condo et al. (2015) observed a food-only correlation of 0.349 that increased to 0.876 with the addition of supplements.

When comparing the differences in iodine and selenium intake between the FFQ1 and 4DDD, the FFQ mostly overestimated intake compared to the 4DDD. The differences between the two medians ranged from -6.9% to 29.7%. Of these differences, total iodine, I-Sal and selenium obtained a significant difference between the FFQ1 and 4DDD. This significant difference means there is poor agreement between the FFQ1 and 4DDD for these groups. For these values, the effect size showed that this significant difference was moderate to medium (0.31 to 0.48). For iodine intake, this difference could be due to how iodised salt intake was calculated. Quantifying salt intake in a dietary assessment can be challenging; the FFQ and 4DDD quantified iodine intake from salt using different methods, which could have led to this significant difference.

The nutrients were also ranked into classification using cross-classification methods, between the comparison of FFQ and 4DDD. The FFQ to 4DDD comparison shows that all iodine values are at least 50% classified into the same tertile. However, selenium is only 32.9% classified into the same tertile. The gross misclassification of nutrients into opposite tertile ranged from

1.4% to 11.0%. In validation studies, the ideal outcome is at least 50% of participants are classified into the correct tertile and less than 10% are misclassified into the opposite tertile (Masson et al., 2003). Most of the nutrient groups obtained >50% classification into the same tertile, except for selenium (FFQ1 to 4DDD). Similar studies obtained similar cross-classification results; Tan et al. (2013) used a 24-hour recall to valid an iodine-specific FFQ, 92.7% of participants were classified into the same tertile or one tertile different, and 6.3% were classified into opposite tertiles. Combet and Lean (2014) found 82% of participants were classified into the same or one tertile different. Kelliher et al. (2023) observed 91% of participants were classified into the same or one tertile different. Thus, the results from the cross-classification were similar to those of other studies. Additionally, the weighted Kappa statistic was calculated as it summarises the cross-classification and provides greater accuracy for the agreement that could have occurred by chance (Masson et al., 2003). The weighted Kappa statistic ranged from 0.007 to 0.421 for the comparison between the FFQ and 4DDD. The iodine and supplements value obtained the greatest Kappa of 0.421, meaning it was of moderate agreement. In contrast, selenium obtained a Kappa of 0.007, which indicates no to slight agreement. Similar studies obtained similar weighted Kappa statistics: 0.229 (Combet & Lean, 2014), 0.28 (Condo et al., 2015) and 0.731 (Fu et al., 2023). Across the comparison of the FFQ to the EIB, the Kappa static showed a slight agreement of 0.115 for iodine and fair agreement for selenium, 0.320.

Of the different Bland-Altman plots for comparing the FFQ1 to 4DDD, three plots showed the mean difference being close to 0: food-only iodine intake, total iodine intake and iodine and supplements intake. These plots showed good agreement, with most of the points being within the LOA. Furthermore, the mean difference was below 0 for both plots; iodine, salt intake, and selenium intake showed bias. Additionally, most of them were observed to be outside the LOA. Looking at other similar studies that have carried out Bland-Altman plots for total iodine, similar trends were observed (Condo et al., 2015; Fu et al., 2023; Tan et al., 2013). Furthermore, Combet and Lean (2014) carried out Bland-Altman plots for comparisons of FFQ – 4DDD and FFQ – EIB; both plots observed similar agreements to what was observed in this study.

The comparison of the FFQ1 to EIB showed a poor correlation between 0.155 (iodine) and 0.146 (selenium). The correlation for comparing the EIB and 4DDD was also poorly correlated, 0.118 (iodine) and 0.051 (selenium). Studies that have used biomarkers to assess a selenium or iodine FFQ are limited. However, studies that have used biomarkers for validation have obtained similar results: 0.362 (Condo et al., 2015), 0.316 (Combet & Lean, 2014), 0.094 (Tan et al., 2013), and 0.123 (Kelliher et al., 2023). In the comparison of the FFQ to EIB, the correlation is smaller. The poor correlation can be linked to the highly variable nature of iodine

intake and with EIB being based on one day of intake. To the best of our knowledge, this study was the first validation study using iodine to use total iodine excretion calculated with BMIC and UIC; therefore, there are limited studies to compare our iodine biomarker results. However, when determining the validity of an FFQ, it is important not just to use correlation independently to measure absolute agreement. In comparing EIB to the FFQ1, selenium obtained a Wilcoxon value ($p<0.05$), meaning poor agreement between the two results. The effect size for selenium was large, meaning that the difference was substantial. When visually inspecting the Bland-Altman plots comparing the FFQ1 to EIB, the LOA has a much greater variance. Iodine shows stronger agreement, with the mean difference sitting closer to 0. Visually inspecting the selenium point, as the difference between the FFQ1 and EIB increased, so did the mean between the two measures.

Overall, these results show that there was a fair to good level of agreement between the FFQ1 and 4DDD. While there were some discrepancies in the results, such as iodine and salt intake, this may be due to systematic bias created by the different techniques used to quantify iodised salt intake. Additionally, selenium showed a lower level of agreement than iodine, which could be due to food sources that contain high levels of selenium being highly variable between different food sources. With a limited number of foods containing naturally high amounts of selenium. While limited studies have specifically looked at selenium when validating an FFQ, some have found poor levels of correlation (Brantsæter et al., 2008).

3.5.2 Reproducibility of the FFQ

For iodine and selenium, there was a 10.3% and 21.4% difference between FFQ1 and FFQ2, respectively. Both of these were found to have a statistically significant difference; the effect size for these values was medium to large. From the results shown by the correlations, iodine and selenium demonstrated good agreement. Other similar reproducibility studies that looked at iodine and selenium observed similar results: 0.63 (Naess et al., 2019), 0.81 (Glabska et al., 2017), 0.79 (Parackal et al., 2021) for iodine and 0.58 (Parackal et al., 2021) and 0.33 (Tenorio et al., 2021) for selenium. After analysing the findings of other validation and reproducibility studies, it can be observed that iodine produces a stronger correlation than selenium. Obtaining results similar to other studies shows these results are expected. Analysing the cross-classifications for reproducibility, iodine and selenium showed 98.3% and 96.6% of participants being classified into the same or one tertile difference between FFQ1 and FFQ2, respectively. However, only 37.3% of the participants were classified into the same tertile for selenium. While selenium did not obtain a result of >50% being correctly classified, it still showed a result of <10% being classified into the opposite tertile. Additionally, the

weighted Kappa statistic further explains the agreement between the two FFQs. Iodine obtained a Kappa statistic of 0.155, meaning there is a fair agreement between FFQ1 and FFQ2. In contrast, selenium obtained a Kappa statistic of -0.006 Masson et al. (2003), stated that negative values in Kappa statistics are unlikely but possible. Furthermore, negative values could occur if the agreement expected by chance is greater than the observed agreement (Cohen, 1968). This result could be due to systematic bias between the two FFQs leading to disagreement.

The Bland-Altman plots for both iodine and selenium show fair agreement between FFQ1 and FFQ2. When visually inspecting both plots, it is observed that most of the points are inside the LOA; the points are also scattered along the mean difference line. Both FFQs were taken nine months apart; diet variation is expected during postpartum. The current breastfeeding recommendations in New Zealand are exclusively breastfeeding until around six months and continuing to breastfeed with complementary feeding for two years (Manatu Hauora: Ministry of Health, 2023). However, some mothers can stop breastfeeding earlier and the nutritional requirements change with this transition. The recommendation is to take iodine supplements during lactation, once lactation has ceased, it is recommended to stop taking the iodine supplement. Therefore, this results in a decrease in iodine intake. In the case of this study, during the nine-month period between the FFQs, participants may have stopped taking an iodine supplement or a supplement containing selenium, causing significant changes between the two FFQs. Additionally, similar studies have had similar results in agreement for selenium; as previously stated, selenium is only naturally high in limited food sources. These changes in food consumption patterns could result in a less significant agreement between dietary assessments taken at different times.

3.5.3 Strengths and Limitations of the Study

This study exhibited key strengths, with it being the first study to validate an iodine and selenium-specific FFQ intended to be used on a breastfeeding population. A key strength of the study was that the FFQ was not just validated against a dietary reference method; additionally, EIB were used to help strengthen the validity. Furthermore, due to the physiology of iodine excretion of the study population, the study utilised both urinary iodine concentrations and breast milk iodine concentration to calculate the total iodine excretion to use as the comparative biomarker for iodine. While validating the FFQ, there is no accurate reference method to determine absolute true dietary intake; the study used a 4DDD as a reference method. This reference method is considered a superior dietary assessment method (Cade et al., 2002). The validity and reproducibility of the FFQ were strengthened by using a range

of statistical analyses; this included Wilcoxon signed-ranked tests, Spearman's coefficients, cross-classifications, Kappa statistics, and the Bland-Altman plot.

The study had several limitations. The participants who took part in the study had a high education level, with many having attended tertiary education (77%). Therefore, due to the education level of the study population, it does not accurately represent the whole population of breastfeeding women, as some of these women may be interested in the topic or be already well-educated on iodine and selenium requirements during lactation. Furthermore, these women may have been more motivated to participate in the study due to their interest in the topic. They may be more likely to take extra consideration of their health. The study's sample size is considered to be small and, all participants were based in Manawatu, New Zealand; increasing the areas where the participants are situated will provide further validity for using the FFQ across New Zealand.

A challenging aspect of the dietary intake was quantifying iodised salt usage. As it is not common to measure salt usage in cooking and adding to a meal, being able to measure the amount consumed from iodised salt was a challenge. Instead of adding participant burden by asking them to measure salt usage in meals, and adding to meal instead 48μg of iodine, (Edmonds et al., 2016) was added to dietary intake when a participant identified that they used iodised salt in the FFQ. For the FFQ habits around iodised salt usage was asked, such as adding to cooking or at the table to gauge the quantifiable amount of iodised salt used. For the 4DDD, the amount of iodine intake from iodised salt was calculated by Food Works as indicated from their diet diary. However, neither technique accurately estimates the amount of iodine consumed through salt.

3.6 Conclusions

In conclusion, the studied Iodine and Selenium Specific Food Frequency Questionnaire showed reasonable relative validity and good reproducibility when compared to the 4DDD for assessing the iodine and selenium intake of breastfeeding mothers in New Zealand. The FFQ could be used in clinical practice or for research as a valid dietary assessment method to assess whether breastfeeding mothers meet their iodine and selenium requirements. This could be a useful tool to track this population's iodine and selenium intake to help identify trends and assess if government initiatives such as subsided iodine supplements are helping this population. To improve the FFQ further, more emphasis could be added to questions around selenium to help increase the strength of agreement. The recommendation for future research around the validity of this FFQ is to aim for it to be used on a larger study population of breastfeeding women across New Zealand with varying education levels and ethnicities.

Chapter Four: Conclusions and Recommendations

4.1 Overview of the study and summary of findings

New Zealand mothers are at increased risk of developing iodine and selenium deficiencies due to their increased requirements during breastfeeding (Thomson et al., 2001). Iodine and selenium have a role in thyroid regulation. Both the mother and her breastfed infant rely on the mother's intake; if the mother is not meeting her requirements, it puts them at risk of iodine and selenium deficiency. This can result in thyroid dysfunction in the mother or her infant (Jin et al., 2022). Dietary assessment tools are important in primary care and research to assess different populations' nutrient intake. Breastfeeding mothers have increased nutrient requirements, and their dietary intake can impact their breastfed infants' nutrient status as well as their own. The breastfeeding population is underrepresented in research, with many studies focusing on pregnancy. However, increased metabolic demands occur during pregnancy and often continue into lactation. A comprehensive literature review revealed that in New Zealand, lactating population have been found to have poor iodine and selenium status (Brough et al., 2015). During breastfeeding, iodine and selenium requirements increase, thus putting these mothers at increased risk of developing an iodine or selenium deficiency. Recent studies have shown that many breastfeeding mothers are not meeting their iodine and selenium requirements in New Zealand, even with the implementation of government initiatives. While other countries' studies have developed and validated iodine or selenium-specific FFQ (Combet & Lean, 2014; Condo et al., 2015; Fu et al., 2023; Kelliher et al., 2023; Naess et al., 2019; Tan et al., 2013), this is the first FFQ developed that assesses both selenium and iodine together, along with this being the first iodine and selenium FFQ to be validated in New Zealand on a breastfeeding mother population.

This study aimed to validate an iodine and selenium-specific FFQ for breastfeeding mothers in New Zealand against a 4DDD and to evaluate the reproducibility by re-administering the FFQ nine months later on the same study population. Validation of dietary assessment tools is important to be accurate and reliable by validating the dietary assessment tool on the intended population. The literature review showed no validated iodine and selenium-specific FFQs exist in New Zealand or worldwide. This study aimed to validate the iodine and selenium-specific FFQ designed to assess the iodine and selenium intake of breastfeeding women living in New Zealand. The 69-item semiquantitative iodine and selenium-specific FFQ were compared against a 4DDD and Estimated Intake from Biomarkers (EIB) to assess validity.

The FFQ's validity was found to have a fair agreement based on the analysis of statistical results using various methods such as the Wilcoxon signed ranked test, correlation coefficients, cross-classifications, weighted kappa statistics and Bland Altman plots. The validation results demonstrated similarities with those observed in other studies; (Brantsæter et al., 2008; Combet & Lean, 2014; Condo et al., 2015; Fu et al., 2023; Karita et al., 2003; Kelliher et al., 2023; Naess et al., 2019; Rasmussen et al., 2001; Tan et al., 2013). The reproducibility of the FFQ was assessed by re-administering the same FFQ nine months later. The results of FFQ1 and FFQ2 were compared and analysed using the same statistical analysis methods as the validation, and the results demonstrated good reproducibility for iodine and fair reproducibility for selenium. From this, it was determined that the iodine and selenium-specific FFQ was a valid and reproducible tool and could be used for breastfeeding mothers in New Zealand to determine iodine and selenium intake and identify individuals with low iodine and selenium intake.

4.2 Strengths and Limitations

This was the first FFQ of its kind to specifically examine selenium and iodine intake in breastfeeding women living in New Zealand. The study had many strengths, including the fact that various statistical methods were used to assess the validity and reproducibility. Literature suggests that using multiple statistical methods increases the strength of the validity (Cade et al., 2002); these include comparing means, Spearman's coefficient, cross-classification, weighted kappa statistics and Bland-Altman plots. Additionally, the reference methods (4DDD) used to assess the validity were another strength of the study. While no gold standard reference method for validating FFQs exists, some studies have recommended using food diaries over other dietary assessment methods (Cade et al., 2002). Furthermore, using relevant biomarkers provided greater strength to the validity of the FFQ. Using EIBs to compare against an FFQ is beneficial as both methods have different measurement errors, decreasing the likelihood of bias in the correlation (Willett, 2012). The EIB used in this study were carefully selected to represent iodine and selenium best, with plasma selenium being the better biomarker of selenium status and an estimated calculation of BMIC and UIC to represent iodine status best.

A limitation of the study would be the population of women who were recruited; most of the women (77%) had a tertiary education. Women participating in these studies are more likely to have an interest in or pre-existing knowledge of iodine or selenium status. This could cause bias because they already have a good iodine or selenium status compared to the average population. Furthermore, the ethnic makeup of this population is not truly a reflection of the

New Zealand population, with most of the study population being NZ European. To better validate the FFQ across the New Zealand population, the validation study could be repeated on a study population that is more representative of the New Zealand population. The sample size of this study was relatively small, with 89 participants. Ideally, the study population would be larger; however, the literature recommends that a validation study have 50 or more participants (Cade et al., 2002; Willett, 1987).

A limitation of the reproducibility is the time between administering the two FFQs. There was a nine-month gap between the two administration dates, with the first occurring at three months postpartum and the second at 12 months postpartum. Participants could have implemented dietary changes during this time, especially during the postpartum period. Food restriction is advised during pregnancy and is no longer applicable. Therefore, this could create bias in the reproducibility results if there are significant dietary changes.

4.3 Recommendations

Some recommendations could be applied to modify the current FFQ and improve the validity and reproducibility determined by the current study. These are outlined below:

- The FFQ could undergo modifications to expand the food items. Including more cultural foods of different ethnicities in New Zealand would allow for the FFQ to be accurately used on a greater population, including more shellfish such as Paua, Kina or Tuatua. Adding these foods would make the FFQ more inclusive of different ethnicities.
- As the selenium and iodine content of foods can be impacted by the area in which they are grown, the FFQ could be validated on a more representative study population from different parts of New Zealand. This would ensure it is a valid tool across different areas in New Zealand.
- The current study's population-ethnic ratio does not reflect the New Zealand ethnicity ratio. If the validation study were to be repeated, it would be beneficial to have more participants from different ethnicities take part to be more reflective of the New Zealand population.
- For the FFQ to more accurately depict iodine and selenium intake, the questions asked about supplement usage and salt could be incorporated into the FFQ itself rather than being separately calculated and added to the iodine and selenium intake.

- Additionally, to reduce reporting errors from participants, providing pictures of portion sizes would increase the accuracy of the answers to the FFQ.
- Two reduce bias within the data by administering the first FFQ at the same visit as taking biomarkers would decrease the risk of bias and random errors from occurring. This would allow for the intake measured by the FFQ to match up with the nutrient status at the given time period.
- There is also a need for a more up-to-date food database in New Zealand. Food Works New Zealand has a limited amount of food products and the nutritional compositions for some products may not be up to date. This could lead to inaccurate calculation of nutrient intakes from food products.

To our knowledge, this is the first FFQ tested for validity and reproducibility to assess iodine and selenium intake in New Zealand breastfeeding women. A literature review revealed that there is a problem in New Zealand and that breastfeeding mothers are at risk of developing iodine and selenium deficiencies. The New Zealand population's iodine and selenium status has changed over the decades. While other countries have developed and validated iodine-specific FFQ, there have yet to be any iodine or selenium-specific questionnaires developed or validated on the New Zealand population. Having validated iodine and selenium-specific FFQ would allow better identification of breastfeeding mothers at risk of developing an iodine or selenium deficiency. In conclusion, the iodine and selenium-specific FFQ demonstrated relative validity compared to a 4DDD and good reproducibility when re-administer nine months later. This tool can be used in primary care to identify breastfeeding mothers at risk of iodine or selenium deficiencies or in research to demonstrate population-level trends in iodine and selenium intake trends.

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Appendices

Appendix A. MINI study General Questionnaire

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Code: _____

MINI Study - Mother and Infant Nutrition Investigation

Date of visit: _____ Day_____ Month_____ Year

General Questionnaire – when your baby is born

I would like to ask you about what you usually eat and your meal preparation.

1. Do you add any **SALT** to your food (either AT THE TABLE or in COOKING)?
   ☐ No (**go to Q 4**)
   ☐ Yes

2. Do you add **SALT** to your food AT THE TABLE?
   ☐ No (**go to Q3)**
   ☐ Yes
   2a. If yes, what type of SALT do you mainly use (more than 60%)?
   ☐ Plain table salt
   ☐ Iodised salt **(go to Q2b.)**
   ☐ Other mineral salt (rock, sea salt)
   ☐ Others_____

   2b.Considering only **IODISED SALT** added AT THE TABLE, please indicate the average amount of your individual portion used DAILY.
   ☐ Less than 1/4 teaspoon
   ☐1/4 teaspoon
   ☐ 1/2 teaspoon
   ☐ 1 teaspoon
   ☐ More than 1 teaspoon

3. Do you add **SALT** to your food in COOKING?
   ☐ No **(go to Q4)**
   ☐ Yes
   3a. If yes, what type of SALT do you mainly use (more than 60%)?
   ☐ Plain table salt
   ☐ Iodised salt **(go to Q3b.)**
   ☐ Other mineral salt (rock, sea salt)
   ☐ Others_____

3b. Considering only **IODISED SALT** added in COOKING please indicate the average amount of your individual portion used DAILY.

☐ Less than 1/4 teaspoon
☐1/4 teaspoon
☐ 1/2 teaspoon
☐ 1 teaspoon
☐ More than 1 teaspoon

4. Which of the following foods do you EXCLUDE from your usual diet? *(Tick all that apply)*
   ☐ Eggs
   ☐ Dairy
   ☐ Fish
   ☐ Seafood
   ☐ Chicken
   ☐ Beef
   ☐ Lamb
   ☐ Pork
   ☐ Other meat or animal products

I would like to ask you what you know about nutrition.

5. Which part of the body needs IODINE to produce hormones?
   ☐ Brain
   ☐ Heart
   ☐ Bone
   ☐ Thyroid gland
   ☐ Do not know

6. What health issues are associated with inadequate intake of IODINE? *(tick all that apply)*
   ☐ Neural Tube Defects
   ☐ Goiter
   ☐ Birth defects
   ☐ Weak bone and teeth
   ☐ Mental retardation
   ☐ Impaired physical development during childhood
   ☐ Blindness
   ☐ Do not know

7. Do you think there is currently a problem with IODINE deficiency in New Zealand?
   ☐ No
   ☐ Yes
   ☐ Do not know

8. From your knowledge, which of the following describes the current fortification in the manufacture of bread in New Zealand? *(Tick all that apply)*

   ☐ Producers must add iodised salt (mandatory fortification)
   ☐ Producers must add folic acid (mandatory fortification)
   ☐ Producers may add or may not add iodised salt (voluntary fortification)
   ☐ Producers may add or may not add folic acid (voluntary fortification)
   ☐ Do not know

9. Since 2010, which target population groups routinely are recommended to take an IODINE supplement? *(Tick all that apply)*

   ☐ Pregnant women
   ☐ Breastfeeding women
   ☐ All women of childbearing age
   ☐ All babies
   ☐ Do not know

10. From your knowledge, which of the following foods contribute good sources of IODINE?

	Good source	Poor source	Do not know
Milk	☐	☐	☐
Potatoes	☐	☐	☐
Fish	☐	☐	☐
Carrots	☐	☐	☐
Bread (excluding organic)	☐	☐	☐
Organic bread	☐	☐	☐
Beef	☐	☐	☐
Seaweed	☐	☐	☐
Lettuce	☐	☐	☐
Eggs	☐	☐	☐
Sea salt	☐	☐	☐
Rock salt	☐	☐	☐

I would like to ask about your supplement usage DURING PREGNANCY.

11. Did you take any supplements?

    ☐ Yes **(go to Q13)**
    ☐ No

Code: _____

11a. If no, which if the following statements are the reasons for not taking any supplements? *(Tick all that apply)*

☐ I was not advised to take them by doctor/nurse practitioner/mid-wife
☐ I could not tolerate them because of nausea (or any other side effects)
☐ I could not afford to purchase them
☐ I did not feel the need to as my health is good
☐ I believed that I could obtain adequate nutrients from my diet
☐ Others _____

12. Please complete the following table with details of any supplements you took.

Brand name (manufacture)	GPs or Midwife's prescription	Start date	Stop date	Frequency Times per week							Dosage each time
				7	6	5	4	3	2	1	
Eg. Blackmores Pregnancy and breastfeeding gold capsule	**Yes**	**12/04/2015**	**12/08/2015**					√			**2 tablets**

I would like to ask about your CURRENT supplement usage SINCE THE BABY WAS BORN

13. Are you taking any supplements?

☐ Yes **(go to Q 14)**
☐ No

13a. If no, If no, which if the following statements are the reasons for not taking any supplements? *(Tick all that apply)*

☐ I was not advised to take them by my doctor/nurse practitioner/mid-wife
☐ I could not tolerate them because of nausea (or any other side effects)
☐ I could not afford to purchase them
☐ I did not feel the need to as my health is good
☐ I believed that I could obtain adequate nutrients from my overall diet
☐ Others _____

14. Please complete the following table with details of any supplements you are taking now.

Brand name (manufacture)	GPs or Midwife's prescription	Start date	Stop date	Frequency Times per week							Dosage each time
				7	6	5	4	3	2	1	
Eg. Blackmores Pregnancy and breastfeeding gold capsule	**Yes**	**12/04/2015**	**12/08/2015**					√			**2 tablets**

Note to the interviewer: If the participant is not able to remember details please ask them to send us an email later with details. (Email requested ☐ Email received☐)

I will now ask you some questions about your smoking habits SINCE YOUR BABY WAS BORN.

15. Have you ever smoked a total of more than 100 cigarettes in your entire life?

☐ No **(go to Q18)**

☐ Yes

16. Did you smoke regularly during **THIS** pregnancy?

☐ No **(go to Q17)**
☐ Yes

16a. If yes, on average, how many cigarettes did you smoke each day?

☐ Less than 1 per day

☐ 1-5 per day

☐ 6-10 per day

☐ 11-15 per day

☐ 16-20 per day

☐ 21-25 per day

☐ 26-30 per day

☐ 31 or more a day

17. Now, after the delivery of your baby, do you continue to smoke?

☐ No **(go to Q18)**

☐ Yes

17a.If yes, on average, how many cigarettes do you now smoke each day?

☐ Less than 1 per day
☐ 1-5 per day
☐ 6-10 per day
☐ 11-15 per day
☐ 16-20 per day
☐ 21-25 per day
☐ 26-30 per day
☐ 31 or more a day

18. Are you regularly exposed to secondhand smoke; for example does someone smoke around you, or in your house or a house you visit often?

☐ No
☐ Yes

18a. If yes, how many hours per day are you exposed to the smoking of others?

_____Hours

I will now ask you some questions about your use of alcoholic drinks SINCE YOUR BABY WAS BORN.

19. Have you had a drink containing alcohol?

☐ No **(go to Q 20)**
☐ Yes

19a. If yes, how often have you had a drink containing alcohol?

☐ Monthly or less
☐ Up to 4 times a month
☐ Up to 3 times a week
☐ 4 or more times a week

19b. How many units do you have on **A TYPICAL DAY** when you are drinking alcohol?

	Beer, cider and RTDs	Wine	Spirits
			
	330ml glass	100ml glass	30 ml short
How many			

About your child

20. What did you give your child to drink routinely during the FIRST WEEK of life? (*Tick all that apply)*
    - ☐ Breastmilk
    - ☐ Water
    - ☐ Sugar water
    - ☐ Infant formula/milk formula
    - ☐ Pasteurized/bottled cow's milk
    - ☐ Soy formula
    - ☐ Hypoallergenic formula
    - ☐ Fruit juices/water down juice/cordial
    - ☐ Herbal drinks
    - ☐ Tea/coffee
    - ☐ Fizzy drinks
    - ☐ Other, specify: _____

21. Do you add sugar to your child's drink?
    - ☐ No
    - ☐ Yes

22. Since the baby was born, have you been breastfeeding your baby, including feeding expressed milk?
    - ☐ No **(go to Q 26)**
    - ☐ Yes

    22a. If yes, which of the choices below most describes your breastfeeding pattern?
    - ☐ Exclusive (100%) breastfeeding
    - ☐ Medium (50-80%) breastfeeding
    - ☐ Partial (less than 50%) breastfeeding
    - ☐ Artificial (less than 10%) breastfeeding

23. On average, how many times a day (during the 24 hour period) do you currently breastfeed your baby?
    _____ Times

24. On average, how long does it take for each breastfeed?
    _____ minutes _____ hours

25. How old was your baby when you stopped breastfeeding?
    - ☐ _____months_____ weeks_____ days
    - ☐ I continue to breastfeed (**go to Q 27**)

26. Tick the reason (s) you chose not to breastfeed, or to stop breastfeeding your baby: *(Tick all that apply)*
    - ☐ Have breastfed long enough
    - ☐ Baby had trouble latching on
    - ☐ Did not have enough milk
    - ☐ Breast milk alone did not seem to satisfy my baby
    - ☐ Painful breast
    - ☐ Baby not gaining enough weight
    - ☐ Baby lost interest/self-weaned
    - ☐ I wanted/needed someone else to feed the baby
    - ☐ Went back to work and expressing breast milk was not convenient/possible
    - ☐ New pregnancy
    - ☐ Baby was old enough that the difference between breast milk and formula was minimal
    - ☐ Other, specify: _____

27. How often do you give your child the following to drink at the moment?

Type of drinks	Never (seldom)	1-3 times/ week	4-6 times/ week	More than once a day
Breastmilk	☐	☐	☐	☐
Pasteurized/bottled cow's milk	☐	☐	☐	☐
Regular Infant formula/milk formula	☐	☐	☐	☐
Hypoallergenic formula	☐	☐	☐	☐
Soy formula	☐	☐	☐	☐
Water	☐	☐	☐	☐
Gripe water	☐	☐	☐	☐
Sugar water	☐	☐	☐	☐
Fruit juices/water down juice/cordial	☐	☐	☐	☐
Herbal drinks	☐	☐	☐	☐
Tea/coffee	☐	☐	☐	☐
Other	☐	☐	☐	☐

Code: _____

Now I am going to ask a few questions about you and your current living situation. The answers to these questions help us to check that we have selected a representative sample of New Zealanders to participate in this survey.

28. Which country were you born in?
    - ☐ New Zealand
    - ☐ Australia
    - ☐ England
    - ☐ Scotland
    - ☐ China (People's Republic of China)
    - ☐ India
    - ☐ South Africa
    - ☐ Samoa
    - ☐ Cook Islands
    - ☐ Other (specify) _____

29. What is your first language? _____.

30. Which ethnic group or groups do you identify with? *(tick all that apply)*
    - ☐ NZ European
    - ☐ Maori
    - ☐ Samoan
    - ☐ Cook Island Maori
    - ☐ Tongan
    - ☐ Niuean
    - ☐ Chinese
    - ☐ Indian
    - ☐ Other (specify) _____

31. If from overseas, in what year did you arrive to live in New Zealand? _____Year

32. What is your date of birth?
    _____ Year _____ Month (range Jan-Dec) _____ Day (range 1-31)

33. How old are you?
    - ☐ <20
    - ☐ 20-24
    - ☐ 25-29
    - ☐ 30-34
    - ☐ 35-39
    - ☐ 40-45
    - ☐ >45

34. What is your highest completed qualification?
    - ☐ School
    - ☐ Trade Certificate
    - ☐ Diploma/Bachelor/Tertiary education
    - ☐ Postgraduate qualification
    - ☐ Other (specify) _____

35. Who do you live with? *(Tick all that apply)*
    - ☐ Husband/partner
    - ☐ Other Children (not including new baby)
    - ☐ My siblings
    - ☐ My parents
    - ☐ Parents in laws
    - ☐ Other relatives
    - ☐ On my own (with my baby)
    - ☐ Others, specify _____

36. What is the total income of your household from all sources, before tax or any other deductions, in the last 12 months?
    - ☐ Loss
    - ☐ Zero income
    - ☐ $1 – $5,000
    - ☐ $5,001 – $10,000
    - ☐ $10,001 – $15,000
    - ☐ $15,001 – $20,000
    - ☐ $20,001 – $25,000
    - ☐ $25,001 – $30,000
    - ☐ $30,001 – $35,000
    - ☐ $35,001 – $40,000
    - ☐ $40,001 – $50,000
    - ☐ $50,001 – $60,000
    - ☐ $60,001 – $70,000
    - ☐ $70,001 – $100,000
    - ☐ $100,001 – $150,000
    - ☐ $150,001 or more

Thanks for completing this questionnaire

Appendix B. Food Frequency Questionnaire – Iodine Selenium

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MINI Study - Mother and Infant Nutrition Investigation

Date of visit: _____ Day_____ Month_____ Year

Food Frequency Questionnaire- Iodine Selenium

Please read the instructions carefully. You should allow approximately 20 minutes completing the questionnaire. You are also provided with sample answers to assist you. Instructions:

- Think about food items you consumed during the **LAST MONTH**.
- Please complete only one box per row. Do not leave any rows blank.
- Look at the food and serving size of each item and write the number of serves that best matches your intake in the corresponding box.
- If the food is eaten daily record this amount in the "**Per Day**" column
- If the food is eaten weekly record this amount of the "**Per Week**" column
- If the food is eaten less than weekly (for example fortnightly or monthly) record this amount in the "**Per month**" column.
- If you did not eat the food in the past month, tick the "**Rarely (<1/month)/Never**" column).
- Many items contain more than one food. Ensure that you read all foods and serving sizes listed and estimate the total average you have consumed.
- **Account for all food eaten, including the ingredients added to recipes, eaten in mixed meals and restaurant or take away meals**. There are prompts under the food items to assist you with this.
- Examples and sample answers are provided

We greatly appreciate your cooperation in this study

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Examples

1. If on average you eat 2 slices of bread and 1 medium roll per day, write **"4"** (2 for bread + 2 for roll) in the box under the "**Per day**" column for 'Bread'.

2. If on average you have fresh salmon for dinner once per week (= 4 times per month) and battered fish from the shop for dinner once per month write **"5**" in the **"Per month"** column for 'Fish'.

3. If on average you have:
□ **1 muffin per day** (= 7 per week) **PLUS** □ **2 slices of cake per week PLUS** □ **2 doughnuts per month** (= ½ per week)

You need to add up the average of these up and write **"9 ½"** (7+2+ ½) in the **"Per week**" column for ''Cake'.
4. If you did not eat canned tuna in the past month **tick** the **"Rarely (<1/month)/never"** column

Foods	Serving size	Per day	Per week	Per month	Rarely (<1/month) /never
Sliced Bread ***(any type)*** **OR** **Bread roll OR** Hamburger bun ***Excluding organic or homemade*** *(Eaten at home or from restaurant/ take-away outlets like McDonalds)*	1 slice (30g) **or** ½ medium bread roll **or** ½ medium bun	4	☐	☐	☐
Fillet of fish (*Eaten at home or from restaurant/take away. Cooked any way including crumbed/ battered or in meals like stir-fry, pasta or soup)*	1 medium fillet (150g)	☐	☐	5	☐
Cake or Baked sweets (*Homemade or purchased including plain and filled cake, cheesecake, muffin, pudding, Danish, pancake, tart)*	1 large serve (100-120g) *(= 1 large slice of cake; 1 large muffin;1large Danish; 2 pancakes)*	☐	9.5	☐	☐
Canned tuna (*Including in sandwiches and salads and in meals like pasta)*	1 small can (100g)	☐	☐	☐	√

Code: _____

Foods	Serving size	Per day	Per week	Per month	Rarely (<1/month)/ never
Dairy products					
Full cream milk (*(plain) (add to coffee, tea, breakfast cereals or on its own)*	1 glass (250mls)	☐	☐	☐	☐
Reduced fat milk (*1-2% fat-plain) (add to coffee, tea, breakfast cereals or on its own)*	1 glass (250mls)	☐	☐	☐	☐
Evaporated milk *(added to meals – only estimated your portion eaten)*	1 glass (250mls)	☐	☐	☐	☐
Flavored milk *(excluding chocolate milk) (add to coffee, tea, breakfast cereals or on its own)*	1 glass (250mls)	☐	☐	☐	☐
Chocolate milk (*add to coffee, tea, breakfast cereals or on its own)*	1 glass (250mls)	☐	☐	☐	☐
Cheese *(all varieties, eaten at home or restaurant or takeaway and added to meals/sandwiches)*	2 slices or 40g = 2 tablespoons grated or 2 wedges)	☐	☐	☐	☐
Yoghurt (all flavor and varieties)	1 pot (200g)	☐	☐	☐	☐
Ice-cream (all flavor and varieties)	2 scoops (60g) or 1 ice cream stick	☐	☐	☐	☐
Dairy dessert (homemade/commercial eg.custard, mousse, rice pudding/crème caramel)	1 cup (200g)	☐	☐	☐	☐
Soy milk (add to coffee, tea, breakfast cereals or on its own)	1 glass (250 mls)	☐	☐	☐	☐
Eggs					
Whole egg (excluding omega-3 enriched) (raw, cooked any ways Raw or cooked any way. Including eggs eaten at home or restaurant/take away and added to meals like sandwiches, salads, hamburgers and sweets)	1 medium egg (60g)	☐	☐	☐	☐

Code: _____

Foods	Serving size	Per day	Per week	Per month	Rarely (<1/month)/ never
Omega-3 enriched whole egg (raw, cooked any ways	1 medium egg (60g)	☐	☐	☐	☐
Fish (including in sandwiches, meals eg. Salad, pizza, soup, curry, stir-fry)					
Fish in batter (deep-fried)	1 medium fillet (150g)	☐	☐	☐	☐
Fresh fish (raw, steamed, pan fried, grilled,baked)	1 medium fillet (150g)	☐	☐	☐	☐
Fish fingers	2 sticks(80g)	☐	☐	☐	☐
Smoked fish	1 medium fillet (150g)	☐	☐	☐	☐
Canned tuna	1 small can (100g)	☐	☐	☐	☐
Canned pink salmon	1 small can (100g)	☐	☐	☐	☐
Canned red salmon	1 small can (100g)	☐	☐	☐	☐
Canned anchovy	1 small can (45g= 10 anchovies)	☐	☐	☐	☐
Canned sardines	1 small can (100g)	☐	☐	☐	☐
Fish paste/spread	1 tablespoon	☐	☐	☐	☐
Seafood (fresh, cooked, tinned, battered/crumbled in meals or soups, pasta)					
Mussels	6 mussels (50g)	☐	☐	☐	☐
Oysters	6 oysters (90g)	☐	☐	☐	☐
Scallops	6 scallops (90g)	☐	☐	☐	☐

Code: _____

Foods	Serving size	Per day	Per week	Per month	Rarely (<1/month)/ never
Prawn/shrimp	6 medium prawn	☐	☐	☐	☐
Squid/calamari	6 calamari rings (120g)	☐	☐	☐	☐
Lobster/crayfish/crab	½ cup meat (70g)	☐	☐	☐	☐
Whitebait (fresh/frozen)	½ cup (70g)	☐	☐	☐	☐
Meat					
Sausages excluding vegetarian sausages	1 sausage	☐	☐	☐	☐
Burger meat/patties	1 meat patty	☐	☐	☐	☐
Lambs liver	1 slice (40g)	☐	☐	☐	☐
Ham/bacon	2 slices (40g)	☐	☐	☐	☐
Chicken	1 serve (150g)	☐	☐	☐	☐
Pork chops	1 chop (150g)	☐	☐	☐	☐
Cereal products					
Sliced bread or bread rolls or Hamburg buns Excluding organic/homemade	1 slice (30g) 1/2 medium roll/bun	☐	☐	☐	☐
Flat bread or Bagel or English muffin	1 bagel	☐	☐	☐	☐
Homemade bread with iodised salt	1 slice (30g)	☐	☐	☐	☐
Homemade bread without iodised salt	1 slice (30 g)	☐	☐	☐	☐

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Foods	Serving size	Per day	Per week	Per month	Rarely (<1/month)/ never
Wheat biscuits cereals	2 biscuits	☐	☐	☐	☐
Museli (all varieties)	½ cup	☐	☐	☐	☐
Noodles or Pasta (all varieties)	1 cup	☐	☐	☐	☐
Rice	½ cup cooked	☐	☐	☐	☐
Vegetables (fresh or frozen)					
Spinach (including baby spinach)	½ cup cooked/1cup fresh (100g)	☐	☐	☐	☐
Bok choy/spring greens/kale	½ cup cooked/1cup fresh (100g)	☐	☐	☐	☐
Broccoli	½ cup cooked/1cup fresh (100g)	☐	☐	☐	☐
Cabbages/Brussel sprouts	½ cup cooked/1cup fresh (100g)	☐	☐	☐	☐
Cauliflowers	½ cup cooked (100g)	☐	☐	☐	☐
Turnip/swede/radish	½ cup cooked (100g)	☐	☐	☐	☐
Sweet potatoes	½ cup cooked (100g)	☐	☐	☐	☐
Tofu, soybean products (Tempeh, bean curd)	½ cup cooked (100g)	☐	☐	☐	☐
Edamame beans	½ cup cooked (100g)	☐	☐	☐	☐
Vegetarian sausages	2 sausages	☐	☐	☐	☐

Code: _____

Foods	Serving size	Per day	Per week	Per month	Rarely (<1/month)/ never
Seaweed (exclude that in sushi, Including dried and cooked in Asian style soups and meals)	2 sheets	☐	☐	☐	☐
Mushrooms (all varieties)	½ cup cooked	☐	☐	☐	☐
Snacks or sweets					
Chocolate or chocolate coated nuts/dried fruit	1 medium bar or ½ cup nuts or fruit (60g=10nuts)	☐	☐	☐	☐
Cake or baked sweets (Homemade or purchased including plain and filled cake, cheesecake, muffin, pudding, Danish, pancake, tart**)**	1 large slice of cake/1large muffin(110g)	☐	☐	☐	☐
Sweet bun (including plain, fruit and finger bun)	1 bun (75g)	☐	☐	☐	☐
Cashew nuts (raw, roasted, salted	10 whole = 10g	☐	☐	☐	☐
Brazil nuts (raw, roasted/salted)	5 whole = 50g	☐	☐	☐	☐
Peanuts (raw, roasted/salted, cooked)	10 medium whole = 50g	☐	☐	☐	☐
Cheese flavoured snacks (twisties, Cheetos, cheese tubes, crackers)	5 crackers = 30g	☐	☐	☐	☐
Chocolate biscuits	2 biscuits	☐	☐	☐	☐
Ready-made food (if you have already accounted for these food in the individual items then DO NOT account for them again)					
Pizza (all flavors, Purchased frozen or from pizza chain**)**	1 large slice (100g)	☐	☐	☐	☐
Quiche (Any flavour. Purchased frozen or commercial)	1 medium slice (120g)	☐	☐	☐	☐

Code: _____

Foods	Serving size	Per day	Per week	Per month	Rarely (<1/month)/ never
Cheese pastry roll/slice or spring rolls	1 individual serve (150g)	☐	☐	☐	☐
Meat Pie, pastie or sausage rolls	1 individual serve (140-180g)	☐	☐	☐	☐
Sushi with seaweed	1 roll	☐	☐	☐	☐
Dim sim	2 dim sims (100g)	☐	☐	☐	☐

Thanks for completing this questionnaire

Appendix C. 4-Day Diet Diary – Maternal

Appendix - MINI Study – 4-Day Dietary Diary (Maternal)

4- day Food Dietary Diary

Date of visit: _____

Day_____ 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 **4 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

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, eg. 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, Hovis, 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:

- 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 tbsps 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!

EXAMPLE

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		
Time	**Where**	**Food/drink description & preparation**	**Brand name**	**Portion size or quantity eaten**
		12noon to 2pm		

12.30am	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
<u>2pm to 5pm</u>				
3pm	Meeting room	Herbal tea Louise slice	Healthiers bakery	1 cup 1 regular slice
Time	**Where**	**Food/drink description & preparation**	**Brand name**	**Portion size or quantity eaten**
<u>5pm to 8pm</u>				

6.30pm	At table with husband and children	Spaghetti, wholemeal Bolognese sauce (see recipe) Courgettes Organe 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	tape	200mls

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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.			

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Code: _____

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

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Code: _____

Spaghetti/noodles

Rice

Chips

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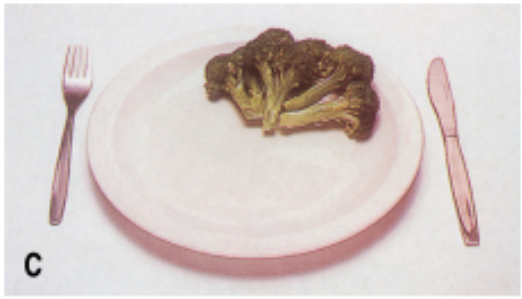
Code: _____

Broccoli or cauliflower



Stew or curry

Battered fish

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COLLEGE OF HEALTH
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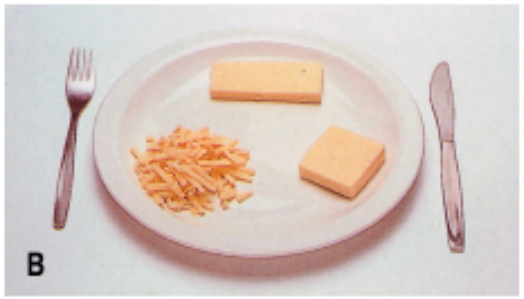
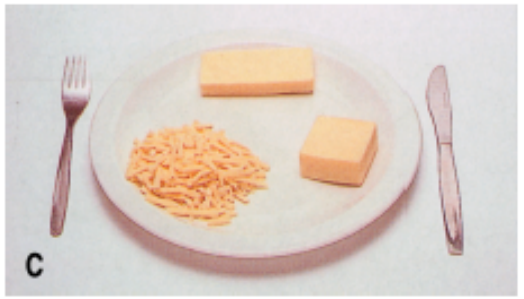
Code: _____

Quiche or pie

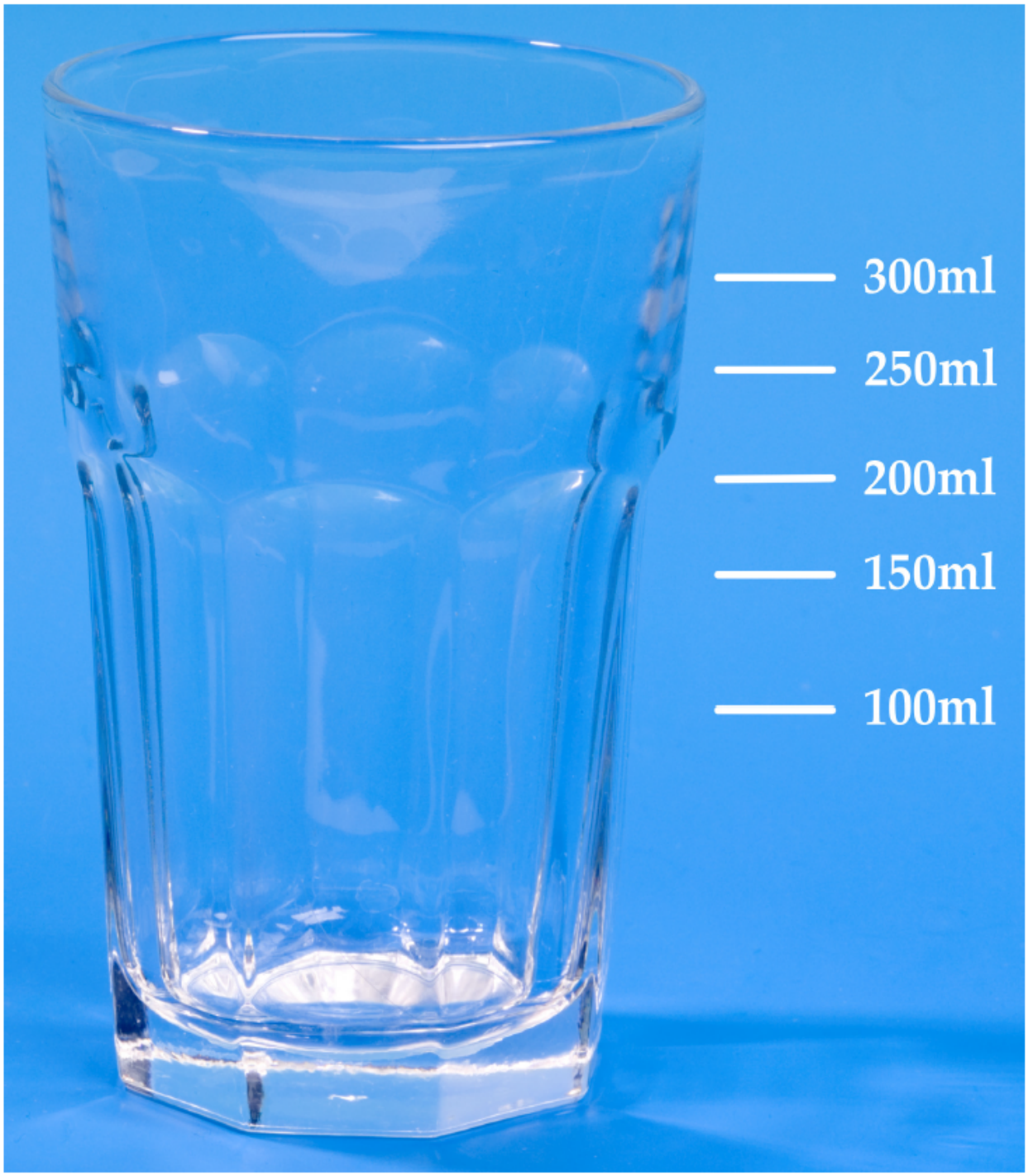
Cheese





Spongy cake

Life Size Glass



Day 1

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.

   Please tell us why you had less than usual

   ☐ No, **more** 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

Please tell us why you had less than usual

☐ No, **more** 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 D: MINI Study Ethical Approval

Health and Disability Ethics Committees
Ministry of Health
Freyberg Building
20 Aitken Street
PO Box 5013
Wellington
6011

0800 4 ETHICS
hdecs@moh.govt.nz

15 December 2015

Ms Ying Jin
School oF Food and Nutriton
Massey University
Private Bag 11222
Palmerston North 4442

Dear Ms Jin

Re:	**Ethics ref:**	**15/NTA/172**
	Study title:	Mother and Infant Nutrition Investigation

I am pleased to advise that this application has been ***approved*** by the Northern A Health and Disability Ethics Committee. This decision was made through the HDEC-Full Review pathway.

Conditions of HDEC approval

HDEC approval for this study is subject to the following conditions being met prior to the commencement of the study in New Zealand. It is your responsibility, and that of the study's sponsor, to ensure that these conditions are met. No further review by the Northern A Health and Disability Ethics Committee is required.

Standard conditions:

1. Before the study commences at *any* locality in New Zealand, all relevant regulatory approvals must be obtained.

2. Before the study commences at *a given* locality in New Zealand, it must be authorised by that locality in Online Forms. Locality authorisation confirms that the locality is suitable for the safe and effective conduct of the study, and that local research governance issues have been addressed.

After HDEC review

Please refer to the *Standard Operating Procedures for Health and Disability Ethics Committees* (available on www.ethics.health.govt.nz) for HDEC requirements relating to amendments and other post-approval processes.

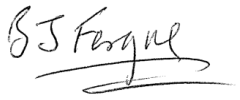
Your next progress report is due by 15 December 2016.

Participant access to ACC

The Northern A Health and Disability Ethics Committee is satisfied that your study is not a clinical trial that is to be conducted principally for the benefit of the manufacturer or distributor of the medicine or item being trialled. Participants injured as a result of treatment received as part of your study may therefore be eligible for publicly-funded compensation through the Accident Compensation Corporation (ACC).

Please don't hesitate to contact the HDEC secretariat for further information. We wish you all the best for your study.

Yours sincerely,



Dr Brian Fergus
Chairperson
Northern A Health and Disability Ethics Committee

Encl: appendix A: documents submitted
appendix B: statement of compliance and list of members

Appendix A
Documents submitted

Document	*Version*	*Date*
PIS/CF	1	20 October 2015
Protocol	1	20 October 2015
Evidence of scientific review	1	20 October 2015
Survey/questionnaire: It is an index to provide an overview of all the questionnaires used in the study.	1	21 October 2015
Survey/questionnaire: V1_M2	1	21 October 2015
Survey/questionnaire: V1_M3	1	21 October 2015
Survey/questionnaire: V1_M4	1	21 October 2015
Survey/questionnaire: Health Screening Questionnaire	1	21 October 2015
Survey/questionnaire: V1_M5	1	21 October 2015
Survey/questionnaire: V1_H1	1	21 October 2015
Survey/questionnaire: V1_H2	1	21 October 2015
Survey/questionnaire: V1_H3	1	21 October 2015
Survey/questionnaire: V2_M1	1	21 October 2015
Survey/questionnaire: V2_M2	1	21 October 2015
Survey/questionnaire: V3_M1	1	21 October 2015
CV for CI	1	21 October 2015
Survey/questionnaire: Ages and Stages Questionnaires_Sample	1	21 October 2015
Investigator's Brochure	1	22 October 2015
Survey/questionnaire: V1_M1	1	22 October 2015
Survey/questionnaire: V2_H1	1	22 October 2015
PIS/CF for persons interested in welfare of non-consenting participant	1	22 October 2015
Application	1	-
PIS/CF: This is the updated version of the Information Sheet.	2	24 November 2015
PIS/CF: This is the updated version of Consent Form.	2	24 November 2015
Investigator's Brochure: This is the updated version of the Advertisement	2	24 November 2015

Appendix B
Statement of compliance and list of members

Statement of compliance

The Northern A Health and Disability Ethics Committee:

- is constituted in accordance with its Terms of Reference
- operates in accordance with the *Standard Operating Procedures for Health and Disability Ethics Committees*, and with the principles of international good clinical practice (GCP)
- is approved by the Health Research Council of New Zealand's Ethics Committee for the purposes of section 25(1)(c) of the Health Research Council Act 1990
- is registered (number 00008714) with the US Department of Health and Human Services' Office for Human Research Protection (OHRP).

List of members

Name	*Category*	*Appointed*	*Term Expires*
Dr Brian Fergus	Lay (consumer/community perspectives)	01/07/2012	01/07/2015
Dr Karen Bartholomew	Non-lay (intervention studies)	01/07/2013	01/07/2016
Dr Charis Brown	Non-lay (intervention studies)	11/11/2015	11/11/2018
Ms Susan Buckland	Lay (consumer/community perspectives)	01/07/2012	01/07/2016
Ms Shamim Chagani	Non-lay (health/disability service provision)	01/07/2012	01/07/2016
Dr Christine Crooks	Non-lay (intervention studies)	01/07/2013	01/07/2018
Dr Kate Parker	Lay (consumer/community perspectives)	11/11/2015	11/11/2018

Unless members resign, vacate or are removed from their office, every member of HDEC shall continue in office until their successor comes into office (HDEC Terms of Reference)

http://www.ethics.health.govt.nz

Appendix E: MDHB Locality Approval

MidCentral District Health Board
Te Pae Hauora o Ruahine o Tararua

MDHB APPROVAL FORM FOR RESEARCH ACTIVITY

Research Practice Title: Mother and Infant Nutrition Investigation (MINI) Study : A cohort of postpartum women
Principal Investigator: Ying Jin
Designation : PHD Candidate Service Area: Womens Health
Research Practice Experience : _____
Other Researchers Involved: Lovise Brough (Massey) Jane Coad (Massey)

Brief description of research study purpose, methodology and reporting:

Purpose:

After the birth of their baby, most women continue to see their health care professionals. However, the focus is often on the infant's health. Only limited attention is given to the mother's mental health. This study will monitor the mothers' health by assessing her nutrient status, thyroid function and mental health. The thyroid is a small butterfly-shaped gland at the base of the neck which produces hormones. How a mother's health status might affect her baby's development during early life is important. The three nutrients we are studying are iodine, selenium, and iron. Understanding these nutrients will help to provide better health care to future mothers. This leads to greater knowledge about the health and wellbeing of both the mothers and their infants.

Methodology:

Advertisements and posters place at selected sites where preganat or post partum women frequently attend. Potential participants will record an expression of interest online or via telephones.Prospective participants will be sent an appropriate study information sheet. Once they indicate their willingness to participate, the researcher will conduct a screening questionnaire to ensure participants are eligible to take part in the study. Informed consent will be obtained. The target number of study participants is 180. Taking

Progress and final reporting:

Section A : Initial Registration and Approval of Research Practice

Documented evidence :

- ☐ Research purpose and parameters
- ☐ Consultation with all MDHB involved parties
- ☐ Risk and indemnity cover
- ☐ Resources required (*eg, staff, equipment, other service involvement)*
- ☐ Approved research budget

Operations Director signature to proceed : Date:

Professional approval

☑ Yes ☐ No ☐ Not applicable

Designation:	Signature:	Date:
ACTING CLINICAL DIRECTOR		14/9/16
CHARGE MIDWIFE	DrNRST	15/9/16

MidCentral District Health Board
Te Pae Hauora o Ruahine o Tararua

MDHB APPROVAL FORM FOR RESEARCH ACTIVITY

External approval *(eg, HDEC, Educational Institution)*

☑ Yes ☐ No ☐ Not applicable

State where from : HDEC

Documented evidence (where applicable):

☐ National application form for ethical review of a research project *(NAF- 2005- v1)*

☐ 'Participants who are unable to give informed consent to participate' form *(NAF- Part 7)*

☑ Locality assessment form

☐ 'Use of human tissue' form *(NAF- Part 5)*

☐ 'Genetic research' form *(NAF - Part 6)*

Section B : Operations Director's Endorsement to Proceed

Proposed start/end dates of research: _____

Operations Director signature _____

Service Line : Women Health Date: 15.9.16.

This submission has been considered to meet ethical and professional requirements, and clearly demonstrate potential clinical, professional and/or strategic benefit to the organisation.

Clinical Board Acknowledgement of Registration

Signed: _____ Designation: CHO Chair Date: 22/9/16

Copy to be retained by Chief Medical Officer's office and details entered onto Register.

To be completed by the Principal Investigator and Operations Director. The Operations Director is to forward a copy of the form to the MidCentral Health Clinical Board, via Quality & Clinical Risk. All relevant supporting documentation is to be included.

MidCentral District Health Board
Te Poe Hauora o Ruahine o Tararua

Locality Assessment Sign Off for Approval of Research/Clinical Trials

Full project title: Mother and Infant Nutrition Investigation

Short project title: MINI

1. Declaration by Principal Investigator

The information supplied in this application is, to the best of my knowledge and belief, accurate. I have considered the potential ethical, resource and cultural issues involved in this research and believe that I have adequately addressed them for this locality.

A formal letter of consultation was sent to the Maori Health Unit on the __/__/__ (date)
Maori Consultation with Dr Maureen Holdaway, Massey University

Name of Principal Investigator (please print): Ying Jin

Signature of Principal Investigator:

Date: 25 July 2016

2. Declaration by Clinical Leader of Service/Department in which the Principal Investigator is located

I have read the application, and it is appropriate for this research to be conducted in this department. I give my consent for this locality to be included in the ethics committee application.

Name (please print): STEVEN GRANT

Signature: | Institution: Palmerston North Hospital/MCH

Date: 14/9/16 | Designation: Acting C.D.

- Where the Clinical Leader is also one of the investigators, the Clinical Leader declaration must be signed by the Clinical Executive Director.

Appendix F: MINI Study ANZCTR Registration

Dear Ying Jin,

Re: Mother and Infant Nutrition Investigation - Investigating micronutrient intake and status in mothers and babies, and their possible effects on thyroid function

Thank you for submitting the above trial for inclusion in the Australian New Zealand Clinical Trials Registry (ANZCTR).

Your trial has now been successfully registered and allocated the ACTRN: ACTRN12615001028594

Web address of your trial: http://www.ANZCTR.org.au/ACTRN12615001028594.aspx
Date submitted: 15/09/2015 1:15:17 PM
Date registered: 1/10/2015 10:29:21 AM
Registered by: Ying Jin

If you have already obtained Ethics approval for your trial, could you please send the ANZCTR a copy of at least one Ethics Committee approval letter? A copy of the letter can be sent to info@actr.org.au (by email) OR (61 2) 9565 1863, attention to ANZCTR (by fax).

Please be reminded that the quality and accuracy of the trial information submitted for registration is the responsibility of the trial's Primary Sponsor or their representative (the Registrant).
The ANZCTR allows you to update trial data, but please note that the original data lodged at the time of trial registration and the tracked history of any changes made will remain publicly available.

The ANZCTR is recognised as an ICMJE acceptable registry (http://www.icmje.org/faq.pdf) and a Primary Registry in the WHO registry network (http://www.who.int/ictrp/network/primary/en/index.html).

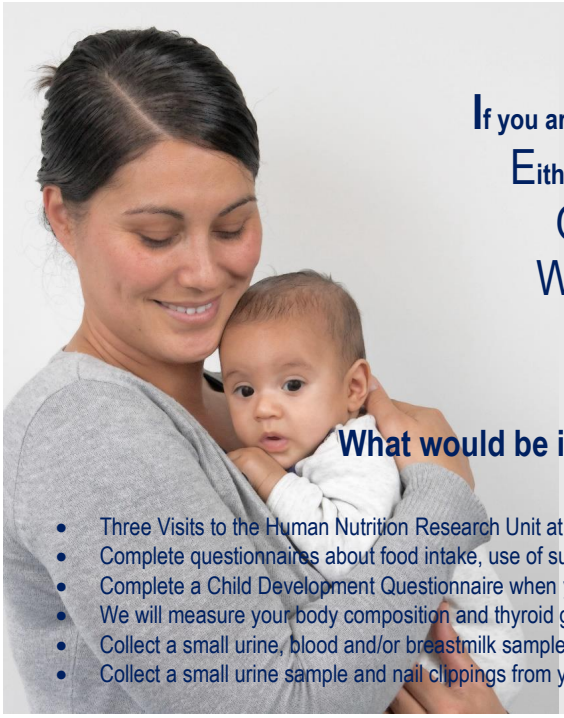
If you have any enquiries please send a message to info@actr.org.au or telephone +61 2 9562 5333.

Kind regards,
ANZCTR Staff
T: +61 2 9562 5333
F: +61 2 9565 1863
E: info@actr.org.au
W: www.ANZCTR.org.au

Appendix G: MINI Study Poster

MINI Study –
Mother and Infant Nutrition Investigation

Would you like to find out more about your dietary intake and nutrient status and its effect on both you and your new-born baby?



If you are a healthy woman aged 16 or older
Either in the late stage of pregnancy
Or have recently given birth
We would like to hear from you

What would be involved if joining this study?

- Three Visits to the Human Nutrition Research Unit at Massey University
- Complete questionnaires about food intake, use of supplements, general health
- Complete a Child Development Questionnaire when your baby reaches 4, 8 and 12 months old
- We will measure your body composition and thyroid gland size
- Collect a small urine, blood and/or breastmilk samples and toenail clippings from you
- Collect a small urine sample and nail clippings from your child

We will continue to follow you and your baby's nutritional health until your child is 12 months old

This project has been reviewed and approved by the Health and Disability Ethics Committee: 15NTA172.

Please contact:
Ms Ying Jin (PhD Scholar) through mini@massey.ac.nz
Or Go to www.massey.ac.nz/ministudy

School of Food and Nutrition, Massey University, 027 399 4138/06-951-7556

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Appendix H: MINI Study Information Sheet with Flowchart

Participant Information Sheet

Study title:	**[MINI - Mother and Infant Nutrition Investigation]**	
Locality:	**Palmerston North**	Ethics committee ref: **15/NTA/172**
Lead investigator:	**Ying Jin**	Contact **email**: **mini@massey.ac.nz** **Register your interest –** **www.massey.ac.nz/ministudy** **Phone: +64 (06) 9517556** **027 399 4138**

Would you like to help us?

We invite you to take part in a research study: Mother and Infant Nutrition Investigation (MINI). This sheet gives detailed information about the study. Please read it carefully before deciding whether you wish to join our study.

We need mothers and their infants to take part. It is important that you understand why we are doing this research, and what it may involve for you. Please take time to read the sheet carefully. Feel free to discuss it with other people, such as your family, whānau, friends, or your health care providers. Please ask us questions if anything seems unclear, or if you wish to know more details.

Introducing the researchers

This research is led by PhD scholar Ms Ying Jin. Ying's supervisors are Dr Louise Brough and Professor Jane Coad. They are human nutritionists in the School of Food and Nutrition, Massey University, Palmerston North. Anne Broomfield, research officer, will also assist in the study.

What is the purpose of this study?

After the birth of their baby, most women continue to see their health care professionals. However, the focus is often on the infant's health. Only limited attention is given to the mother's mental health. This study will monitor the mothers' health by assessing her nutrient status, thyroid function and mental health. The thyroid is a small butterfly-shaped gland at the base of the neck which produces hormones. How a mother's health status might affect her baby's development during early life is important. The three nutrients we are studying are iodine, selenium, and iron. Understanding these nutrients will help to provide better health care to future mothers. This leads to greater knowledge about the health and wellbeing of both the mothers and their infants.

Do I have to take part?

No. It is entirely up to you to decide whether you wish to take part. If you do agree, you will be asked to sign a Consent Form. You will be given a copy of both the Participant Information Sheet and the Consent Form to keep.

Should you change your mind about being in the study, you are free to withdraw from the study at any time without giving any reason.

What would your participation involve?

If you are interested in taking part in the study, please phone or email us. You can also enter your details on this study's "Express of Interest" webpage. We will reply immediately and arrange a brief telephone conversation. We will ask you some questions to ensure that you are eligible. You must feel totally comfortable about taking part in the study.

Soon after, we shall make an appointment for you and your baby to come into the Human Nutrition Research Unit at Massey University. If this is not possible, we may visit you either at home, at a local community Centre, or at a health professionals' clinic.

During the first visit, we shall

- ask you some questions about your nutrient supplement use, and your nutrition knowledge. We will also ask you about your health, diet and some personal information;
- measure your weight, height, and body composition;
- ask you to provide small samples of urine and breast milk which we will use to assess your nutrient status;
- measure your baby's weight, length and head circumference.
- collect a small urine sample from your baby to assess his/her nutrient status.

Your first visit should take no more than two hours.

After the first visit, you will be given

- two small paper bags for you to collect nail clippings from yourself and from your baby to assess selenium status.
- a 4-day food record diary to measure your nutrient intake.

Within a month after your first visit, at a convenient time, we will collect the samples and food diary from you at home.

The second visit will be when your baby is 6 months old. The last visit will be when your baby is 12 months old. We will ask you to complete a questionnaire to assess your child's development at 4, 8 and 12 months.

A detailed Flow Chart is on pg. 6 of this Information Sheet.

How would the required samples be collected?

A clear detailed instruction of how to collect infant or adult nail clippings would be given at the first visit. Infant urine samples will be collected by placing a pad inside the nappy, checking every 10 minutes until wet, and then urine aspirated (extracted) with a syringe. Blood samples will be drawn by experienced phlebotomist. The collected biological samples will be frozen, labelled with a unique code (no personal information will be displayed on the samples), and then stored for 10 years to allow a number of analyses to take place. After 10 years, the samples will be properly disposed in biohazard bags to be incinerated (burned) by a professional company who specialise in destroying biological samples. We acknowledge that the use and storage of tissue is a cultural concern for some Māori people. We are unable to return body fluids such as blood, urine and breastmilk due to safety (microbiological) issues. However, if you wish, the nail clippings, after analysis, will be returned to you if you request this in advance.

What are the possible risks to you?

There are small risks when taking blood samples such as discomfort, bruising, infection or fainting. To minimise any risk, your blood will only be taken by experienced and fully trained research staff.

Any risks involved in this study are very minor. All of the checks are routinely made. If you have any concerns during the study you may discuss these with any of the study team.

Any complaints you may make will be fully investigated. If you have any concerns about any aspect of this study, you should speak immediately to a member of the study team. They will do their best to answer all of your questions fully.

What are the advantages of taking part in the study?

- Your thyroid gland size, thyroid function and iron status will be monitored during the study. These are not normally covered by primary health care services;
- Repeated screening for postnatal depression during the first year after delivering a baby;
- Based on your food diary, you will receive feedback on your intake of nutrients within a month after we receive the dietary diary. This will be compared to New Zealand standard dietary guidelines.
- You will also receive information about your child's development assessments at 4, 8 and 12 months.

Will my participation in the study be kept confidential?

Yes. All information collected about you and your baby during the study will be kept strictly confidential. Each mother will be given a unique code which will be used on all data collected. No identifying details will be recorded on the interview sheets or other records.

When the study results are presented, you will not be named or recognised from any of the information given. All information will be entered into a protected database at Massey University. Information collected about you and your baby will be kept strictly confidential and secure in a locked filing cabinet. All electronic files on computers will have passwords and restricted access. Only the named members of research team will have access to detailed personal information.

Massey University maintains a central record of all research projects undertaken. This does not include personal information about those who take part. The data (without containing personal information) will be held for 10 years after the youngest person in the study has reached the age of consent or 16 years old.

What will happen to the results?

Should you wish, you will receive all the results about you and your baby. Should your results be, in any way, unusual, you will be encouraged to contact your general practitioner and seek appropriate medical advice. Once the whole study has ended, we can send you a summary of the study results, should you wish to have it. The results will also be presented at scientific meetings or published in peer reviewed journals. This ensures that a wider community, including health professionals, can know and read about the findings. You and your baby will not be identified by any of these publications or presentations.

What would happen if you were injured in the study?

If you were injured in this study, which is unlikely, you would be eligible for compensation from ACC. This would be the same as if you were injured in an accident at work or at home.

If you have private health or life insurance, you may wish to check with your insurer that taking part in this study will not in any way affect your cover.

Who has reviewed the study?

This project has been reviewed and approved by the Northern A Health and Disability Ethics Committee through the full review pathway.

Contact for further information:
If you have any further questions or if you have any concerns whilst taking part in the study then please contact:

Ms Ying Jin, Lead Investigator/PhD Scholar
Email: mini@massey.ac.nz, or go to www.massey.ac.nz/ministudy
Cell phone: 027 399 4138
Telephone: +64 (06) 9517556

Dr. Louise Brough, Principle Supervisor/Senior Lecturer
Telephone: +64 (06) 356 9099 ext. 84575

Email: L.Brough@massey.ac.nz

Where can you go for more information about the study, or to raise concerns or complaints?

If you have any questions, concerns or complaints about the study at any stage, you can contact:

Ms Anne Broomfield, Research Technical Officer
Human Nutrition Research Unit
Massey Institute of Food Science and Technology
Telephone: +64 (06) 356 9099 ext. 84566
Email: A.M.Broomfield@massey.ac.nz

If you want to talk to someone who is not involved with the study, you can contact an independent health and disability advocate on:

Phone: 0800 555 050
Fax: 0800 2 SUPPORT (0800 2787 7678)
Email: advocacy@hdc.org.nz

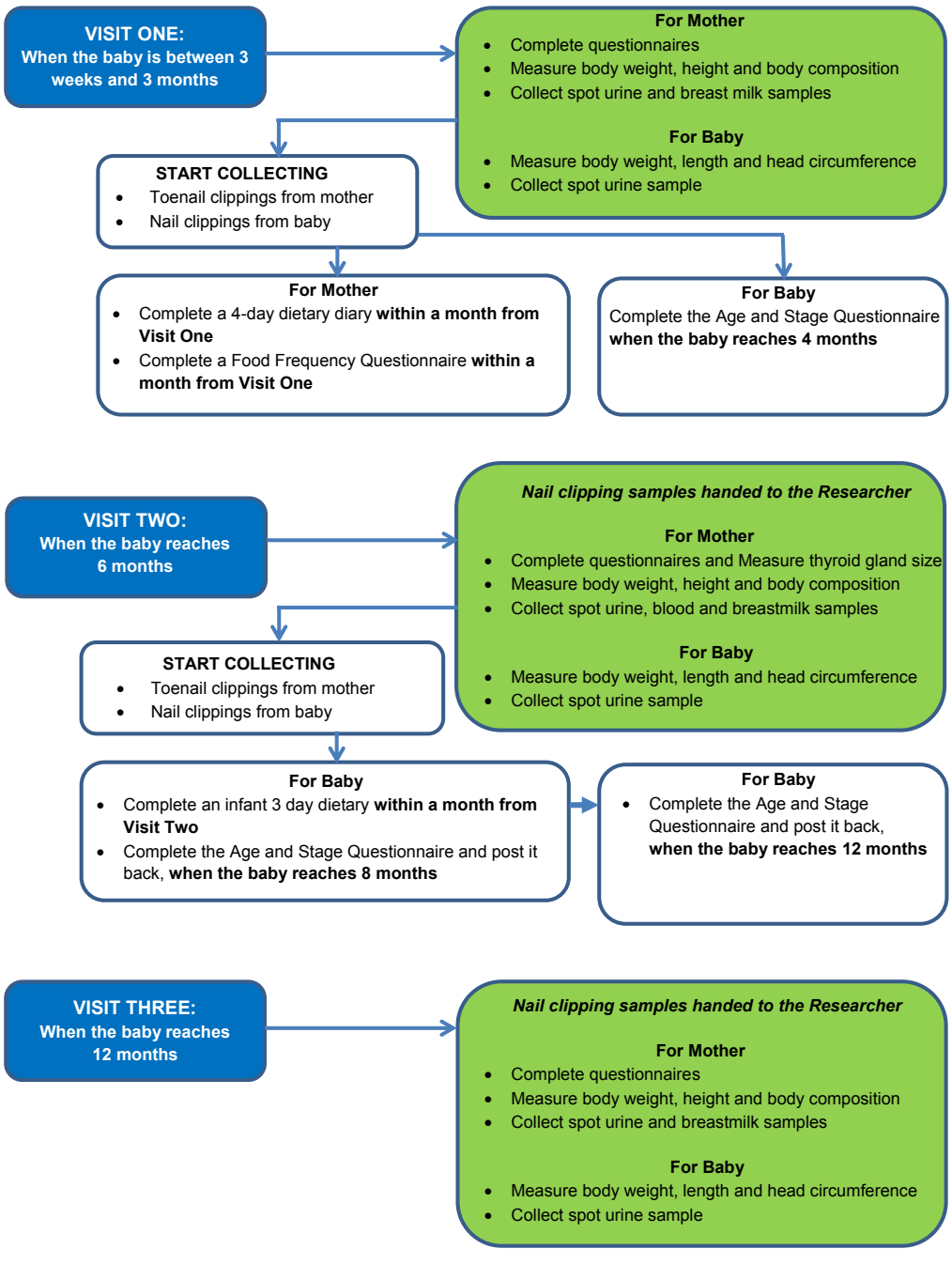
If you feel you would like to talk to a Māori health support person, please contact:

Dr Maureen Holdaway
Associate Director, Research Centre for Maori Health & Development
Telephone: +64 (06) 356 9099 ext. 85092
Email: M.A.Holdaway@massey.ac.nz

You can also contact the health and disability ethics committee (HDEC) that approval this study on:

Phone: 0800 4 ETHICS
Email: hdecs@moh.govt.nz

MINI STUDY FLOW CHART



Appendix I: MINI Study Consent Form

MINI Study - Consent Form

Please tick to indicate you consent to the following

I have been given sufficient time to consider whether or not to participate in this study.		
I have had the opportunity to use a legal representative, whanau/ family support or a friend to help me ask questions and understand the study.		
I am satisfied with the answers I have been given regarding the study and I have a copy of this consent form and information sheet.		
I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time without this affecting my medical care.		
I consent to the research staff collecting and processing my information, including information about my health.		
If I decide to withdraw from the study, I agree that the information collected about me up to the point when I withdraw may continue to be processed.	Yes ☐	No ☐
I consent to my GP or current provider being informed about my participation in the study and of any significant abnormal results obtained during the study.	Yes ☐	No ☐
I understand that my participation in this study is confidential and that no material, which could identify me personally, will be used in any reports on this study.		
I know who to contact if I have any questions about the study in general.		
I wish the nail clippings to be returned to me after analysis	Yes ☐	No ☐
I wish to receive a summary of the results from the study.	Yes ☐	No ☐

Declaration by participant:

Participant's name: _____

Signature: _____ Date: _____

Declaration by a member of the research team:

I have given a verbal explanation of the research project to the participant, and have answered fully any of the participant's questions concerning this study..

I believe that the participant fully understands the details of this study and has given informed consent to participate.

Researcher's name: _____

Signature: _____ Date: _____

Appendix J: MINI Study Health Screening Questionnaire

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Code: _____

MINI Study - Mother and Infant Nutrition Investigation

Date of visit: _____ Day_____ Month_____ Year

First name_____ Surname_____

Primary Contact Address

Street number and name:

Suburb:

City:

Postcode [if known]:

Primary Contact Phone Number(s)

Email address

Secondary Contact information

Street number and name:

Suburb:

City:

Postcode [if known]:

Subject Identifier

This page will be detached from remainder of the questionnaire at the end of the interview. Confidential information will be stored separately.

Participants information

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Code: _____

MINI Study - Mother and Infant Nutrition Investigation

Date of visit: _____ Day_____ Month_____ Year

Health Screening Questionnaire

Thank you volunteering to take part in this study. I would like to ask you a few questions to check that you are a suitable subject and provide you with an opportunity to ask any questions that you may have about the study.

What is your age?

Are you currently breastfeeding?

When was your baby born?

Do you have any contagious blood borne disease, eg. Hepatitis A or HIV?

Do you currently have any medical conditions?

Have you ever been diagnosed with thyroid disease such as thyroid enlargement or goiter/ hyperthyroidism/ hypothyroidism?

If yes, are you currently receiving any treatment or consuming medication containing iodine? Or, are you now fully recovered?

Are you taking iodine contain supplements due to other reasons rather than pregnancy or lactation?

Are you taking any other medication? ***If yes, can you please indicate the type or name of the medication(s) that you are taking?***

Does your baby have any health complications, eg. Preterm?

Preferred method of contact:

Appendix K: Supplementary Results

Bland-Altman plots for validity

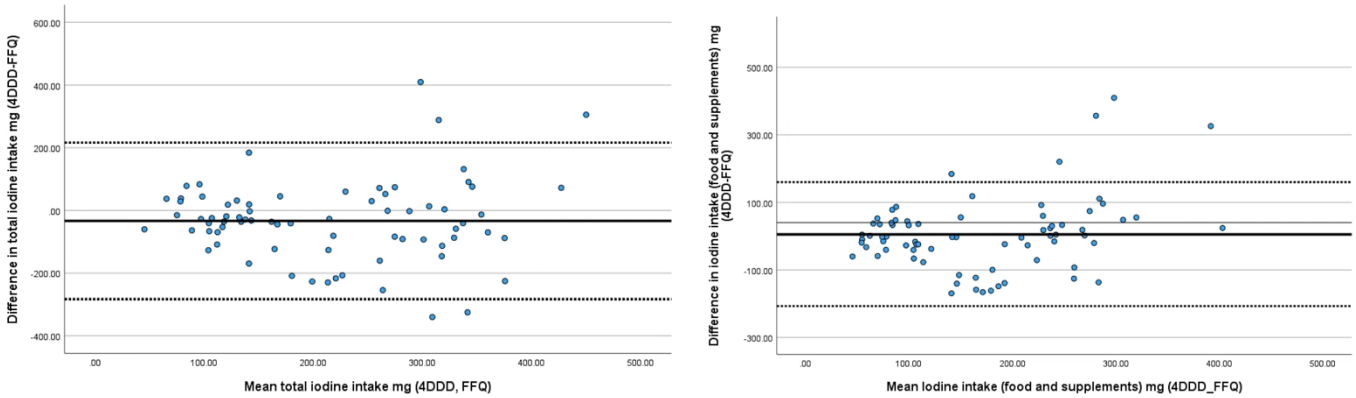


Figure 5. 1 Bland-Altman plot of the agreement for iodine intake (food, supplements, and salt), iodine intake (food and supplements).