# Maternal thyroid function, postnatal depression, the intake and status of iodine, selenium, and iron in postpartum women and their infants

A thesis presented in partial fulfilment of the requirements for the degree of

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# Abstract

**Background:** Thyroid dysfunction is a common health issue in women, with a higher prevalence found in postpartum women. Postnatal depression (PND) is a maternal health issue which can exacerbate negative health effects on their newborns. Iodine, selenium, and iron are three essential nutrients for the synthesis of thyroid hormones. Historically, dietary insufficiency of iodine and selenium exist in New Zealand. To improve iodine status, the New Zealand government introduced mandatory fortification of bread with iodised salt (2009), and recommended iodine supplementation (150 μg/day) for all pregnant and breastfeeding women (2010). Mostly, the iron status of postpartum women in New Zealand is rarely medically examined, unless high levels of blood loss during childbirth are recorded.

**Objectives:** The overall aim of this PhD thesis was to investigate maternal thyroid function, postnatal depression, and the intake and status of iodine, selenium and iron in mothers and infants during their first postpartum year.

**Method:** This observational longitudinal cohort study was conducted in Palmerston North, New Zealand, from June 2016 to December 2017. Mother-infant pairs attended study visits at three, six and twelve months postpartum (3MPP, 6MPP, and 12MPP). Online questionnaires investigated maternal iodine knowledge, supplement use, mode of infant feeding, and sociodemographic characteristics. Weighed four-day dietary diary, with urine/blood/breastmilk samples, were taken to measure maternal iodine, selenium, and iron intake/status. Infant iodine and selenium concentrations were determined in spot urine samples. The Edinburgh Postnatal Depression Scale was used to screen for PND. At 6MPP, serum thyroid hormones [free triiodothyronine, free thyroxine, thyroid stimulating hormone (TSH), thyroglobulin (Tg) and anti-Tg and thyroid peroxidase antibodies] and thyroid volume were measured.

**Results:** At 3MPP, 87 breastfeeding mother-infant pairs were recruited, followed up at 6MPP (n = 78) and 12MPP (n = 71). At 6MPP, 18% of women had thyroid dysfunction.

Median total thyroid volume was 6.1 mL. Median (p25, p75) Tg was 11.4 (8.6, 18.6) μg/L, above 10 μg/L. Median maternal plasma selenium was 105.8 (95.6, 115.3) μg/L; 23% (17/74) being below 95 μg/L; with 4% of women experiencing iron deficiency without anaemia. Women with marginally lower plasma selenium were 1.14% times more likely to have abnormal TSH concentrations.

Over the first postpartum year, maternal median urinary iodine concentration (MUIC) was 82 (46, 157) μg/L, 85 (43, 134) μg/L, and 95 (51, 169) μg/L, all below 100 μg/L; median BMIC was 69 (52, 119) μg/L, 59 (39, 108) μg/L, and 35 (26, 54) μg/L, all below the recommended 75 μg/L. Median maternal iodine intake was 151 (99, 285) μg/day, with 58% below the Estimated Average Requirement (EAR). At 3MPP, 46% of women took iodine-containing supplements, this reduced to 11% at 6MPP, and 6% at 12MPP. Women who used iodine-containing supplements had significantly higher MUIC (111 vs 68 μg/L) and BMIC (84 vs 62 μg/L) than non-users ($P < 0.001$). Infants fed by women using iodine-containing supplements had a higher MUIC (150 vs 86 μg/L, $P = 0.036$) than those of non-users. Infant MUIC at 3MPP [115 (69, 182) μg/L] and 6MPP [120 (60, 196) μg/L] were below 125 μg/L (suggested cut-point for iodine adequacy in infants). Median maternal selenium intake was 62 (51, 85) μg/day and 56% had intakes below the EAR. Median infant selenium intakes at 3MPP and 6MPP were 9 and 8 μg/day. Median maternal urinary selenium concentrations were 22, 22, and 26 μg/L across three time points, respectively. The highest prevalence of minor depression was observed in women with mean plasma selenium at 106 μg/L.

**Conclusions:** A high prevalence of thyroid dysfunction was observed in a cohort of postpartum women who were iodine deficient, with suboptimal selenium intake, but having mostly adequate iron status. Women with low plasma selenium were likely to experience thyroid dysfunction. Iodine deficiency of lactating women remains, particularly for those who did not use iodine-containing supplements. The low use of iodine-containing supplements is concerning during later breastfeeding. Maternal selenium intake/status was suboptimal. Relation between selenium status and risk of PND was inconclusive. Iodine/selenium intake and status of infants were suboptimal.

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# Abbreviations

| | |
|---|---|
| AI | Adequate Intake |
| Anti-Tg | Anti-Thyroglobulin antibodies |
| Anti - TPO | Anti-thyroid peroxidase |
| AP diameter | Anteroposterior diameter |
| ASQ | Ages and Stages Questionnaire |
| BIA | Bioelectrical Impedance Analysis |
| BMIC | Breastmilk iodine concentration |
| BMSC | Breastmilk selenium concentration |
| CRM | Certified reference material |
| CRP | C-reactive protein |
| CMIA | Chemiluminescent microparticle immunoassay |
| CV | Coefficient of variance |
| 4DDD | four-day diet diary |
| DMT | Divalent metal ion transporter |
| EAR | Estimated Average Requirement |
| EBF | Exclusively breastfeeding |
| EPND | Edinburgh postnatal depression scale |
| FFQ | Food Frequency Questionnaire |
| $fT_4$ | free Thyroxine |
| $fT_3$ | free Triiodothyronines |
| GPs | General practitioners |
| GPx | Glutathione peroxidases |
| HAZ | Height-for-age Z-score |
| Hb | Haemoglobin |
| ICCIDD | International Council for Control of Iodine Deficiency Disorders |
| ICP-MS | Inductively coupled plasma mass spectrometry |
| ID | Iron deficiency |
| IDA | Iron deficiency anaemia |
| IQR | Interquartile range |

| | |
|---|---|
| MINI | Mother and infant nutrition investigation |
| MPP | Month postpartum |
| MUIC | Median urinary iodine concentration |
| MUSC | Median urinary selenium concentration |
| NBF | None-breastfeeding |
| NIS | Sodium-iodide (Na/I) symporter |
| NZ | New Zealand |
| PBF | Partially breastfeeding |
| PND | Postnatal depression |
| PPT | Postpartum thyroiditis |
| SD | Standard Deviation |
| SPSS | Statistical Package for the Social Sciences |
| RDI | Recommended Dietary Intake |
| sTfR | Soluble transferrin receptor |
| SF | Serum ferritin |
| $T_3$ | Triiodothyronine |
| $T_4$ | Thyroxine |
| Tg | Thyroglobulin |
| Thg-DIT | Thyroglobulin 3,5-diiodotyrosine |
| Thg-MIT | Thyroglobulin 3-monoiodotyrosine |
| TPO | Thyroid peroxidase |
| TSH | Thyroid stimulating hormone |
| TRH | Thyroid releasing hormone |
| UIC | Urinary iodine concentration |
| UL | Upper level of intake |
| UNICEF | United Nations Children's Fund |
| USA | United States of America |
| USC | Urinary selenium concentration |
| WAZ | Weight-for-age Z-score |
| WHO | World Health Organization |

# List of Articles and Conference Presentation

In this thesis, the following six articles have been written in manuscript format being incorporated as different chapters. Therefore, in some cases, there may be repetition.

| | |
|---|---|
| **Article I** | Jin, Y.; Coad, J.; Weber, JL.; Thomson, JS.; Brough, L. Selenium intake in iodine-deficient pregnant and breastfeeding women in New Zealand. Nutrients 2019, 11, 69<br><br>*Incorporated as Chapter 3* |
| **Article II** | Jin, Y.; Coad, J.; Zhou, SJ.; Skeaff, S.; Benn, C.; Kim, N.; Pond, RL.; Brough, L. Mother and Infant Nutrition Investigation in New Zealand (MINI Project): Protocol for an observational longitudinal cohort study. JMIR Research Protocols. 2020, 9, 8<br><br>*Incorporated as Chapter 4* |
| **Article III** | Jin, Y.; Coad, J.; Zhou, SJ.; Skeaff, S.; Ramilan, T. Brough, L. Predictors of thyroid dysfunction in postpartum women with suboptimal iodine and selenium and adequate iron status. (Submitted to the Clinical Endocrinology, in review).<br><br>*Incorporated as Chapter 5* |
| **Article IV** | Jin, Y.; Coad, J.; Zhou, SJ.; Skeaff, S.; Benn, C.; Brough, L. Use of iodine supplements by breastfeeding mothers is associated with better maternal and infant iodine status. Biological Trace Element Research. 2020.online<br><br>*Incorporated as Chapter 6* |
| **Article V** | Jin, Y.; Coad, J.; Zhou, SJ.; Skeaff, S.; Brough, L. Iodine status of postpartum women and their infants aged 3, 6 and 12 months - Mother and Infant Nutrition Investigation (MINI). (Submitted to British Journal of Nutrition, in review).<br><br>*Incorporated as Chapter 7* |
| **Article VI** | Jin, Y.; Coad, J.; Pond, R.; Kim, N.; Brough, L. Selenium intake and status of postpartum women and postnatal depression during the first year after childbirth in New Zealand – Mother and Infant Nutrition Investigation (MINI) Study. Journal of Trace Elements in Medicine and Biology. 2020. 61 (126503).<br><br>*Incorporated as Chapter 8* |

# Conference Presentation

**Oral Presentation.**

Jin, Y.; Coad, J.; Brough, L. Inadequate iodine status in breastfeeding women and their infants in Aotearoa New Zealand- Mother and Infant Nutrition Investigation (MINI). The 13th European Nutrition Conference – FENS 2019: The Convention Centre Dublin, Ireland, 15-18 October.

**Receiving the "Zonta Club of Manawatu Science and Technology Award" (July 2019) to support presenting at the above conference – FENS 2019, Dublin, Ireland**

# Chapter 1 Introduction

*This Chapter introduces the background of the thesis and provides justification for the research work undertaken. It also presents the study aims, objectives and hypotheses of the thesis. The thesis structure is outlined, then concluded by a table describing each researcher's individual contribution.*

## 1.1 Introduction

Women's health during the first year after childbirth is important for their newborn infants' growth and development, as well as for their own long-term health. Maternal endocrine hormones adjust to support foetal development during the pregnancy period, and continuous readjustment is needed to maintain homeostasis after parturition. Thyroid hormones play an essential role in metabolic function within the human body, and are responsible for adequate myelination, neuron maturation, and central nervous system development in a human developing brain (1). These hormones are tightly controlled by the hypothalamic-pituitary axis with a negative feedback system. Women with limited thyroidal reserves or iodine deficiency may develop thyroid dysfunction during the postpartum period (postpartum thyroiditis) (2); this remains one of the most common endocrine disorders that postpartum women experience (3). In 2017, the American Thyroid Association reported from different studies the prevalence of postpartum thyroiditis ranged from 1.1% to 16.7% (4). Maternal thyroid dysfunction has been linked to the development of postnatal depression (3, 5), is weakly associated with reduced breastmilk production and milk-ejection reflex ("let-down") (4), and potentially affects the infant's neurodevelopment due to thyroid dependent processes, such as neural proliferation and differentiation(6).

Optimal thyroid function relies on adequate biosynthesis of thyroid hormones, which depends on three trace elements: iodine, selenium, and iron (7, 8). Mostly, pregnant women made dietary changes to support foetal health, such as increase dietary intakes of iron and calcium, either through carefully selected food choices for food cravings and safety, or dietary supplements encouraged by health care providers (9). However, after childbirth, the centre of attention shifts considerably to nurture the newborn infants, rather than remaining with the new mothers themselves. There is some published research investigated breastfeeding women and their infants' iodine and selenium status. There are only a few studies which has examined women's iron status postnatally. Maintaining optimal maternal nutrition during the post-partum period is

important to enable women to meet both the demands of their breastfed infant and their own needs. More importantly, having adequate iodine, selenium and iron intakes ensures optimal thyroid function.

In New Zealand, both iodine and selenium are insufficiently contained within local food supplies. Iodine deficiency was a concern in New Zealand in the early 20th century, but its prevalence was mostly reduced through the introduction of iodised salt from the 1930s (10). However, mild iodine deficiency re-emerged in the 1990s (9, 11, 12, 13, 14). This has resulted in mandatory addition of iodised salt in commercially made New Zealand bread and bread products, introduced in September 2009 (15); and in 2010, a New Zealand government subsidised iodine only supplement (150 μg/day) was recommended to all pregnant and breastfeeding women (16). Subsequent studies have demonstrated that most school children (17, 18) and adults (19, 20) in New Zealand have achieved adequate iodine intake and status, but the status of pregnant and breastfeeding women is unclear.

Low selenium status in New Zealand has been partially reversed by increased consumption of imported flour from Australia (which generally has higher selenium concentrations than flour produced in New Zealand) (21, 22). Previous local research, which investigated selenium status in postpartum women and their infants in 2001 (23), found these women were at that time at risk of selenium deficiency (after the importation of flour from Australia). With continuous dietary practice changes in the recent decade (including consuming plant-based diets) (24), limited research has since investigated selenium intake and the status of postpartum women and their newborn infants in New Zealand.

Health professionals closely monitor the iron status of women during pregnancy (25). However, after childbirth, management of iron status can be inconsistent (25). The general belief is that lactational amenorrhea lowers the risk of iron deficiency without anaemia (ID) or iron deficiency anaemia (IDA) for breastfeeding women, and their

iron status recovers (26). Consequently, the iron status of postpartum women remains largely unreported, due mainly to infrequent research studies.

Most previous research in women of childbearing age (27) and postmenopausal women (28) have investigated iodine, selenium, and iron intake/status separately, or a combination of any two of them. However, there is little up to date data on investigating the status of iodine, selenium, and iron together, as all three nutrients are important for the synthesis of thyroid hormones.

## 1.2 Thyroid function and its relation to iodine, selenium, and iron

Studies have shown mild-to-moderate iodine deficiency could impact on thyroid hormone synthesis (29). Both insufficient and excess iodine intake may be associated with a development of goitre (enlarged thyroid gland) and thyroid autoimmune disease (30). Both deficient and excessive selenium intake have been linked to increased risk of thyroid disorders in epidemiological studies (31). Lower serum selenium was reported to increase risk for goitre, or possible development of multiple thyroid nodules, in women, rather than men (32, 33). However, the effects of selenium supplementation on thyroid status are not yet conclusive after several selenium supplementation studies on elderly populations (34, 35, 36), and pregnant women (37, 38). The degree of effect from selenium supplementation would depend on selenium status at the baseline, and the dose and duration of selenium supplementation implemented.

Both animal and human studies have suggested ID or IDA may impair thyroid metabolism (39, 40, 41). A series of intervention study investigated the thyroid responses of goitrous-children to one oral dose of iodised oil containing 200 mg iodine, where, after 30 weeks, prevalence of goitre reduced dramatically within non-anaemic children (12%) when compared to children with IDA (64%) (42); a following-up trial provided oral iron supplementation (60 mg, 4 times/week for 12 weeks) to children with IDA and observed a further reduction in goitre prevalence to 20%. It

suggested that goitrous children with IDA may benefit from concurrent supplementation of iodine and iron (42).

Most women return to normal thyroid function at three months postpartum after prenatal increase of thyroid hormone production (4). However, globally, postpartum women experience abnormalities in thyroid function at twice the prevalence of the general population (43). Pregnant women with positive antithyroid peroxidase antibodies (TPOAb) are at increased risk of experiencing postpartum thyroiditis (PPT). Although other micronutrients, such as zinc, copper, vitamins A and D, may also support thyroid hormone synthesis, only iodine, selenium and iron are preeminently essential, and therefore, all three were selected for this study among postpartum women in New Zealand.

## 1.3 Iodine intake and status in New Zealand

Iodine is the key component of thyroid hormones which play an essential role in brain development (44). Animal studies suggest that normal brain development requires optimal thyroid hormones and adequate supply of iodine (39). The World Health Organization (WHO) has estimated that iodine deficiency is the most preventable cause of compromised neurodevelopment which affects around two billion people spread over 130 countries. Most research has been focused on children's development (45, 46) rather than younger infants (aged less than two years old). Alongside the recognised importance of prenatal development, achieving adequate iodine status is crucial for postnatal development (47).

Due to the persistent inadequacy of iodine intake, a government subsidised iodine only supplement (150 μg/day) has been recommended for all breastfeeding women in New Zealand since the year 2010. The effectiveness of such prophylaxis was investigated in 2011, and a pilot study indicated a low awareness of the government subsidised iodine supplements for breastfeeding women, with only a 35% reported usage of any iodine-containing supplements (48). Investigation of the current use of

iodine-containing supplements by breastfeeding women, and their impact on the iodine status of women and their infants is warranted as the most recent data was collected in 2011 and was only a small sample.

## 1.4 Selenium and postnatal depression

A meta-analysis of 59 studies from across the globe has reported that 13% of women are affected by postnatal depression (49). A 1999 postal survey of New Zealand women at four months postpartum (n = 224) found a higher prevalence of PND (16%) than the global average (50). The same study reported that approximately one quarter of affected women were still depressed when their infant reached their first birthday. Mothers were often reluctant to seek available help (50), which echoes a recent report from the 2015 New Zealand new mothers' mental health cross-sectional survey (n = 805) (51). This survey indicated a prevalence of PND was 14% and those women were less likely to seek help (51). Such under-diagnosed and, at times, untreated mental health conditions (50, 51) affect both the mother and their children's ongoing cognitive, emotional and behavioural development (52).

In addition to socioeconomic status (53), dietary patterns (54), and thyroid hormones, micronutrient deficiencies have been suggested to adversely affect mental health (7, 55, 56). Results from a longitudinal prospective Canadian study (Alberta Pregnancy Outcomes and Nutrition, n = 475) found that women who used selenium supplementation during their pregnancy experienced a lower risk of having future postpartum depressive symptoms (57), but details on the dose, frequency and duration of selenium supplements were unreported, and neither was selenium status assessed. A few studies have examined women's selenium status in relation to the risk of PND, particularly during the first postpartum year.

## 1.5 Study aims and hypotheses

The overall aim of this PhD thesis is to investigate maternal thyroid function, postnatal depression, and the intake/status of iodine, selenium and iron among mothers and infants during their first postpartum year.

**Study 1 – Secondary analysis on the Mother and Baby Study**

Study 1 was a secondary analysis of existing data collected from the 2009/2011 Mother and Baby Study. The study aim was to investigate selenium intakes of pregnant, lactating women, and their breastfed infants, in Palmerston North, in the North Island of New Zealand.

**Study 2 - Mother and Infant Nutrition Investigation (MINI)**

Study 2 was a longitudinal observational study following a cohort of mother-infant pairs at three, six and twelve months postpartum. The study's aims included an investigation of thyroid function, and its relation to the intake and status of iodine, selenium, and iron of postpartum women, conjointly with the risk of developing postnatal depression.

The objectives of Study 2 were to:

- investigate the role of iodine, selenium, and iron status in maternal thyroid function;
- compare breastfeeding women's iodine intake and status in iodine-containing supplement users and non-users;
- examine iodine and selenium intake and status of mothers and their infants at three, six and twelve months postpartum;
- determine maternal selenium status in relation to the risk of postnatal depression during the first postpartum year;
- explore maternal iron status at six months postpartum.

***Hypothesis 1*:** Suboptimal iodine, selenium or iron status will impede maternal thyroid function at six months postpartum.

***Hypothesis 2*:** Breastfeeding women who used iodine-containing supplements will achieve better iodine status for themselves and their infants.

***Hypothesis 3*:** Postpartum women and their infants will remain iodine deficient, despite two New Zealand government initiatives to improve iodine status.

***Hypothesis 4*:** Suboptimal selenium intake and status exist among New Zealand postpartum women.

***Hypothesis 5*:** Selenium intakes of breastfed infants aged three and six months are suboptimal.

***Hypothesis 6*:** Low plasma selenium will increase the risk of postnatal depression at three, six and twelve months postpartum.

***Hypothesis 7*:** High prevalence of iron deficiency and iron deficiency anaemia exist in women at six months postpartum.

## 1.6 Structure of thesis

The thesis is presented in nine chapters. Six of those chapters are either published or forthcoming articles, with two submitted for publication to peer review journals which are currently under review. All six articles are written in the style of the journals they have been submitted to, however, referencing and text formatting have been modified to match the flow of the thesis.

This thesis begins with an introduction (Chapter One). Chapter Two provides a review of literature on the prevalence of thyroid dysfunction in postpartum women, and the central roles of iodine, selenium, and iron in thyroid function. It reviews the intake and status of the three nutrients in published studies both in New Zealand and globally, and summarises literature focused on biological factors associated with an increased risk of postnatal depression. Perceived research gaps are highlighted. This

is followed by Chapter Three which was published in the journal ***Nutrients***, and presents the results from an analysis of the data collected in the 2009/2011 Mother and Baby study (Study 1), which explored selenium intake in iodine-deficient pregnant and breastfeeding women in Palmerston North, in the North Island of New Zealand. Chapter Four presents the MINI (Study 2) research method which has been published in the ***JMIR (Journal of Medical Internet Research) Research Protocols***, detailing the study cohort recruitment and data collection procedure, together with methods used in this longitudinal follow-up cohort study investigation.

Results from the MINI study (Study 2) are presented in Chapters Five (Hypotheses 1 and 7), Six (Hypothesis 2), Seven (Hypothesis 3), and Eight (Hypotheses 4, 5 and 6). The findings of thyroid function, including thyroid hormone concentrations and thyroid volumes of postpartum women, together with their interactions with iodine, selenium, and iron status, are discussed in Chapter Five. Chapter Six focuses on iodine intake and the status of breastfeeding women who used iodine-containing supplements, making comparisons to those who were non-users; in addition, current iodine knowledge and practice are discussed. This chapter has been published in the ***Biological Trace Element Research***. Chapter Seven examines iodine status of postpartum women and their infants at three, six and twelve months postpartum. Further, it explores iodine partitioning in urine and breastmilk among exclusively breastfeeding women at three months after parturition. Chapter Eight which has been published in the ***Journal of Trace Elements in Medicine and Biology***, investigates maternal and infant selenium intake and status during the first postpartum year and the relationship with postnatal depression and anxiety.

The final discussion in Chapter Nine presents a summary of the main findings, strengths, and limitations from two studies, and considerations of the significant contribution and relevance to the subject field. Final concluding points are summarised, together with relevant implications for public health practice and the identification of future research priorities.

## 1.7 Researchers' contributions

| Researchers | Contributions |
|---|---|
| Ying Jin<br>PhD researcher | Responsible for all aspects of the study including: Ethics approval, Recruitment and participants management, Data collection, Statistical analysis, and Writing of the thesis.<br>Responsible for all aspects of the articles including: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing-Original draft preparation, Writing-reviewing and Editing, and Submission. |
| Dr Louise Brough<br>Primary supervisor | Conceptualization, Methodology, Funding acquisition, Formal analysis, Reviewing all articles, and Reviewing the thesis. |
| Professor Jane Coad<br>Secondary supervisor | Conceptualization, Methodology, Funding acquisition, Reviewing all articles, and Reviewing the thesis. |
| Professor Sheila Skeaff<br>Collaborator | Resources and Reviewing<br>(Articles II, III, IV and V) |
| Dr Shao J Zhou<br>Collaborator | Resources and Reviewing<br>(Articles II, III, IV and V) |
| Dr Rachael Pond<br>Collaborator | Funding acquisition, Advice on postnatal depression and Ages and Stages Questionnaires, and Reviewing<br>(Articles II and VI) |
| Dr Nick D Kim<br>Collaborator | Funding acquisition, Advice on selenium and its analysis, and Reviewing<br>(Articles II and VI) |
| Dr Cheryl Benn<br>Collaborator | Methodology and Reviewing<br>(Articles II and IV) |
| Dr Thiagarajah Ramilan | Formal analysis and Reviewing<br>(Article III) |

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# Chapter 2 Review of the Literature

*Chapter 2 begins by outlining the framework of the literature review. It provides a detailed synthesis of important early and current published literature on the prevalence of thyroid dysfunction in postpartum women, the physiology of thyroid hormone regulation and synthesis, and the central roles of iodine, selenium, and iron in thyroid function.*

*This is followed by a review of each of the three individual micronutrients and their pivotal importance to health; it contains details relating to the intake and status of iodine, selenium and iron in published studies, both in New Zealand and globally, with specific reference to postpartum women and their newborn infants. The prevalence of postnatal depression worldwide, including New Zealand, is presented, and literature centred on inadequate thyroid hormones, selenium, and iron in relation to the risk of postnatal depression is reviewed.*

*Lastly, this review summarises notable research gaps across a wide research area. These feed into the research hypotheses and overall design of the Mother and Infant Nutrition Investigation (MINI). The research objectives of the MINI study aim to direct attention to such research gaps.*

## 2.1 Introduction

Thyroid dysfunction is a common health issue among women (1), with a higher prevalence found in postpartum women (2, 3). Most of what is understood regarding the role of thyroid hormones, their regulation, synthesis, and release in the body, comes from experiments with animals, which will be described first in detail. Current knowledge of the prevalence of thyroid dysfunction in postpartum women will be reviewed. A wealth of research has emerged investigating the central roles of micronutrients in optimal thyroid function. Specific literature in this section will give insight into the contributions made by iodine, selenium, and iron in maintaining adequate thyroid function.

Literature relating to iodine, selenium and iron intake and status will then follow, each in turn, within the global context. For each nutrient examined in the MINI study, literature involving postpartum women and their infants will be carefully reviewed. Literature discussing the impact of iodine deficiency on brain development also will be included.

One important step in investigating nutrient intake and status is the selection and justification of choosing assessment methods. This literature review includes dietary assessment and measurement of nutrient intake methods related to iodine, selenium, and iron. The advantages and limitations of a range of biological assessment methods of iodine, selenium and iron status will be discussed to clarify the choice of methods used in the MINI study.

It is well established that local food supplies in New Zealand contain low iodine and selenium, leading to low dietary intake (4). An abundance of literature has scrutinised knowledge gained in the study of iodine and selenium intake and status within New Zealand. This review will cover available major research work completed to date and emerging studies currently examining the effectiveness of two government iodine initiatives in New Zealand populations. These initiatives include mandatory fortification of all bread (except organic and unleavened) with iodised salt (2009) (5);

and the provision of subsidised 150 μg daily iodine supplement for all pregnant and breastfeeding women (2010) (6).

Finally, the literature review examines previous research regarding the prevalence of postnatal depression and the role played by micronutrients. The suspected role of thyroid function in affecting the risk of postnatal depression will also be discussed.

## 2.2 Thyroid function

Optimal thyroid function relies on the biosynthesis of adequate thyroid hormones, which are essential in maintaining the human body's metabolism, temperature (thermoregulation), and psychological mood (7). In a developing brain, thyroid hormones are responsible for adequate myelination, neuron cells maturation, and the central nervous system development (8). Three dietary minerals: iodine, selenium and iron play a pivotal role in the synthesis of thyroid hormones (9, 10). As one of the main research objectives, this thesis examines iodine, selenium, and iron status in relation to thyroid function.

### 2.2.1 Thyroid dysfunction in postpartum women

Thyroid function can be altered which results in hypothyroidism (underactive thyroid) or hyperthyroidism (overactive thyroid). A 2014 meta-analysis of 17 studies from European countries reported a higher prevalence of thyroid dysfunction in women than men, including both overt (6% vs 1%) and subclinical (8% vs 5%) categories (1). Globally, new mothers are more likely to experience thyroid dysfunction (7-23%), when compared to a prevalence of 3-4% in the general population (2, 3). Maternal hypothyroidism and hyperthyroidism have been suggested to negatively impact on breastmilk production and milk ejection reflex ("let-down") (11). Although evidence was weak, the American Thyroid Association continues to recommend thyroid function testing for those who experience poor lactation without obvious identifiable reasons (11).

Postpartum thyroiditis (PPT) refers to thyroid dysfunction occurring in the first year after parturition, including transient (temporary) hyperthyroidism, hypothyroidism, or both, excluding Graves' disease (11). The prevalence of PPT, ranging from 1.1% to 16.7%, has been reported by the American Thyroid Association in 2017 (11). Increased risk of PPT has been observed for those with other autoimmune conditions, such as diabetes mellitus type 1 (12). Women with positive thyroid peroxidase antibodies (TPOAb) in early pregnancy may have an 50% increased risk in developing PPT (13). It was postulated that the presence of thyroid antibodies such as TPOAb may link to an increased risk of developing PPT due to the activation of the complement system (14). Antenatal screening of TPOAb and/or antithyroglobulin antibody (TgAb) status have been suggested to be part of a cost-effective strategy in identifying risks for PPT (15). Measuring TgAb may be more sensitive in detecting thyroid autoimmune abnormalities although further evaluation on the usefulness in predicting PPT is required (16).

An 80-90% of women with PPT experience a transitional period of hypothyroidism and have normal thyroid hormones concentrations by the end of their first postpartum year (11). However, women with PPT might develop permanent hypothyroidism or experience PPT again in each subsequent pregnancy (16, 17). A large prospective study screened 4384 women at six and twelve months postpartum from an area of mild iodine deficiency in Southern Italy and reported the incidence of PPT was 3.9% (169/4384), and, of these, 54% (92/169) of women continued to be affected by hypothyroidism at the end of their first postpartum year (18). Combined data from 54 Welsh women who participated in three studies between 1983 and 1995 showed those women who recovered from the initial PPT had a 70% (9/13 examined) risk of experiencing a recurrence in a subsequent pregnancy (14). After examining a cohort of Iranian women with subclinical (abnormal TSH concentrations but fT4 within the normal reference range) and overt (abnormal TSH and fT4 concentrations) PPT, undergoing T4 therapy, Azizi found that slightly more women with subclinical PPT developed permanent thyroid dysfunction than those with overt PPT, although

it was non-significant (19). However, Azizi suggested that identifying both subclinical and overt cases of PPT early allows timely interventions. To the best of our knowledge, there are no current data available regarding the prevalence of postpartum thyroid dysfunction in New Zealand.

### 2.2.2 Thyroid hormone regulation and biosynthesis

The thyroid gland is a butterfly-shaped endocrine organ and consists of left and right lobes joined by the isthmus, which is located anterior to the trachea. This gland is responsible for synthesising and secreting thyroid hormones, including triiodothyronine ($T_3$) and thyroxine ($T_4$). Since the receptors for both $T_3$ and $T_4$ are abundant on most human body cells, thyroid hormones can stimulate protein synthesis, and promote the usage of lipids and glucose for adenosine triphosphate (ATP) production, thus effectively accelerating the human body's metabolism (20). To maintain the body's metabolic homeostasis, thyroid hormone synthesis and secretion are tightly regulated by the Hypothalamic-Pituitary-Axis. For example, low levels of thyroid hormones in the blood stimulate the hypothalamus to release a thyroidtropin-releasing hormone (TRH) which promotes the secretion of thyroid stimulating hormones (TSH) from the pituitary gland. An increased level of TSH stimulates the thyroid gland to secrete thyroid hormones controlled by a tightly regulated negative feedback mechanism; any increase of thyroid hormones detected in the blood will inhibit the secretion of both TRH and TSH (Figure 2.1). Therefore, the levels of thyroid hormones remain continually at an optimal range for body function (20).

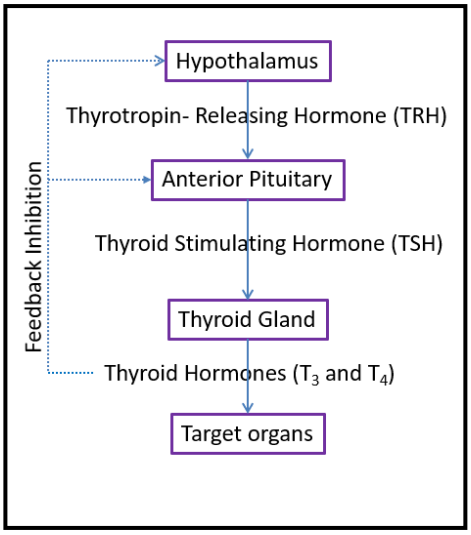



**Figure 2.1 Illustration of the regulation of thyroid hormone secretion**

Thyroid hormone synthesis involves a series of reactions which require several essential micronutrients, notably, iodine, selenium, and iron [Figure 2.2 (20)]. Initially, iodine absorbed from the diet is converted to iodide circulating in blood plasma. Iodide is actively transported into thyroid cells via a sodium-potassium ATPase pump. Upon entering the thyroid cells, iodide is oxidised into iodine by the reduction of hydrogen peroxide ($H_2O_2$). At this moment, iodine combines with tyrosine residues of thyroglobulin, a large glycoprotein, to produce precursors of thyroid hormones. If one iodine atom binds to a thyroglobulin, it produces monoiodotyrosine (Thg-MIT). If two iodine atoms bind, diiodotyrosine (Thg-DIT) is produced. The process of forming Thg-MIT and Thg-DIT is catalyzed by the thyroid peroxidase (TPO) which requires iron for its activity (21). Excess $H_2O_2$generated from thyroid hormone synthesis is neutralised by the actions of selenium-containing enzymes, such as glutathione peroxidase (GPx). Following this, Thg-MIT and Thg-DIT are then transported into a thyroid colloid where Thg-DIT condenses with another Thg-DIT molecule to form Thg-thyroxine (Thg-T4), or alternatively condenses with a Thg-MIT molecule to form Thg-triiodothyronine (Thg-T3).

Lastly, newly formed Thg-T4 and Thg-T3 are released to thyroid cells via exocytosis, and their amino acid components are removed before T3 and T4 are released into

blood circulation. The concentration of T4 in blood plasma is 40 times higher than that of T3, but T3 is the more metabolically active form of thyroid hormone. Most T3 and T4 are bound with transport proteins in blood; a very small portion circulates as free forms, which can actively bind with target cell receptors. At the target tissue, T4 is deiodinated into T3, with the removed iodine from T4 returned to blood for later use (20). To enable the conversion from T4 to T3, a selenium dependent iodothyronine 5'-deiodinase is required (20).

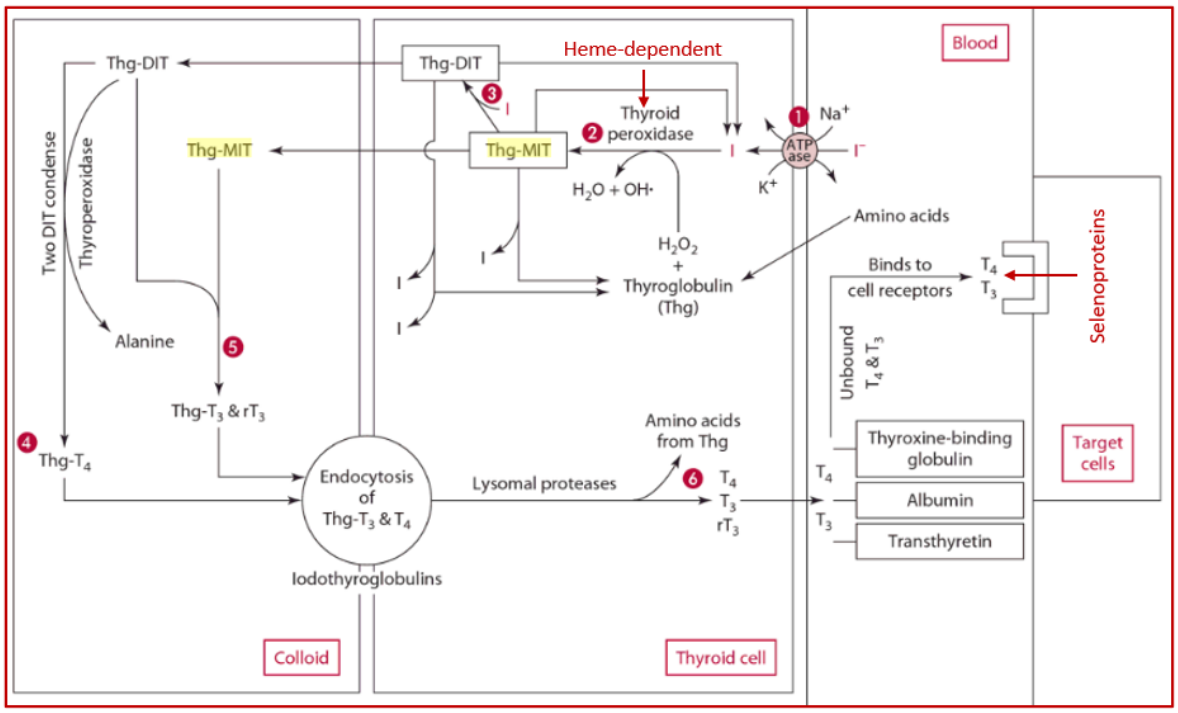



**Figure 2.2 Thyroid hormone synthesis pathways [Gropper, Smith, Groff, 2005 (20)]**

## 2.2.3 Contribution of iodine, selenium and iron to optimal thyroid function

### *2.2.3.1 Iodine in thyroid function*

Thyroid function can be impaired by deficient iodine intakes. A thyroid gland from a healthy adult resident in an iodine sufficient area can trap 60 μg of iodine per day to provide adequate thyroid hormone synthesis (22). At a low threshold of approximately 50 μg iodine intake per day, the thyroid gland also maintains adequate synthesis of

thyroid hormones (22). When iodine deficient, TSH secretion from the pituitary gland is raised, which increases the available sodium-iodide symporters on thyroid cells to allow a maximum iodine update. Consequently, renal clearance of iodine is reduced (23). However, when iodine deficiency is severe, the depleted iodine stores continuously stimulate the thyroid gland for hormone synthesis. This process may result in hypothyroidism and, at extreme levels, the development of a goitre (enlarged thyroid gland) via hyperplasia.

Thyroid function can be impaired by excessive iodine intakes. Generally, excessive iodine intake can be tolerant by euthyroid adults (up to 2g/day without any clinical signs) (24). The acute Wolff-Chaikoff effect (down regulation of NIS then decreasing organic binding of iodine to synthesise thyroid hormones) is described as a protective mechanism to prevent large quantities of iodine uptake. Most people escape from this effect without long-term adverse consequences, and their thyroid hormone productions resume (25). However, people with autoimmune thyroiditis, or history of PPT, continue to experience the Wolff-Chaikoff effect which results in hypothyroidism (24, 25). A five-year epidemiological survey investigated the incidences of thyroid diseases presenting in three communities in China (n= 3761) (24). Based on the median urinary iodine concentrations (MUIC), iodine status of three Chinese communities was classified as mildly iodine deficient (88 μg/L), more than adequate iodine (214 μg/L) and severe iodine excess (634 μg/L). This Chinese study found no significant differences in the cumulative incidence of hyperthyroidism (1.4%, 0.9%, and 0.8%, respectively, $P > 0.05$) related to their iodine status, indicating that high iodine intakes may not increase the risk of hyperthyroidism (26). However, excessive intakes were more likely to result in hypothyroidism as the incidence of subclinical hypothyroidism significantly increased from 0.2%, to 2.6% and 2.9% ($P < 0.001$) (27).

To correct iodine deficiency, mandatory legislation of the provision of iodised salt programs were reported in 108 countries prior to May 2018 (28). Increasing iodine intake from fortification in a mild-to-moderate iodine deficient population may

temporally increase the incidence of overt-hyperthyroidism [the Jod-Basedow effect (28)]. Possible explanations include the existence of autonomous thyroid nodules in the elderly population and the presence of autoimmune disease in young adults (23, 29). This phenomenon was demonstrated in Danish adults with mild-to-moderate iodine deficiency (MUIC ranged 45 -61 μg/L) by an increased incidence of hyperthyroidism from 102.8 (baseline in 1997-1998) to 140.7 cases per 100, 000 people annually soon after the introduction of iodised salt (2001-2002) (30). Further, a 20% increase in the incidence of overt-hypothyroidism was reported in a seven-year follow-up study in Denmark (the period of mandatory iodine fortification) within a mildly iodine deficient female population aged 20-59 years (Overall population MUIC 86 μg/L in Aalborg and 99 μg/L in Copenhagen) (31). It has been suggested to gradually introduce iodised salt fortification to an optimal level in a population of existing mild and moderate iodine deficiency (23). Even a small change of iodine intake may result in increasing or decreasing prevalence or incidence of thyroid dysfunction. This suggests the importance of continuously monitoring thyroid function after the establishment of an iodine fortification programme.

Iodine supplementation has been implemented to reduce iodine deficiency for pregnant and lactating women, as they are at greater risk of iodine deficiency due to increased requirement. A few interventional studies have evaluated its effects on the prevalence of PPT; however, the results are inconsistent. Swedish postpartum women in a 1990 interventional study were allocated into two treatment groups: 0.1 mg of levothyroxine daily for 38 weeks (n = 18), or a 150 μg/day iodine supplement for 40 weeks (n = 20), and a separate control group (n = 20) (32). Women in the iodine supplementation group developed more severe episodes of PPT when compared to the control, suggesting the extra iodine aggravated thyroid dysfunction, however the iodine status of these women was not examined at baseline. To further examine the effect of iodine supplementation, a randomised placebo-controlled trial was conducted in Denmark, a moderately iodine-deficient area (33). Euthyroid pregnant women (n = 72) were assigned into three groups: a 150 μg/day iodine supplementation

during both pregnancy and postpartum, or only during pregnancy, or given a placebo (supplement without iodine). No significant differences in PPT prevalence, severity, or duration among three groups was reported. Danish authors suggested both prenatal and postpartum iodine supplementation of 150 μg/day were safe (33). Findings may differ if these women at the beginning of the study were iodine deficient, a sudden increase of iodine may trigger the Wolff-Chaikoff effect which results in hypothyroidism (24, 25).

Enlarged thyroid and changes in thyroid hormone levels are related to iodine status. The prevalence of 5% or more goitres in school children may indicate a public health issue of iodine deficiency within a population, based on the WHO guideline updated in 2014 (34). A recent population study in Denmark [the Danish investigation on iodine intake and thyroid disease (The DanThyr)] found that the prevalence of goitre (thyroid volume more than 18 mL examined by ultrasound) was as high as 33% within women aged 60-65 years old who had mild-to-moderate iodine deficiency (29). Iodine fortification has greatly reduced the rates of thyroid enlargement in the Danish adults who live in areas of moderate iodine deficiency pre-fortification, when compared to those residing in the mild iodine deficiency areas (35). Similar effects were reported from a study in an Italian village fifteen years after the implementation of a voluntary iodised salt programme, with a lower goitre rate observed at 10% post-fortification compared with 34% within the same cohort pre-fortification (36). Thyroid hormone levels (serum T3 and T4) can remain within normal ranges despite of presenting mild-to-moderate iodine deficiency in a population (37); however, with the presence of moderate-to-severe iodine deficiency, TSH concentrations may be elevated, but would appear to be lower within a population demonstrating mild iodine deficiency (38). Furthermore, serum T4 decreases while T3 may be normal or slightly increased, because T3 is more likely to be secreted than T4 during iodine deficiency.

### *2.2.3.2 Selenium in thyroid function*

The thyroid gland is one of the endocrine tissues containing the highest level of selenium per mass unit (39), and a hierarchical system exists in the thyroid to maintain its selenium content, even in conditions of limited supply (40). The functional role of selenium rests with its integral part of specific enzymes - iodothyronine 5'-deiodinases (a family of selenocysteine-containing proteins). These enzymes then convert the inactive form of thyroid hormone ($T_4$) into active triiodothyronine ($T_3$), which is then subsequently released into blood circulation (20). Of the three types of deiodinase (type I, II and III), type I is the most sensitive to selenium deficiency (41). Selenium as a component of the glutathione peroxidase (GPx) protects the thyroid from oxidative damage (41).

Experimental studies in rats have been undertaken to determine the role of selenium deficiency interacting in iodine metabolism, which continues to affect thyroid function. An animal study from Dumont's group suggested selenium and iodine status both contribute in thyroid hormone synthesis but fail to model co-deficiency in thyroid degeneration (42). Their data reported selenium deficient rats had decreased thyroid GPx activity, but a protective effect from selenium deficiency was observed on the thyroid gland (reduced thyroid weight and increased serum $T_3$ and $T_4$) in the animal model (42). Further histological analysis on the thyroid tissue of rats having iodine and selenium co-deficiency, evidenced an increased thyroid fibrosis and proliferation of thyroidal epithelial cells. This phenomenon suggested the existence of a possible connection between selenium deficiency and myxedematous cretinism (a type of cretinism characterised by mental retardation and hypothyroidism) (43).

Previously, human evidence was reported of the coexistence of selenium deficiency and iodine deficiency in Zaire, Central Africa in the 1980s (44, 45, 46). In an area of high endemic goitre and cretinism, lower serum selenium and plasma GPx were recorded, compared to those residing in non-endemic areas. This presented evidence that selenium deficiency may modulate negative effects from any iodine deficiency.

Combined iodine and selenium deficiency may be an etiologic factor in the pathogenesis of endemic myxedematous cretinism, which remains highly prevalent in Central Africa (46, 47). During iodine deficiency, the production of hydrogen peroxide is increased due to the stimulation from TSH on the thyroid during hormone synthesis. Deficiency of selenium results in a negative impact of the GPx defense mechanism (protecting the thyroid gland from excessive hydrogen peroxide production). Concurrent selenium deficiency reduces the negative effect of low active thyroid hormone from iodine deficiency affecting the brain, the proposed diagram (Figure 2.3) suggests a mechanism for the development of myxedematous cretinism in combined selenium and iodine deficiency (41).

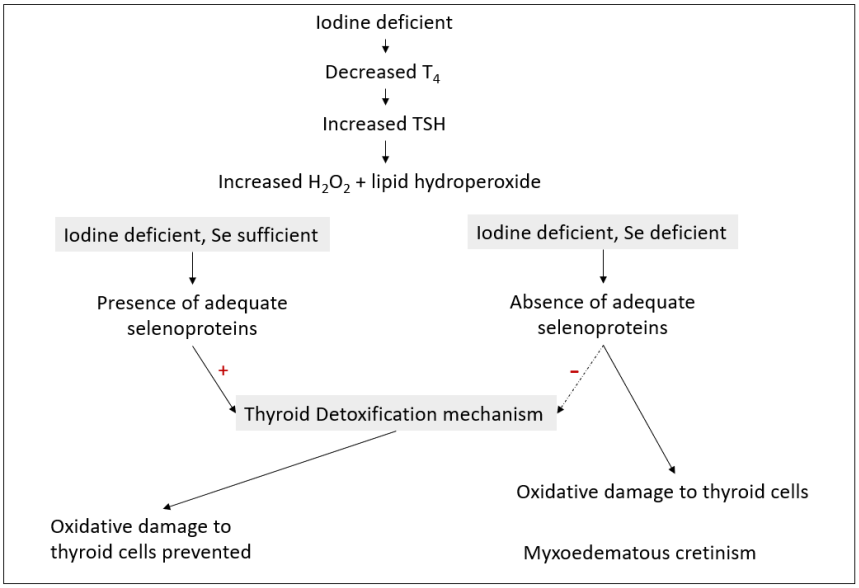



**Figure 2.3 A mechanism for the development of myxoedematous cretinism [Arthur, Beckett, Mitchell, 1999 (41)]**

Both cross-sectional and interventional studies have investigated the relationship between selenium status and thyroid function in adult, elderly, and pregnant populations. Within mild iodine deficiency areas, lower serum selenium was reported to increase the risk of enlarged thyroid glands or possible development of multiple thyroid nodules in women, rather than in men (48, 49). A small sample placebo-controlled Italian trial of elderly persons aged over 85 years (n = 36) found a marked decrease in serum total T4 (67 vs 62 nmol/L), but not in free T4 (9.4 to 9.3 pmol/L)

after supplementation of 100 μg selenium (sodium selenite) daily for three months, giving the baseline mean serum selenium as 66 μg/L, raised to 106 μg/L after supplementation (50). However, a randomised double-blinded placebo-controlled trial of selenium supplementation in UK elderly participants (n = 501) reported no significantly measurable beneficial effect on the conversion from T4 to T3, despite consumption of 100, 200 or 300 μg daily for six months (51). This observation could be explained by selenium status at baseline (mean plasma selenium of 91 μg/L) being less likely to impact on the activity of the iodothyronine deiodinases. Pregnant women with positive TPOAb are more likely to develop PPT, selenium supplementation may reduce such incidence by decreasing inflammation activity (52). A randomised placebo-controlled Italian study reported women in their first trimester with positive TPOAb had a lower incidence of PPTs (28.6%), compared to 48.6% in the non-supplementation group, after supplementing with selenium as selenomethionine at 200 μg/day during both pregnancy and their postpartum period; however, the result still warrants further examination concerning the aspect of reverse-effects after stopping supplementation (52). Of interest, study subjects were advised to use iodised salt, but iodine status was not measured and remained unconfirmed, as deficiency of both iodine and selenium may impair thyroid function.

Low levels of selenium are found in New Zealand soils, leading to low levels in the food supply (53). The effects of selenium on thyroid status have been examined in New Zealand following instances of inadequate selenium status reported by Robinson's trace element research group from Dunedin (53, 54). Based on the data from five different local studies of adults aged 18-68 living in the South Island, New Zealand, between 1993 and 2001 (55), it was suggested that selenium levels were borderline in maintaining optimal function of selenoproteins, as they exerted only a minor effect on serum T4 levels and T3:T4 ratios after supplementation. To explore further the effect of iodine and selenium supplementation on thyroid function, a randomised placebo-controlled trial was conducted in a group of moderately iodine deficient (MUIC as 48 μg/L) volunteers aged 60-80 years (n = 102) (56). The

participants were given a treatment of selenium solely (100 μg L-selenomethionine); or of 80 μg iodine solely; or with a combination of both iodine and selenium supplements; or a placebo for three months. No additional beneficial effect from either selenium supplementation in improving the thyroid hormone status was observed, apart from an increase in plasma selenium and whole blood GPx activity (56). The mean plasma selenium at baseline was 95 μg/L, so the deiodinases activities were likely maintained with less effect on thyroid function (56). Any effect of selenium supplementation on thyroid status among the elderly is yet to prove conclusive. Further studies are required to identify and understand as to what extent current selenium status might interact with mild iodine deficiency in relation to thyroid function.

#### *2.2.3.3 Iron in thyroid function*

Iron is another essential trace element believed to play an important role in thyroid hormone metabolism. Animal studies of iron deficient anaemic rats by Beard, Tobin and Green (1989) suggested an impaired response in thyroid hormone regulation, where, for example, rats failed to respond appropriately to TRH stimulation, resulting in decreased plasma levels of $T_3$ and $T_4$ (57). In addition, decreased activity of hepatic iodothyronine 5'-deiodinase was observed in severely affected iron deficient anaemic rats (57), which may result in a decreased production of $T_3$. Furthermore, a randomly controlled trial provided strong evidence that the activity of TPO (a haem-iron dependent enzyme which enables the addition of iodine to tyrosine residue for thyroid hormone synthesis) reduced markedly within food-induced iron deficient anaemic male rats when compared to the controls in situ. The study used pair-fed controls to distinguish lower effects of serum thyroid hormones from reduced food intake due to iron deficiency anaemia (IDA) (58).

Human studies have also suggested that iron deficiency without anaemia (ID) or IDA may impair thyroid metabolism. For example, lower $T_3$, $T_4$ and increased TSH were observed in subjects with moderate-to-severe IDA (haemoglobin < 75 g/l) (59) and

mild IDA (75 g/l < haemoglobin < 110 g/l) (60). Observational studies from Ethiopia and Philippines found no differences in goitre rate assessed by palpation of an enlarged thyroid gland (one of the measures for iodine deficiency) between anaemic and non-anaemic children (61, 62), pregnant and lactating women (62). However, anaemic status in these two studies were not classified as IDA which may due to deficiencies in folate, zinc, or vitamin B 12. Research has investigated the interaction between iron deficiency and goitre rate. An Iranian school children study reported a higher goitre rate (3.8 times, measured by palpation) in children with low serum ferritin (< 12 μg/dL), when compared to those with normal serum ferritin (63).

One interventional study in the Ivory Coast investigated the thyroid responses of goitrous-children with IDA to iodine supplementation. Firstly, goitrous-children aged 6-12 years were supplemented with one dose of 200 mg oral iodine; after 30 weeks, children without anaemia gained a higher percentage of thyroid volume reduction (45%) compared to children with IDA (22%), and the prevalence of goitre was 12% compared to 64% in children with IDA (64). This result indicated IDA may reduce the effectiveness of iodine prophylaxis. From the beginning of 30 weeks, children with IDA were provided with 60 mg oral iron supplementation (4 times/week for 12 weeks), and, at 65 weeks, thyroid volume reduction was observed to be 38%; the overall prevalence of goitre was reduced to 20% when compared to non-anaemic children (12%) (65). However, the diets of the children were not controlled, although the nutritional status (height, weight, and growth) was measured during the trial; there was no control group arranged for anaemic children without iron treatment. By the end of the trial, the group of goitrous children with IDA remained at a higher goitre rate, and the research results suggest that goitrous children with IDA may benefit from concurrent supplementation of both iodine and iron.

A subsequent nine month randomised double-blind control trial by the Zimmermann group provided dual-fortified salt (DFS) containing 25 μg iodine and 1mg iron per gram of salt to iodised salt given to 6-15 years-old goitrous Moroccan children who had a high prevalence of IDA at 25-35% (such fortification achieving 150-250μg/day

iodine or 7-12 mg/day iron) (66). The study found a higher reduction of thyroid volume (38%) at 40 weeks among children receiving DFS, when compared to the iodised salt-only group (18%) (66). This dual fortification trial confirmed that concurrent supplementation with iron and iodine may achieve a reduction of both goitrous and iron deficiency in such vulnerable groups of children. A recent study of reproductive aged women with IDA in Turkey (a country with mild iodine deficiency) subsequent to a six-month oral iron supplementation (100 mg elemental iron per capsule, twice a day) reported a decreased mean thyroid volume at 13 mL, when compared to the baseline of 17 mL (67). However, selenium status remained untested during the clinic trials on school children and women detailed above.

Although other micronutrients such as zinc, copper, vitamin A and vitamin D may also support thyroid hormone synthesis. In New Zealand, traditionally, iodine and selenium intakes are inadequate, and iron deficiency and iron deficiency anaemia are present in women at reproductive ages. Therefore, these three nutrients were selected for investigation among postpartum women in this MINI study.

## 2.3 Iodine

Iodine plays a role in adequate thyroid hormone synthesis enabling optimal thyroid function in the body. Iodine deficiency can result in a broad spectrum of disorders – Iodine Deficiency Disorders (IDD), including hypothyroidism, hyperthyroidism, goitre, physical development retardation, and cretinism) (68, 69). IDD are prevalent in many countries of the world and is mostly associated with inadequate dietary iodine intake, which affects all ages and stages of human life.

### 2.3.1 Iodine and health

Knowledge of the impact of iodine deficiency on brain development has expanded over the last 30 years (70). Animal studies have confirmed that normal brain development requires optimal thyroid hormones which rely on an adequate supply of iodine (70). In humans, iodine is essential for growth and development during the

prenatal period and the first two to three years postnatally. Iodine deficiency has resulted in 11.2 million individuals in the world experiencing cretinism (a condition of impaired growth, defects of hearing and speech, and brain damage) and a further 43 million people with different levels of mental impairment in the 1990s (71). The latest evaluation of global iodine nutrition (2020) from 194 WHO members reported that 28 countries remained iodine deficient (72). Where there is severe iodine deficiency occurring in utero, children and adolescents may develop neurological cretinism (severe mental retardation with euthyroid) rather than hypothyroid cretinism (dwarfism, myxedema and skeletal retardation with severe hypothyroidism) (22); even mild iodine deficiency in uterus can have long-term negative effects on brain development, such as poor academic performance in school children (73, 74).

Meta-analysis enquiries from 18 observational and experimental studies (published between 1969 and 1990) reported populations aged from infancy to adulthood who were living in iodine deficient areas have been reported to have a mean of 13.5 intelligence quotient (IQ) points lower than those present in non-iodine deficient groups, based on the assumption that the scores in both groups exhibited normal distributions (75). Authors of this meta-analysis questioned whether the score for the iodine-deficient group was able to be normally distributed and called for further studies. Another meta-analysis based on 37 studies of Chinese children aged under 16 years, reported a reduction of 12.5 IQ points among children being exposed to severe iodine deficiency, when compared to children living in iodine sufficient areas (76). Results from the Chinese meta-analysis also showed an increment of the IQ points (12 Raven points or 17.25 Binet IQ points) of children born 3.5 years after the introduction of an iodine supplementation programme (75).

A number of human cohort studies have reported that mild or moderate iodine deficiency during pregnancy has resulted in impaired/delayed neurodevelopment in utero and the consequences were detected at infancy (77) and also at ages of eight to nine years (73, 74). A 2018 population-based prospective cohort study of 851 Norwegian mother-infants measured maternal urinary iodine concentrations (UIC)

and infant neurodevelopment at 6, 12 and 18 months (assessed by Bayley Scales of Infant and Toddler Development, Bayley-III) (77). The authors found evidence of poor communication skills by infants aged 18 months who were born to mothers with mild iodine deficiency. Studies from two other large cohorts – the Avon Longitudinal Study of Parents and Children (ALSPAC) in the United Kingdom (UK) (73) and the Gestational Iodine Cohort Australia (74) investigated maternal UIC during early gestation in relation to children's later educational outcomes. In the first study, children's long-term neurodevelopment was assessed by verbal IQ at eight years of age, reading accuracy, and comprehension assessed at nine years of age (UK); and, in the second study, school-based Australian academic performance was assessed based on the Australian numeracy and literacy curriculum, only literacy measures were associated with maternal UIC. In both studies, many adjustments were made including maternal and paternal education and occupation, home and family situations, life events as well as maternal age, gestational age, and birth weight, however, maternal IQ was not adjusted for evaluating neurodevelopment of their children. In contrast, another Australian study in Adelaide, after adjusting for maternal IQ, failed to find any association between maternal UIC and children's neurodevelopment outcomes (Bayley-III) at the age of 18 months (78). This Adelaide study also found children born to women with both high (≥ 391 μg/day) and low (< 220 μg/day) iodine intake in pregnancy (assessed by a validated iodine food frequency questionnaire) showed poor neurodevelopment outcomes (cognitive, language and motor scores) (78). A large Norwegian study (MoBa) found that inadequate maternal iodine intake during early gestation (assessed from a food frequency questionnaire) was associated with delayed children's language skills at the age of three years (79) and poor school performance at the age of eight years based on maternal self-reporting scores (78). These epidemiological studies discussed are well-designed and have shown clear association between mild iodine deficiency in utero and impaired neurodevelopment remained after adjusting a comprehensive range of confounders. However, assessing neurodevelopment is challenging as different tools (both objective

and subjective) are used. Iodine is required for the neurodevelopment during gestation, it is inconclusive how much iodine is sufficient or excessive in terms of optimal neurodevelopment.

Most research studies have focused on school children's development rather than investigating younger infants (those less than two years old). The World Health Organization estimates that iodine deficiency is the most preventable cause of brain damage which affects around two billion people living in over 130 countries (70). The most recent up-to-date meta-analysis combined data from population-based birth cohorts - Generation R (Netherlands), INMA (Spain), and ALSPAC (United Kingdom) between 1990 to 2008, using iodine-to-creatinine ratio (μg/g) as an indicator of maternal iodine status during the first trimester. Findings suggest that infants born to women with a low urinary iodine creatinine ratio until 14 weeks gestation, were more likely to have a low verbal IQ score (80). The researchers indicated achieving optimal iodine status during the early gestational period remains critical in neurodevelopment. However, the first 1000 days of life is designated a "window of opportunity" for optimal growth and development (81). Therefore, preconception period and the first two years after birth are also crucial in support optimal neurodevelopment by achieving adequate iodine supply, which may require further research investigation.

### 2.3.2 Iodine intake and status

An individual's iodine intake depends on the type and amount of iodine-containing food and/or water regularly consumed. The personal choice of consuming iodised salt can vary considerably from day to day, excluding those living in countries where iodisation of salt is mandatory. Food sources and water may vary in their iodine content due to natural reasons, such as region of origin, seasonal variation, and fortified food versus unmodified (82). Regular consumption of iodine-containing supplements may contribute to daily iodine intake.

### *2.3.2.1 Food sources*

Iodine content in food can vary between countries and differ when sourced from different regions of the same country. In general, fish, seafood, and plant material from the sea (high concentration of iodide in sea water, 50 µg/Liter) usually contain higher levels of iodine in comparison to other food sources, such as poultry and meat. Milk and dairy products are the major contributors to iodine intake due to their moderate concentration of iodine and frequent daily consumption in many Westernised populations. The iodine contribution from plant and animal foods depends on iodine taken up from local soil, water used for irrigation, fertilisers and livestock feed. However, soils in New Zealand typically provides low levels of available iodine, which results in low iodine concentrations in locally grown foods (83).

In New Zealand, important dietary sources are fish and shellfish containing varied iodine concentrations ranging from 24 to 130 µg/100 g (84). The next highest iodine concentration occurs in eggs, due to most poultry feeds being supplemented by trace minerals including different farming types (battery, free-range, barn laid) (85). From the 1960s, iodophors were used to sanitise milking machines and related equipment in the local dairy industry; as a result, milk and other dairy products made a significant contribution to the iodine intake within a typical New Zealand diet. Concern over contamination led to reduced use of iodophors from the early 1970s (86), for example, the iodine concentration in trim milk decreased from 0.44 mg/kg in 1978 to 0.10 mg/kg in 2009 (84). The reduced iodine content in milk has contributed to the more recent inadequate iodine intake re-emerging within New Zealand in the 1990s (83, 85).

Table 2.1 illustrates the changes of iodine concentration in food from the 1920s through to the 2000s, reported from an ongoing series of the New Zealand Total Diet Survey (84, 87, 88, 89). The changes observed over the time (between 1920s and later times) may be that recent methods are more sensitive in detecting iodine, or changes in iodine concentrations in the samples of individual food items assessed, for example,

lamb-mutton composite sample examined in 2016, or mandatory fortification of iodised salt in bread.

Iodised table salt was introduced from the 1930s in New Zealand, which had reduced the goitre rates by the 1950s (85). However, public health messages have led to reduced salt consumption to prevent high blood pressure and reduce the risks of other chronic medical conditions. Another possible contributor is the recent shifting from traditional home cooking with iodised salt to commercially prepared food containing non-iodised salt (90). Modern dietary choices have expanded to include other conventional salts containing minimum levels of iodine, for example, pink salt, rock salt, and herbal salt, reducing the iodised salt intake even further. A proportionate to population sized survey of New Zealand school children from Dunedin and Wellington found half of the children did not use iodised salt at the table, and this was coupled with a low use of iodised salt in cooking within the household (85). Although iodised salt is not considered to be a major contributor to iodine intake, for those who regularly consume iodised salt, its contribution cannot be ignored.

More recently, to combat iodine deficiency, mandatory fortification of bread with iodised salt (25-65 mg iodine/kg salt) was introduced in New Zealand in September 2009, applying to all commercial bread other than organic and unleavened. A post-fortification study from the Ministry for Primary Industries measured iodine concentrations in all bread in 2012 (91). The median iodine concentration of bread requiring fortification ranged between 30.1 and 45.9 µg/100 g; this was in contrast to 1.6 µg/100 g within bread without fortification (91). Comparison of fortified bread with pre-fortification values based on the Concise New Zealand Food Composition Tables 8$^{th}$ edition (2009) (92) (Table 2.2) shows a dramatic increase of iodine concentration in the listed bread and bread products post-fortification.

**Table 2.1 Iodine content (µg/kg) of New Zealand foods**

| Food | 1920s | 2003-2004 | 2009 | 2016 |
|---|---|---|---|---|
| **Animal food** | | | | |
| Fish, canned | 360 | 130 | 180.1 | 182 |
| Fish, fresh | 50-96 | 216 | 237 | 211 |
| Oysters | 880 | 970 | 1298 | 1171 |
| Meat, bacon | 15 | 11 | 16 | 35 |
| Beef | 13 | 7-10 | 9-16 | 5-11 |
| Mutton/Lamb | 10 | 32 | 10.6 | 294-297[a] |
| Eggs | 94 | 519 | 465 | 544 |
| Dairy, milk | 20 | 86-96 | 94-103 | 78 |
| Cheese | 31 | 63 | 61.4 | 44 |
| **Cereal foods** | | | | |
| Bread, white | 2 | 3.2 | 2 | 388[b] |
| Bread, brown | 12 | 4.8 | 13 | 414[b] |
| Rice, white | < 8 | 3.1 | 1.2 | 2-11 |
| **Fruits and vegetables** | | | | |
| Apple | 6 | 2 | 14 | 3 |
| Orange | 2 | 2.1 | 2.3 | 1 |
| Tomato | 8 | 1.1 | 1.0 | 0-2 |
| Carrot | 8 | 4.4 | 17 | 7 |
| Kumara | 3 | 2.9 | 2.3 | 3-4 |
| Lettuce | 17 | 10.9 | 2.2 | 1-2 |
| Potato with skin | 22 | 6.8 | 6.1 | 4-5 |
| Silver beet | 27 | 27 | 16 | 9 |

[a] This result was from a single lamb-mutton composite sample, and sheep may receive iodine supplementation, but meat from this area is rarely for human consumption.

[b] Data presented is after the mandatory fortification of bread with iodised salt in New Zealand.

**Table 2.2 Iodine content (µg/100 g) of New Zealand foods (pre- and post-fortification).**

| Bread and bread product category | Median Iodine (µg/100 g) Pre-fortification | Median Iodine (µg/100 g) Post-fortification |
|---|---|---|
| Bread rolls, white | 0.3 | 53.5 |
| Bread rolls, mixed grain | 1.2 | 41.1 |
| English style muffin | n/a | 43.9 |
| Fibre white | n/a | 49.3 |
| Hamburger buns | n/a | 51.1 |
| Organic and unleavened | n/a | 1.6 |
| Pita bread | 0.5 | 27.7 |
| Pizza bases | 0.3 | 0.5 |
| White | 0.3 | 41.3 |
| Whole meal | 0.5 | 38.8 |

After the government iodine fortification initiative, studies of school children in New Zealand found that bread contributed 28% of iodine intake immediately after fortification (93), it increased to 51% six years later (94). These studies concluded that mandatory fortification successfully improved iodine intake assisting New Zealand school children to reach adequate levels. The data from New Zealand Ministry for Primary Industries in 2016 have suggested that iodine intake for women of childbearing age also achieved adequacy (95). Consequently, bread has been estimated currently to be the primary dietary source of iodine now contributing to most adult diets, as evidenced in the 2016 New Zealand Total Diet Survey (89).

However, such fortification may not always provide sufficient iodine for pregnant and breastfeeding women due to their increased requirements. To assist, in 2010, the use of government subsidised iodine-only supplements (150 µg/day) was recommended to all pregnant and breastfeeding women in New Zealand (96). A 2011 study in Palmerston North, New Zealand, indicated that there was a low awareness of this supplementation initiative for breastfeeding women, with only 35% of the women reporting use of any iodine-containing supplements (97). Low use of iodine-

containing supplements among both pregnant and lactating women was similarly reported in the United States (98). Recently, a cross-sectional study of over 110 pregnant Australians reported that only 58% consumed iodine-containing supplements (150, 200, 250 or 300 µg/day) (99), which was similar to 66% reported by a Western Australian study of 425 pregnant participants in 2012/2013 (ranging from 38-500 µg per day) (100). In New Zealand, government subsidised iodine-only supplements can be purchased on prescription and a range of dietary supplements containing iodine are available over the counter. Consumers, however, easily may become confused as to which may be the most appropriate product for their own personal circumstances. Women on a tight budget may not be able to afford the cost of government subsidised iodine-only supplements outside the free antenatal care period of six weeks after birth as offered by their registered General Practitioners (GPs). A New Zealand supplementation study reported that 13% of women who were prescribed with government subsidised iodine-only supplements did not fill their individual prescription (need to be paid) (101). Further investigation is warranted to determine current use and barriers to use iodine-containing supplements and their effect on iodine intake.

#### *2.3.2.2 Dietary inhibitors of iodine metabolism - goitrogens*

Goitrogenic compounds can block iodine uptake by the thyroid gland, thus interfering with thyroid hormone synthesis, release and utilisation. These compounds are found in several natural food items. High consumption of cruciferous vegetables such as cabbage, kale, broccoli, cauliflower, turnip, brussels sprouts, cassava, lima beans, linseed, sorghum, and sweet potato are associated with a higher consumption of goitrogens. Diets rich in these foods may contribute to an increased risk of goitre and other IDD among people who reside permanently in iodine-deficient areas (22, 102). Isoflavones are other substances which may impair TPO activity and thus inhibit thyroid hormone formation (103). The main dietary sources of isoflavones include soy-based food, peas, and millet. In animal models, it has been shown that soy's anti-thyroid effects are more marked when iodine deficiency exists (104). In humans,

infants fed with soy-formula without added iodine have been shown to develop goitre or hypothyroidism which can be alleviated and corrected by iodine supplementation (104). However, isoflavones are believed to have negligible effects on the thyroid function of healthy adults (22, 103), but their impact on other groups, such as people who have mild iodine deficiency, or those who have an extremely high level of consumption of soy products, remains unclear (105).

In addition, another goitrogenic exposure is tobacco smoke. It is believed that thiocyanate content in tobacco smoke decreases breastmilk iodine concentrations in women who smoke during their pregnancy (106), or during lactation (107). This phenomenon was explained by smokers accumulating thiocyanate in their blood and tissues, which inhibit the sodium-iodide symporters (NIS) in the mammary gland and result in blocking iodine from being secreted into breastmilk. Overall, it is important to assess goitrogenic food consumption/tobacco smoke exposure when evaluating iodine intake, since the negative effects from goitrogenic factors may depend on the quantities of consumption/exposure and their background iodine intake.

#### *2.3.2.3 Requirement of iodine intake*

The Estimated Average Requirement (EAR) of iodine for adult men and non-pregnant non-lactating women are 100 μg/day to adequately maintain iodine in blood plasma and sufficient storage in the thyroid gland (86). The Nutrient Reference Values (NRVs) for Australia and New Zealand recommends the EAR and Recommended Dietary Intake (RDI) are 160 μg/day and 220 μg/day respectively for pregnant women (86) (Table 2.3).

During lactation, iodine is required to fulfil maternal thyroid function, and be secreted into breastmilk to ensure adequate iodine for optimal infant thyroid function. Studies have suggested that the mammary glands are able to concentrate 20-50 times more iodine than maternal blood due to the active NIS (108). The secretion of iodine into breastmilk is influenced by prolactin, oxytocin and oestradiol (108). A lactating woman may secrete 75-200 μg iodine a day via 0.5-1.11 L breastmilk daily up until six

months postpartum; thus, the daily iodine requirement is estimated at 225-350 μg/day (71). However, the NRVs from Australia and New Zealand suggested an extra 90 μg/day secretion of iodine throughout breastmilk and suggest 190 μg/day as the EAR value for lactating mothers (86) and an RDI of 270 μg/day (Table 2.3).

Breastfed infants depend on the iodine content in breastmilk for thyroid function and to build up reserves in their thyroid gland during the first six months of life (108). There are slight differences in the recommendations for adequate intake of infants aged 0-6 months among advice from the WHO (109), the Institute of Medicine (IOM) (110), and the NRVs from Australia and New Zealand. For example, the NRVs assume an average breastmilk intake of 0.78 L/day and an average iodine concentration in breastmilk at 115 μg/L, and this suggests that 90 μg/day is adequate to ensure a positive iodine balance in growing infants (86), when compared with the higher IOM recommendation of 110 μg/day (109, 110).

For a population to have a very low prevalence of inadequate dietary intake, the mean/median intake should be above the RDI; while the percentage below the EAR approximates the proportion that is at risk of dietary inadequacy, according to the EAR cut-point method (111, 112).

Table 2.3 Dietary Reference Intakes for iodine (ug/day) by age or population group

| Age or population group | NRVs Australia and NZ (86) | | Institute of Medicine (110) | | World Health Organization (WHO) (109) |
|---|---|---|---|---|---|
| Infant 0-6 months | 90 (AI) | | 110 (AI) | | 90 |
| Infant 7-12 months | 110 (AI) | | 130 (AI) | | 90 |
| Children 1-8 yr | 65 (EAR) | 90 (RDI) | 65 (EAR) | 90 (RDA) | 120 |
| Children 9-13 yr | 75 (EAR) | 120 (RDI) | 73 (EAR) | 120 (RDA) | 120 |
| Children 14-18 yr | 95 (EAR) | 150 (RDI) | 95 (EAR) | 150 (RDA) | 150 |
| Adults >19 yr | 100 (EAR) | 150 (RDI) | 95 (EAR) | 150 (RDA) | 150 |
| Pregnancy | 160 (EAR) | 220 (RDI) | 160 (EAR) | 220 (RDA) | 250 |
| Lactation | 190 (EAR) | 270 (RDI) | 209 (EAR) | 290 (RDA) | 250 |

AI = Adequate Intake; RDA = Recommended Dietary Allowance.

### *2.3.2.4 Assessment of iodine intake*

The following discussion highlights how iodine intake can be assessed by using several dietary assessment methods, including estimated/weighed food records (dietary diaries), food frequency questionnaires (FFQs) and repeated 24-hour dietary recalls.

Food records are used to measure actual food intake at the time of consumption rather than relying on respondents' memory. Weighed food records are commonly treated as a gold standard method to accurately assess the dietary intake and can be frequently used as a validation tool for other methods (113, 114). However, it adds a huge burden to respondents as they are required to record all food items including names, brands, preparation methods, and accurately measured quantities at the time of consumption over a specific given period (115). It is more expensive to administer since adequate measurement equipment needs to be used as well as hard copies of the food record instruction booklets. It can be difficult when respondents dine at restaurants, buy takeaways, or simplify the recording by altering their normal diet. Furthermore, motivated study subjects are more likely to collect precise dietary information. Most

recently, since the availability of mobile-based devices increased dramatically, new approaches have been developed to assess dietary record digitally, for example e-CA (an electronic mobile-based food record) (116). Such a tool has been suggested by researchers to reduce respondents' burden and food data entry errors. However, further validation studies are required to assess and confirm their frequent usability.

FFQs have been developed and validated to assess iodine habitual intake in a range of populations in different countries as it reduces burden on respondents and is less expensive to administer, when compared to dietary recalls and food records. High iodine content food items and foods containing iodine that are known to be frequently consumed by a certain population are included in the FFQ. Frequency is assessed by providing categories based on days, weeks, and months. Standard portion sizes are usually well-defined in a questionnaire so that total iodine intake per day can be estimated. FFQs are either administered by a personal interviewer or made available as a self-completion online or paper-based questionnaire. For instance, researchers have used iodine specific FFQs to assess dietary iodine intake among New Zealand school children (117) and adults (118), and pregnant women in Australia (113). However, information collected from FFQs relies heavily on respondents' memory and cognitive ability to record retrospective data (115). Consequently, the overall accuracy of FFQs needs to be validated in conjunction with other more accurate dietary methods or biomarkers, for example, urinary iodine concentration or serum thyroglobulin

The 24-hour recall is another dietary method carrying a low respondent burden and limited opportunity to change diet, especially if respondents are not pre-warned of the interview. However, this approach requires respondents having a good memory and some suitable ability to estimate portion sizes, along with an experienced interviewer to conduct the procedure. Extra assistance in estimating portion sizes is usually given by using photographs of foods, food models and providing household measuring cups, spoons and rulers which may all improve the quality of dietary data collected via 24-hour dietary recalls (119). This method has been often used with large representative population studies to assess average usual intake, for example, the New

Zealand National Nutrition Surveys (120) and the U.S. National Health and Nutrition Examination Survey (121).

Despite the strengths and weaknesses of each dietary assessment method as described above, both under- and over-reporting remain of concern. To eliminate under-reporting, the Goldberg cut-off method is commonly used to compare an individual's energy intake with their basal metabolic rate based on age, weight, gender; excepting those who are purposely restricting food intake to manage body weight (122). Here, it is also important to note that adequate administration of any dietary assessment method and appropriate nutrient levels in an up-to-date food composition table is essential to achieve relatively adequate dietary intake data.

It can be challenging to select an appropriate dietary assessment method which best suits the objectives of the study and a specific participant group, and to then balance the respondents' burden with the required accuracy of the data collection. In the current study, FFQs were carefully selected to collect habitual iodine and selenium intake data. Weighed food records (dietary diaries) were used to evaluate participants' current whole dietary intake, including iodine, selenium, and iron intake. The food records also may be used to validate the iodine-selenium specific FFQ which was adapted from one previously used in an Australian study (113).

There are further challenges in estimating iodine intake. Due to a wide coverage of the iodised salt fortification programme, iodised salt may be a major contributor to overall iodine intake. However, it is difficult to accurately measure iodised salt consumption; accordingly, often a set amount is usually added. In New Zealand, 48 µg iodine [equivalent to the consumption of 1 g discretionary iodised salt (48 mg iodine/kg salt)] was added to an individual's daily dietary intake (118), for those who reported regular use of iodised salt at the table, or in cooking. Although the Concise New Zealand Food Composition Tables (12$^{th}$ Ed) has included iodine content in food items (90), the iodine content in bread has yet to be updated (91). In addition, this database has not been updated frequently to match the fast-changing food items more

recently available in the market and contains mostly data from the United Kingdom and the United States. Iodine content in those overseas databases may not accurately reflect local New Zealand food items. A call for a well-constructed iodine food content national database (including major dietary contributors from food, beverages, and supplements) has been made to improve the understanding of iodine intake patterns for both individuals and wider populations (90).

### *2.3.2.5 Assessment of iodine status*

#### Measurement of urinary iodine

Iodine absorbed through the diet is excreted mostly via urine, and the urinary iodine content reflects recent iodine intake. Iodine concentrations in spot urine (single void) samples (expressed as UIC μg/L) and a 24-hour urinary collection (expressed as UIE μg/day) can be measured to evaluate iodine status. Both measures may not be used as an indicator of an individual' iodine status due to the variations of daily (123) and seasonal iodine intake (124). Iodine concentration in spot urine samples are also influenced by fluid intake (125, 126) and diurnal iodine secretion (127). Therefore, a single UIC value is limited in assessing an individual's current iodine status. Ten repeated spot urine samples of UIC are needed to obtain an accurate measure of individual iodine intake (128). In comparison to spot urine iodine concentration, the 24-hour urinary iodine excretion is not affected by diurnal and hydration variations over the day of measurement, but daily variation and seasonal difference of intakes remain. Obtaining a complete collection of urine over a single 24-hour period is challenging, due to the personal inconvenience for subjects and difficulty in ensuring accuracy, particularly in field research settings. Proving that compliance criteria have been fully met over a 24-hour period, urine sample collection is not easy to monitor, as subjects may unintentionally miss completing some collections. Spot urine samples are easier to obtain and cause less respondent burden during data collection. Recently, age and gender adjusted iodine creatinine ratio has been recommended for use by adults to allow correction for hydration status (129, 130). For example, in a Danish

pregnant and lactating women's study, urine creatinine concentration was used to level out the fluctuations in the UIC caused by the timing of spot urine samples (131) and to estimate total 24-hour urinary iodine excretion (126).

At population levels, UIC is a useful biomarker when specimens are collected from a large representative sample of a target group to lessen any inter- and intra-individual variations. The median value of UIC (MUIC) as μg/L is more commonly published, since most urinary iodine values are not normally distributed. In this respect, the World Health Organization, International Council for Control of Iodine Deficiency Disorders (ICCIDD), and the United Nations Children's Fund (UNICEF) (2007) have summarised the epidemiological criteria for assessing iodine status based on the MUIC established for different subgroups [see Table 2.4, (132)]. Of note, lactating women, and children less than two years old, with MUIC less than 100 μg/L, will be classified as having insufficient iodine intake.

### Measurement of thyroid hormones

As part of the body's negative feedback system used to maintain homeostasis, decreased thyroid hormones ($T_3$ and $T_4$) trigger a higher secretion of TSH from the anterior pituitary gland directly into the blood. As inadequate iodine intake directly affects the thyroid function, thyroid hormone levels in serum are considered as functional measures. Increase or decrease in TSH, $T_3$ and $T_4$ are observed among iodine deficient populations, but concentrations can also overlap with the iodine sufficient populations, due to tight physiological homeostatic regulation (69). Therefore, serum $T_3$, $T_4$ and TSH are not considered sensitive indicators for assessing iodine status in adults (69). However, serum TSH has been suggested as a sensitive measure for newborn infants' iodine status (132).

**Table 2.4 Epidemiological criteria for assessing iodine nutrition for different groups (132)**

| MUIC (μg/L) | Iodine intake | |
|---|---|---|
| **Pregnant women** | | |
| < 150 | Insufficient | |
| 150-249 | Adequate | |
| 250-499 | Above requirements | |
| ≥ 500 | Excessive | |
| **Lactating women**[a] | | |
| < 100 | Insufficient | |
| ≥ 100 | Adequate | |
| **Children < 2 years of age** | | |
| < 100 | Insufficient | |
| ≥ 100 | Adequate | |
| **Children ≥ 6 years of age** | Iodine intake | Iodine Status |
| < 20 | Insufficient | Severe iodine deficiency |
| 20-49 | Insufficient | Moderate iodine deficiency |
| 50-99 | Insufficient | Mild iodine deficiency |
| 100-199 | Adequate | Adequate iodine nutrition |
| 200-299 | Above requirements | A slight risk of more than adequate intake in the overall population. |
| ≥ 300 | Excessive[b] | Risk of adverse health consequences (iodine-induced hyperthyroidism, autoimmune thyroid disease) |

[a] Although lactating women have the same requirement as pregnant women, the MUIC is lower because iodine is excreted in breastmilk.

[b] The term "excessive" means in excess of the amount required to prevent and control iodine deficiency.

Thyroglobulin (Tg) is a glycoprotein which plays an important role in the synthesis of thyroid hormones. Previously, the measurement of serum Tg has been used to monitor thyroid cancer treatment (133). A small amount of this protein (< 10 μg/L) is constantly present in the blood of healthy individuals with adequate iodine status. Serum Tg concentrations increase with enlarged or inflamed thyroid, or rising TSH

secretion (133, 134). Iodine supplementation studies suggest serum Tg responds rapidly to the changes in iodine supply (135, 136), which in turn indicates that serum Tg might be a more sensitive indicator than other thyroid hormone measurements (134, 137). Measuring serum Tg is acknowledged as being sensitive in assessing both iodine deficiency and excess at population levels (134, 137, 138). Measuring TgAb is recommended to avoid an underestimate of serum Tg (139). However, limitations exist in using serum Tg as a biomarker including the uncertain prevalence of TgAb in iodine deficiency, and large variations in assays for analysis and poor reproducibility which result in limited ability to establish normal ranges (140).

Research has attempted to establish a reference cut-off of serum Tg (< 13 μg/L) for iodine status in school-aged children (141), and suggests iodine sufficiency if less than 3% of the population have Tg > 40 μg/L. This cut-off has been validated in a 24-week supplementation study (150 μg/day) of an adult population in New Zealand (142). However, the effectiveness of the suggested cut-off for pregnant women remains unclear (143). For example, two recent observational cohort studies have investigated the use of serum Tg as a biomarker for iodine status among mildly iodine deficient pregnant women. One study in the United Kingdom of pregnant women (n = 230), reported a higher serum Tg (20 μg/L) in the group of women at 35 gestational weeks with the iodine-to-creatinine ratio < 150 μg/L, when compared to the others (16 μg/L); suggesting that serum Tg was a useful functional marker to evaluate iodine status during pregnancy (144). However, a study of 299 pregnant women in Greece did not support using serum Tg as a biomarker of iodine status during pregnancy, although serum Tg concentrations were weakly correlated to the UIC within mild iodine deficiency only in the third trimester, which suggests a possible stimulating effect from a decreased human chorionic gonadotropin (145). It remains debatable whether trimester specific serum Tg concentrations would be more useful in evaluating iodine status (143). No studies have investigated the value of Tg in evaluating the iodine status of lactating women.

### Measurement of the thyroid gland

Measurement of the thyroid gland size is another assessment method for evaluating iodine status. Palpation by experienced physicians is used to detect an enlarged thyroid gland (goitre). While palpation is non-invasive, the method is subjective and can result in a large inter-observer error. Palpation of goitre was considered insensitive and less specific in populations with mild iodine deficiency (140). Since the late 1990s, to improve diagnostic precision, ultrasound is used as a safe, quick and non-invasive technique in assessing thyroid gland size. However, ultrasound measurement on thyroid volume may introduce inter-observer variability due to subjective judgement being applied on finding and measuring the maximum diameters on the thyroid gland image, which indicates a standardisation of technique may increase the accuracy of sonographic measurement of thyroid volume (146). Possible intra-observer variations on the measure of children's thyroid glands also have been reported, but the mean value was relatively smaller for intra-observer (8.4%) than the inter-observer (30%) errors (147), which may result in difficulties in comparing outcomes to other studies.

There is often a long lag time taken to normalise the thyroid gland volume after adequate iodine intake is achieved (148). This is evident in the discrepancy shown in a normalisation of UICs and the remaining goitre rate after the introduction of the mandatory salt iodisation programme. A five-year consecutive study of children with moderate iodine deficiency in West Africa found that UICs were normalized within a year, with only an 8% reduction of the goitre rate; after four years, 29% of the children remained goitrous (148). A previous one-year study of South African children examined the short-term effectiveness of an iodised salt programme, and they found children achieved adequate intake based on the UIC, but the goitre rate assessed by palpation did not decline (149). Unlike UICs, which respond rapidly to changes in dietary iodine intake, thyroid volume changes primarily reflect historical iodine status. Therefore, when interpreting results from the measurement of thyroid volume, the current iodine status of the population should be taken into consideration.

The methods described above have been used in both observational and interventional studies to monitor iodine status among different population groups. In summary, the UIC values reflect recent iodine intake (within 24-hour periods), serum Tg displays a relatively slower response to dietary iodine intake, and the long-term iodine status being reflected in measuring thyroid volume.

### 2.3.3 Iodine intake and status in postpartum women and their infants

Iodine concentration in breastmilk is an additional indicator of iodine status/intake for lactating women and their breastfed infants. Breastfed infants rely on iodine from breastmilk to support their thyroid hormone synthesis, which is critical in their neurodevelopment (108). It is important that lactating mothers achieve adequate iodine intake. Hence, an increased expression of NIS raises iodide uptake by the mammary glands, culminating in an increased secretion of iodine in breastmilk (107). Maternal iodine intake needs to increase to allow for that secreted into breastmilk.

Several factors may affect breastmilk iodine concentrations (BMIC), including the stages of lactation (150); the timing of breastmilk collection; maternal iodine intake; maternal fluid intake (126), and exposure to cigarette smoking (107). A 2018 systematic review of five longitudinal studies of BMICs in non-supplemented women summarised that the BMIC dropped from colostrum (200-400 μg/L) to mature milk (100-150 μg/L) (151), and continually decreased until nine months after birth (152). A New Zealand study between 2004 and 2005 observed a significant reduction of mean BMIC from week one (43 μg/L) to week 24 postpartum (25 μg/L) for breastfeeding women not consuming iodine-containing supplements (150). In addition, maternal fluid intake has less influence on breastmilk iodine than the urinary iodine concentrations (126). Importantly, smoking has been suggested to impair the NIS transporter in the mammary gland due to the presence of thiocyanate; smoking mothers showed a much lower level of BMIC when compared to non-smokers (107).

Observational studies have been conducted on the iodine status of breastfeeding women among iodine-deficient and iodine-sufficient countries. A systematic review

included 42 published studies between 1964 and 2013 (153) and reported that iodine deficiency in lactating mothers remained irrespective of mandatory or voluntary fortification strategies implemented in their countries. For these reasons, authors supported the provision of 150 μg/day to pregnant and breastfeeding women (153). The table 2.5 summarises observational studies conducted in the past 10-year on postpartum women's iodine status (2009-2020). As shown, most of the countries listed have implemented either mandatory or voluntary salt iodisation programmes, with levels of iodisation between 20 and 70 mg iodine/kg salt. Denmark introduced the mandatory fortification of bread with iodised salt (9-25 μg/100 g bread) from 2000 (154); followed by Australia and New Zealand (14-28 μg/100 g bread) in 2009 (155). Bread fortification was projected to increase iodine intake to an average of 84 μg/day in New Zealand adults (156). Most studies recruited non-exclusive breastfeeding women (supplementing infants with formula or complementary foods), whereas some used data from exclusively breastfeeding women (infants solely fed from breastmilk). The timing of postpartum data varied from four days to 12 months, thus, the iodine concentration in breastmilk may reflect changes in the stages of lactation from colostrum to mature milk.

The current cut-off for UIC for iodine adequacy in a population of lactating women is above 100 μg/L, and data from most countries (Table 2.5) indicated insufficient iodine intake. Based on the Global Scorecard of iodine nutrition in 2020 (72), iodine intakes stated from each country studied did not accurately reflect breastfeeding women's iodine status, as the scores were based on the MUIC of school-aged children. The appropriate cut-off for BMIC has not yet reached a scientific consensus. Further study in examining BMIC and its relation to iodine status will be required in both iodine sufficient and insufficient regions.

Some interventional studies have investigated the effects of mother and infant iodine supplementation on their iodine status. In regions of moderate to severe iodine deficiency (maternal MUIC as 30-37 μg/L), in Morocco, a randomised double-blinded placebo-controlled trial (n = 241 mother-infant pairs) compared the effectiveness of

using maternal supplementation, either with a single dose of 400 mg iodised oil or supplementing infants (aged ≤ 8 weeks) directly with a single dose of 100 mg (157). This study found that maternal supplementation is more effective in ensuring adequate infant iodine status and maintaining BMIC levels until at least six months postpartum (157). A recent randomised trial in Southern Ethiopia showed that continuous use for six months of iodine supplementation of 225 µg daily was equally effective at maintaining UIC and BMIC when compared to a household iodised salt programme (35 mg iodine/kg salt), as the median UIC/BMIC in the iodine supplementation group showed adequate iodine status (150 µg/L/104 µg/L) and 110 µg/L/111 µg/L in the iodised salt group (158). In the regions of moderate iodine deficiency (maternal MUIC as 42 µg/L), in New Zealand, prior to mandatory fortification of bread with iodised salt, a randomised double-blind placebo-controlled trial reported that maternal supplementation of 150 µg and 75 µg iodine daily for the first six months postpartum improved BMIC. It is important to note, however, that both dosages were not sufficient to enable achieving adequate iodine status for mothers and their infants (150).

### 2.3.4 Iodine intake and status in New Zealand

In New Zealand, soils contain low levels of iodine, resulting in low concentrations in the local food supply (88). Consequently, people living in New Zealand have a low dietary intake of iodine. Iodine deficiency was a concern in New Zealand in the early years of the 20th century, but its prevalence was mostly reduced through the introduction of iodised salt in the 1930s (85). However, since the 1990s, several studies in New Zealand have shown iodine deficiency has re-emerged within adults (159), pregnant and breastfeeding women (150, 160), school children (85) and breastfed infants and toddlers (161). To overcome iodine deficiency in New Zealand, two government initiatives were introduced: mandatory iodised salt in commercially made bread and bread products from September 2009 (5), and iodine supplementation (150 µg/day) for all pregnant and lactating women was recommended in 2010 (96).

Although recent studies suggested that adults (95, 118) and children (94) in New Zealand may now have adequate iodine intake/status, women aged 40 to 63 years with low bread intake (162), and both pregnant and breastfeeding women remain deficient (97). A pilot study of a small sample of self-selected highly educated pregnant (n = 34) and breastfeeding women (n = 36) assessed UIC, BMIC and serum Tg, and results suggested iodine deficiency (97). There is a need to investigate iodine status of a representative sample of postpartum women and their infants, after the establishment of government iodine interventions.

**Table 2.5 A summary of recent ten-year observational studies on postpartum women's iodine status (2009-2020).**

| Country | Country iodine fortification | Sample size (BF) | Timing Postpartum | MUIC (μg/L) | Median Urine Creatinine (ug/g) | Median BMIC (μg/L) | % using iodine supplement | Iodine supplement users vs non-users | Authors |
|---|---|---|---|---|---|---|---|---|---|
| Australia | MF of **IS** in bread 2009 | 50 | 4 days | 46 | 81 | 84 | n/a | n/a | Chan et al. 2003 (163) |
| Australia | MF of **IS** in bread 2009 | 60 | 6 months | 123 | n/a | n/a | BF:45 | UIC: 206 vs 97 *P*=0.029 | Axford et al. 2011(164) |
| Australia | MF of **IS** in bread 2009 | 291 (2006) 653 (2012) | 7 days | n/a | n/a | Pre: 187 Post: 103 (*P*<0.001) | P: Pre: 47 Post: 90 | BMIC: pre: 100 vs 105 *P*=0.93 post: 195 vs 137 *P*<0.001 | Huynh et al. 2016(165) |
| Australia | MF of **IS** in bread 2009 | 55 | 39 days | n/a | n/a | 167 | BF: 57 | BMIC: With **IS**: 272 vs 156 W/O **IS**: 151 vs 98 *P*=0.028 | Jorgensen et al. 2016(166) |
| Australia | MF of **IS** in bread 2009 | 538 | 3 months | 125 | n/a | 127 | n/a | n/a | Huynh et al 2017(167) |
| Britain | No **IS** | 168 | 26 weeks | 79 | n/a | n/a | 28 | UIC: no difference *P*=0.600 | Bouga et al. 2015(168) |

| Country | Country iodine fortification | Sample size (BF) | Timing Postpartum | MUIC (µg/L) | Median Urine Creatinine (ug/g) | Median BMIC (µg/L) | % using iodine supplement | Iodine supplement users vs non-users | Authors |
|---|---|---|---|---|---|---|---|---|---|
| China (Linfen) | MF of **IS** 1993 | 298 Excl. | 15 weeks | 107 | n/a | n/a | zero | n/a | Dold et al. 2017(169) |
| China (Henan) | MF of **IS** 1993 | 747 Excl. | 4 months | 177 | n/a | n/a | n/a | UIC: 192 vs 174 *P*<0.001 | Yang et al. 2017(170) |
| China (Tianjin) | MF of **IS** 1993 | 88 | 4, 8 and 12 weeks | *4 weeks 152 *8 weeks 112 *12 weeks 109 | n/a | mean 4weeks 222 8weeks 175 12weeks 148 | n/a | n/a | Wang et al. 2018 (171) |
| Croatia | Iodine sufficient No **IS** | 73 Excl. | 12 weeks | 35 | n/a | 124 | zero | n/a | Dold et al. 2017 (169) |
| Denmark | MF of **IS** in bread 2000 **IS** raised from 13 to 20 mg/kg in July 2019 | 127 | 22 days | 72 | n/a | 83 | BF: 47 | MUIC 83 vs 65 *P* =0.004 BMIC 112 vs 72 *P*<0.001 | Andersen et al. 2014 (126) |

| Country | Country iodine fortification | Sample size (BF) | Timing Postpartum | MUIC (µg/L) | Median Urine Creatinine (ug/g) | Median BMIC (µg/L) | % using iodine supplement | Iodine supplement users vs non-users | Authors |
|---|---|---|---|---|---|---|---|---|---|
| Iceland | Iodine sufficient - high intake of fish and dairy in the adult Icelandic diet (172) | 57 | 5.5 months | n/a | n/a | 84 | 4-6 | n/a | Petersen et al. 2020 (173) |
| India | Iodine-replete No **IS** | 128 Excl. | 30 to 90 days | 185 | n/a | 230 | n/a | n/a | Pal et al. 2018(174) |
| Iran (Gorgan) | MF of **IS** 1994 | 100 | 30-180 days | 259 | n/a | 93 | n/a | n/a | Azizi 2007(175) |
| Iran (Tehran) | **IS** 1990 | 147 | 4 days | 68 | n/a | n/a | n/a | n/a | Nazeri et al. 2016(176) |
| Iran (Tehran) | **IS** 1990 | 148 | 3-5 days | BMIC ≥100 70 BMIC < 100 37 | n/a | 218 | n/a | n/a | Nazeri et al. 2018(177) |
| Korean^ | No **IS** fortification | 50 | 2-5 days 4 weeks | n/a | n/a | mean 2-5 days 2170 4 weeks 892 | n/a | n/a | Moon et al. 2009(178) |
| Morocco | iodine deficient No **IS** | 74 Excl. | 16 weeks | 33 | n/a | 30 | n/a | n/a | Dold et al. 2017(169) |

| Country | Country iodine fortification | Sample size (BF) | Timing Postpartum | MUIC (µg/L) | Median Urine Creatinine (ug/g) | Median BMIC (µg/L) | % using iodine supplement | Iodine supplement users vs non-users | Authors |
|---|---|---|---|---|---|---|---|---|---|
| Nepal | 80% household had **IS** | 500 | 6.8 months | 230 | n/a | 250 | n/a | n/a | Henjum et al. 2018(179) |
| Netherlands | **IS** since 1945<br>**IS** in bread | 33 | 4 weeks | *112 | n/a | 152 | P: 61 | n/a | Stoutjesdijk et al. 2018(180) |
| Norway | **IS** with 50 mg Iodine/kg salt | 175 | 11 weeks | 64 | n/a | 68 | 29 | habitual users<br>99 vs 60<br>*P*<0.001<br>used last 24h<br>140 vs 61<br>*P*<0.001 | Henjum et al. 2017(181) |
| Norway | **IS** with 50 mg Iodine/kg salt | 133 | 1 to 12 months | 80 | n/a | 71 | 23 | n/a | Groufh-Jacobsen et al. 2020 (182) |
| New Zealand | MF of **IS** in bread in 2009 + iodine 150ug/day | 35 | 3 months<br>6 months<br>12 months | *3months 37<br>*6months 25<br>*12months 47 | n/a | n/a | n/a | n/a | Thomson et al. 2001(183) |
| New Zealand | MF of **IS** in bread in 2009 + iodine 150ug/day | 68 | Pre: 81 days<br>Post: 135 days | *Pre: 34<br>*Post:74 | n/a | Pre: 55 (48)<br>Post: 63 (44) | Pre: 19<br>Post: 36 | BMIC<br>Post: 126 vs 58<br>*P*<0.001 | Brough et al. 2013(97) |

| Country | Country iodine fortification | Sample size (BF) | Timing Postpartum | MUIC (µg/L) | Median Urine Creatinine (ug/g) | Median BMIC (µg/L) | % using iodine supplement | Iodine supplement users vs non-users | Authors |
|---|---|---|---|---|---|---|---|---|---|
| Philippines | **IS,** 26% household uses(184) | 281 Excl. | 8 weeks | 89 | n/a | 185 | zero | n/a | Dold et al. 2017(169) |
| South Africa | **IS**, but a third lacks access | 100 | 3 months | 118 | 126 | 179 | n/a | n/a | Osei et al. 2016(185) |
| Sudan | **IS,** 14% household uses(186) | 47 | 3 months<br>6 months<br>9 months | 3months 51<br>6months 30<br>9months 63 | n/a | n/a | n/a | n/a | Eltom et al. 2000(187) |
| Sweden | **IS**, with 40-70 mg iodine/kg salt (188) | 84 | 0.5 months<br>4 months<br>12 months | 0.5 months n/a<br>4 months 78<br>12 months 107 | n/a | 0.5 months 90<br>4 months 12<br>12 months n/a | 0.5 months 19<br>4 months 12<br>12 months 10 | 0.5 months BMIC 140 vs 71, *P* = 0.001 | Manousou et al. 2020 (189) |
| Switzerland | **IS,** 90% household uses | 196 | 6 months<br>12 months | 67 | n/a | 6months: 51<br>12months: 42 | 3 | n/a | Andersson et al. 2010(190) |

BF: breastfeeding; BMIC: breastmilk iodine concentration; P: pregnancy; MF: mandatory fortification; IS: iodised salt; Pre: pre-fortification; Post: post-fortification; UIC: urinary iodine concentration.

*samples used were 24-hour urinary collection.

^In Korean, traditionally new mothers daily consume seaweed

## 2.4 Selenium

Selenium plays a key role not only in the synthesis of thyroid hormones, but also is involved in multiple areas of human health, including immune function, mental health, and infant neurodevelopment. There is a complex relationship between selenium intake and health outcomes, as both selenium deficiency and excess may result in adverse health outcomes (191). Numerous research studies have investigated selenium and human health, and endeavoured to understand the mechanisms involved (192).

### 2.4.1 Selenium and health

Through selenoproteins, selenium plays an antioxidant and anti-inflammatory role against oxidative stress and regulates the redox reactions of several micronutrients (192). Severe selenium deficiency results in clinical effects such as Keshan (an endemic disease of myocardium necrosis and fibrosis) and Kashin-Beck diseases (an endemic disease of osteoarthropathy) within populations living in selenium-deficient areas (193). Selenium status is associated with pregnancy-induced hypertension, preeclampsia, pre-term birth, and recurrent miscarriage. These topic areas have been extensively researched by Rayman's group (192, 194, 195) and Barrington (196), both are based in the United Kingdom. Selenium supplementation has shown protective effects for some selected cancers, such as prostate cancer, but only among those presenting with low selenium status initially (192). Excessive intake also can be detrimental, for example the human selenosis outbreak (selenium intoxication, such as loss of hair and nails, and lesions of skin) which occurred in Enshi, China, between 1961-1964 (197). Therefore, like many essential nutrients, selenium presents a U-curve in its health effect (191, 198) (Figure 2.4).

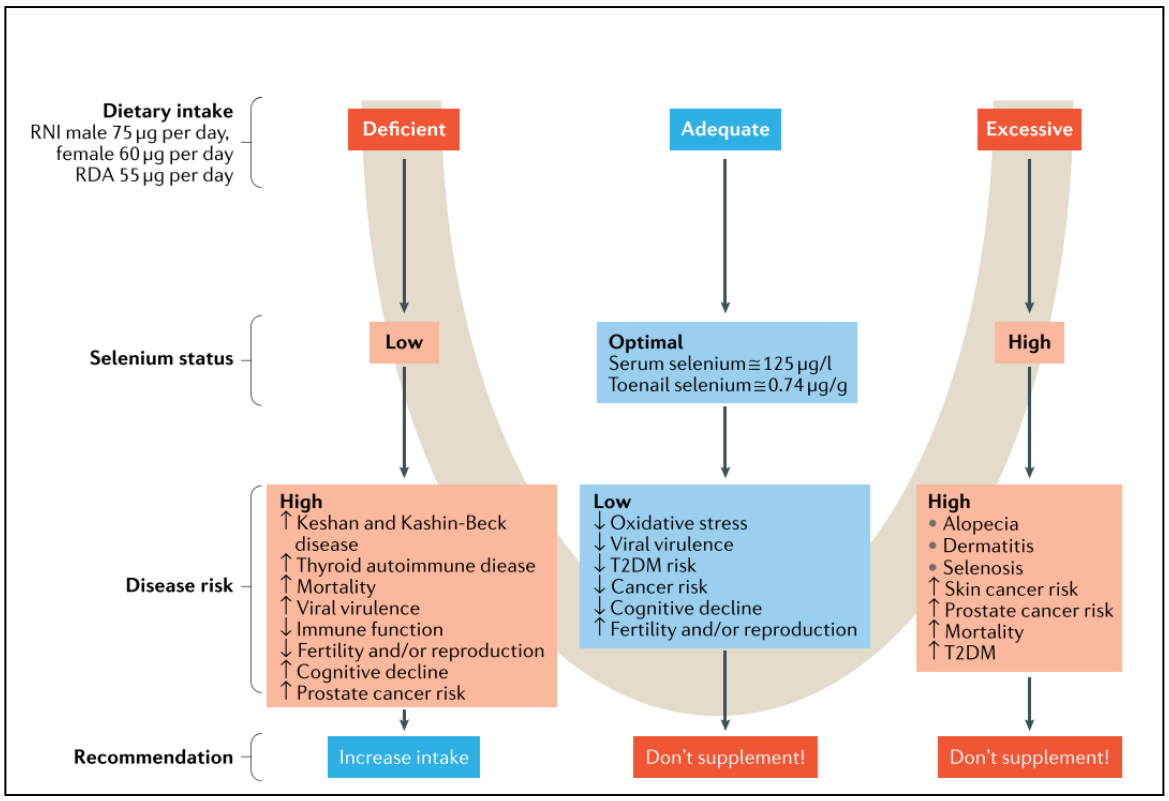



**Figure 2.4 U-shaped relationship between selenium status and disease risk [Winther, Rayman, Bonnema, and Hegedüs, 2020 (198)]**

Selenium plays an important role in normal brain development, although the exact mechanism is unclear. Two recent large cohort studies from Poland (n = 410) (199) and Spain (n = 490) (200) found maternal selenium status in the first trimester (mean serum selenium concentration in Polish mothers at 48.3 µg/L; in Spanish mothers at 79.1 µg/L) was positively associated with neuropsychological development, which was measured by the Bayley Scales of Infants and Toddler Development at one year and two years of age (199), and the McCarthy Scales for Children's Abilities (MSCA) at five years of age (197). A Norwegian study of 114 pregnant and lactating women and their infants examined the effect of maternal selenium status on infant neurodevelopment as measured by the Ages and Stages Questionnaire (ASQ), and reported that low maternal serum selenium concentration (< 71 µg/L) at 18 gestational weeks was associated with lower infant psychomotor scores (total, problem solving, personal-social functioning and fine motor function) at six months of age (201). It should be

noted that infant neurodevelopment in relation to postnatal selenium intake and status will warrant ongoing investigation, as brain development continues up to two years of age (202).

### 2.4.2 Selenium intake and status in postpartum women and their infants

#### *2.4.2.1 Selenium intake*

Most organic selenium occurs in human diets as selenomethionine and selenocysteine, which are absorbed and stored in pools to reserve selenium for the tissues (198). Inorganic forms selenite and selenate commonly used in dietary supplementation and infant formula may be less bioavailable than organic forms of selenium (198). The selenium content in food sources depends on the soils where plants are grown, and the nature of animal feed. Geographic variations of selenium in local soils are reflected in subsequent human dietary intake, with worldwide selenium intakes ranging from seven to 4990 μg/day, varying between deficient to toxic intakes (192). About 50-60% of selenium intakes are excreted into urine to maintain selenium homeostasis (203).

Newborn infants are born with some selenium reserves; however, breastfed infants rely on continuous supplies from breastmilk to achieve optimal selenium status. Breastmilk is recommended as the best food for infants during the first six months of life (204). Selenium present in human breastmilk is significantly associated with maternal selenium intake (205, 206). Variation in dietary selenium intakes of people living in different geographic areas is widely reflected in breastmilk selenium concentrations (BMSC). For example, Shearer and Hadjimarkos observed that mature breastmilk from American women living in a high selenium area had a selenium concentration of 283 μg/kg, compared to only 2.6 μg/kg from women living in an area with endemic Keshan disease which indicates selenium deficiency (207).

The assessment of selenium requirements for adults has been based on maximising plasma GPx activity, rather than the prevention of Keshan disease (208). During

lactation, selenium requirements are necessarily increased to meet the growing infants' needs. The EAR for lactating women is at 65 μg/day to include an allowance of 12 μg/day selenium secreted into breastmilk and the RDI is 75 μg/day by assuming a coefficient of variation of 10% for the EAR (since insufficient data are available to determine the standard deviation). Final EAR and RDI recommendations are rounded up to the nearest 5 μg (86). The Upper Level of Intake (UL) is suggested as 400 μg/day for all women without amendment for lactation. Infants' dietary intake is calculated from selenium concentration in human milk multiplied by the volume of breastmilk needed at different ages (86). Adequate Intakes (AI) for selenium are suggested to be 12 and 15 μg/day for birth to six months, and seven to twelve months, respectively (86). UL for Infants are 45 and 60 μg/day for birth to six months, and seven to twelve months, respectively, based on a previous study of Shearer & Hadjimarkos (207).

#### *2.4.2.2 Selenium status*

Selenium status of postpartum women and infants have been examined. A comparative observation study from Levander et al. (1987) reported on plasma selenium from a small sample of 23 North American women at one, three and six months postpartum (136, 137, and 138 μg/L) (206). Although these concentrations did not change during the stages of lactation, they were lower than those in a non-lactating group (151, 152, and 144 μg/L, above 110 μg/L to maximise expression of selenoprotein P )(203, 207). BMSCs of these American women at all three time points (20 μg/L, 15 μg/L, and 15 μg/L respectively) were found to be adequate to meet the needs of infants aged 0-6 months (10, 12 and 13 μg/day, based the US National Research Council recommendation 10-40 μg/day) (206).

Recent studies examined both maternal plasma and cord blood selenium in postpartum women in Greek mothers along with Albanian mothers who had immigrated to Greece before birth (209), and Spanish mother-infant pairs (210). Maternal blood/plasma selenium concentrations were determined in labour, so, in consequence, they cannot be directly compared to the 1987 North American study.

Much lower serum selenium concentrations were observed in Albanian immigrant mothers (37 μg/L) compared to Greek women raised and living in the same region (68 μg/L). Furthermore, Albanian infants showed much lower serum selenium (measured in the cord blood) when compared to Greek infants (34 vs 37 μg/L, $P < 0.05$). Authors suggested this phenomenon could be due to the low socioeconomic status of Albanian mothers who found selenium rich food unaffordable, particularly meat and fish (209). A cross-sectional study with 83 Spanish mother-infant pairs attempted to identify selenium varieties in maternal blood (during 24 hours before giving birth) and cord blood by measuring plasma selenium, selenoprotein P (SeP), serum GPx, and Se metabolites (SeMetab). Also reported was the low selenium status of these mothers to optimise GPx activity (< 70 μg/L) (210). Even though 25% of participating women consumed selenium-containing supplements during pregnancy, no differences in selenium measures were detected (median total plasma selenium 67.4 vs 69.7 μg/L, $P = 0.54$; SeP 41.4 vs 44.9 μg/L, $P = 0.16$; GPx 10.8 vs 11.6 μg/L, $P = 0.43$; SeMetab 4.4 vs 3.2 μg/L, $P = 0.18$). This observation may be due to low dose of supplementation at 55 μg/day as selenite, which is an inorganic form of selenium with less bioavailability. In addition, it would be useful to include supplement compliance when reporting results.

Three clinical trials examined the effects of selenium supplementation on status. Common forms of selenium supplementations include selenium-Methionine (Se-Met), yeast containing selenium (Se-yeast), together with inorganic form of selenite or selenate which may be less bioavailable than organic forms of selenium (198). In a randomised double-blind placebo-controlled trial, American women were supplemented 200 μg daily selenium as Se-Met or as Se-yeast at four weeks postpartum for four weeks (211). The participating women were followed at eight and twelve months postpartum. They found Se-Met significantly increased plasma selenium of lactating women (98.7 μg/L at four weeks, 194.3 μg/L at eight weeks postpartum), but a declined plasma selenium was observed in those lactating women who were in the placebo group (137.4 μg/L at four weeks, 119.3 μg/L at eight weeks postpartum) (211). This American study had a small sample size (31 lactating and 22

non-lactating), and even smaller numbers were allocated to each treatment group; breastfeeding patterns (exclusively or partially) were unreported. In addition, the baseline plasma selenium concentrations were statistically different between the control and Se-Met treatment group (137.4 vs 98.7 μg/L, $P < 0.05$). Such differences at baseline may suggest instability in the data (211). In a larger sample size interventional study (n = 167), 100 μg of selenite daily, or 100 μg of Se-yeast daily or no supplementation were randomly assigned to Finnish women who were exclusively breastfeeding (212). The participating women were followed at two, four and six months postpartum. The findings showed the Se-yeast, when compared to selenite, more effectively increased selenium in serum (increment of 86 μg/L vs 51 μg/L at four months of lactation) and breastmilk, which may have increased the infants' selenium intake (11.5 vs 8.9 μg at age of six months based on 800 mL daily breastmilk consumption) (212). Authors suggested one of the available compounds in selenium yeast was Se-Met, therefore, organic forms of selenium supplementation showed clear effectiveness in improving maternal selenium status and infant selenium intake (212). In contrast, sub data generated from the Breastfeeding, Antiretrovirals and Nutrition study [randomised intervention with antiretroviral drugs (ARV), lipid-based nutrient (LNS) containing 75 μg selenite, LNS and ARV, and the control)] reported that the plasma selenium of HIV-infected Malawian mothers was unchanged after supplementing 75 μg selenite daily from birth till 28 weeks postpartum (213). Women's HIV status may have had an impact on selenium uptake. The authors acknowledged selenite, as an inorganic form of selenium, is less readily absorbed, thus this may also have had a minimum impact on selenium status (213). Variable findings in these studies may be due to factors such as the type of selenium source, dosage, original selenium status before supplementation, or variable stages of lactating, so further research is needed to investigate the effects of selenium supplementation in improving maternal status. Interestingly, all three studies observed a similar decline of BMSC from early to later stages of lactation, despite appropriate supplementation.

It is thought that such gradual decreases of selenium in breastmilk concentrations may be a natural physiological process (the changing of needs of the infants) (210).

### *2.4.2.3 Assessment of selenium intake and status*

Selenium intake can be assessed by dietary assessment methods, including estimated/weighed food records, food frequency questionnaires (FFQs), and repeated 24-hour dietary recalls (see Section 2.3.2). Weighed food records is recognised as the gold standard measure of food and nutrient intake (119). However, this often presents challenges when estimating selenium intake solely from food records. Selenium values contained in the updated food composition data may not reflect regional variations of selenium in the food items consumed, since not all data bases may contain accurate selenium data.

Methods used to assess selenium status for both breastfeeding women and their infants include urinary selenium excretion, blood and breastmilk selenium concentrations, and selenium concentrations obtained from hair/nail clippings.

Urinary selenium excretion is the portion of absorbed selenium that has not been retained by body tissues. It is considered as a proxy measure reflecting short-term dietary selenium intake, since increased dietary intake results in raised urinary excretion of selenium (214). Historical data from China showed a 400 times higher urinary selenium excretion in people with selenosis compared to those living in an area of endemic Keshan disease (215). Urinary selenium excretion is commonly used to estimate daily dietary selenium intake for adults, based on 50-60% of the excretion rate. However, it is not possible to use the urinary selenium excretion rate to estimate selenium intake with lactating women, since selenium is also secreted into breastmilk.

Selenium concentrations can be measured in both spot and 24-hour urine samples. Collecting 24-hour urine samples requires motivated participants and may not be practical in large studies. Diurnal variation and hydration status are the main challenges in evaluating selenium excretion in spot urine samples (215). Urinary

creatinine can correct for the changes in hydration status. In healthy Polish subjects (n = 199), the urinary selenium to creatinine ratio was positively correlated the whole blood selenium (216), which suggests the ratio may be a better method to estimate selenium status than urinary selenium excretion alone.

Measuring the selenoproteins in blood, including selenoprotein P, GPx1(plasma GPx) and GPx3 (GPx in red blood cells) has been suggested, as these are useful functional markers for selenium status (200). Results may be misleading if only one of the selenoproteins is measured, due to the hierarchal arrangement of selenoproteins in body function. In a randomized double-blind trial in New Zealand in 1987, women aged 18-23 years were given either 200 μg Se-yeast enriched in Se-Met, or brewer's yeast mixed with selenite, or a placebo for 32 weeks (217). At the conclusion of supplementation, plasma selenium in the Se-Met group (190 μg/L) was 1.7 times more than that in the selenite group (110 μg/L), both being effectively increased from the baseline values of 53 μg/L (217). A 2009 systematic review reported that plasma selenium was increased significantly by selenium supplementation (218). However, differences observed in gender, supplement type/dosage/duration, or the subject's baseline selenium status, as well as an inconsistency of measurement assays raised difficulties in the meta-analyses of the results (218). The review found that plasma selenium is the most widely measured biomarker in the published supplementation studies, due to its effectiveness in reflecting levels of selenium intake (218).

A range of plasma selenium values in adults has been suggested for optimal activity of iodothyronine 5' deiodinases (65 μg/L) (208), nutritional adequacy (70 μg/L) (219), or maximal GPx activities (95 μg/L) (217), but no reference values are available at this time for plasma selenium to define adequate or deficient selenium status for lactating women. Furthermore, plasma selenium has been used to estimate selenium intake, as recorded from an extensive data analysis of Chinese studies, where a proposed formula was suggested to calculate selenium intake daily ($\log Y = 1.623 \log X + 3.433$; X = plasma selenium, mg/L; Y = selenium daily intake μg/day) (220). It is useful to compare such results to the estimated dietary intake from dietary assessment.

Measuring selenium concentrations in nail clippings is another established method of assessing selenium status (220, 221). Selenium is incorporated into hair or nails (keratinised tissues) and, as they grow slowly, this reflects long-term (up to 52 weeks) selenium status; however, hair and fingernails are easily contaminated by selenium-containing shampoo or other environmental exposures, and toenails are recognised as having less exposure to these contaminants. Toenail clippings were used to determine selenium concentrations in large cohort or epidemiological studies, such as the risk of preeclampsia in pregnant women (195). In comparing selenium concentrations in toenails to other biomarkers including whole blood, serum and urine, twelve healthy American males were randomly assigned to either consuming two slices of study bread containing selenium at 206 μg/day, or 388 μg/day, or the control group over a one-year period; and their toenail clippings were collected for analysis at a twelve-week interval over a two-year period (222). The trial found toenail selenium concentrations started to reflect dietary change after the first three months, while whole blood and serum selenium increased after two weeks, and consequently, measuring selenium in nail clippings could be an important biomarker representing exposure up to 52 weeks (221, 222). The advantages of measuring nail clippings include that collecting nail clipping samples are less invasive and convenient for participants (221). Additionally, measuring selenium concentrations in maternal toenail clippings after childbirth can be used to estimate fetal selenium exposure (222).

### 2.4.3 Selenium intake and status in New Zealand

In New Zealand, local food supply contains low levels of selenium. Historical data (1966-1986) collected by Robinson's group reported low selenium intake in the New Zealand population, particularly those living in the South Island (223). Even after the importation of selenium-rich wheat from Australia (containing 10 times higher levels of selenium than that contained in New Zealand wheat), low selenium intake and status of people who live in the North and South Island continued to be present (53). Differing selenium intake between the two islands are due to locally produced wheat being preferred for breadmaking in the South Island of New Zealand (83).

Based on self-reported 24-hour diet recall data from the 2008/09 New Zealand Adult Nutrition Survey, a median selenium intake of women aged 31-50 years old was 51.9 μg/day, with 58% below the EAR, indicating the presence of inadequate intake (224). An increase in median selenium intake among females aged over 25 years from 2009 (56 μg/day) to 2016 (68 μg/day) was observed in the 2016 New Zealand Total Diet Survey (percentages below the EAR were not reported) when compared to the previous national Adult Nutrition Survey (89). The authors suggested that the increased selenium intake was due to increased consumption of selenium rich food since selenium concentrations of key food contributors had remained constant (89). Low selenium intakes continue to be reported in New Zealand women of childbearing age (225), and postmenopausal women (226). Before the year of 2000, several research studies in New Zealand examined selenium status in adults, with serum or plasma selenium ranging from 43 to 69 μg/L, which was lower than the suggested minimum level of plasma selenium for nutritional selenium adequacy (70 μg/L) (227). It was clearly established that New Zealand adults had low selenium status.

Pregnant and lactating women who live in low selenium areas remain of concern, owing to their increased requirement for meeting both foetal and infant growth. A few studies in New Zealand have investigated the selenium intake and status within these populations. An early double-blind supplementation study recruited thirty-five pregnant women from the South-Island of New Zealand (either receiving 50 μg selenomethionine daily, or a placebo) and they were followed up until 12 months postpartum, with the addition of 17 non-pregnant women who received a selenium supplement forming a positive control group (183). The focus of this South-Island study was to compare the responses of selenium supplementation for non-pregnant, pregnant and postpartum women. The data reported that levels of plasma selenium for postpartum women rose at three and six months postpartum but fell at 12 months postpartum. This confirmed an overall higher level of plasma selenium recorded for those women who received supplementation over the 12-month period, when compared to the non-supplemented pregnant group. Even so, the study, when

published, offered no detailed data report for the selenium status of these postpartum women, therefore their selenium status remained inconclusive (183).

Between May 1998 to March 1999, an observational study by McLachlan et al. was conducted on infants aged 6-12 months, toddlers aged 12 to 24 months, and their postpartum mothers in the South Island of New Zealand (228). The study measured maternal plasma selenium and children's serum selenium, and maternal dietary intake from three-day weighed food records. The mean selenium plasma concentration in lactating women (72.9 μg/L), was close to that of non-pregnant/non-lactating women (73.5 μg/L, $P$ = 0.055). Estimated dietary selenium intake was significantly higher in lactating women (46 μg/day) than non-pregnant/non-lactating women (36 μg/day, $P$ = 0.004), despite their similar plasma selenium status. The loss of selenium in breastmilk may account for this difference. However, this study did not examine selenium concentration in breastmilk, therefore the total intake for breastfed infants was unable to be firmly established.

More recent reported data was from a cross-sectional study of 53 exclusively breastfeeding mother-infant pairs at eight weeks postpartum in Dunedin, New Zealand between 2012 and 2013 (229). Results showed mean selenium intake was 47 μg/day based on the three-day weighed food records; and mean maternal serum selenium concentration at 75 μg/L with 39% lower than 70 μg/L, suggesting suboptimal selenium status. However, the sample size was small, and the information as to the use or not of selenium-containing supplements during pregnancy or lactation was not forthcoming. To our knowledge, there have been no recent studies in New Zealand investigating selenium status in breastfeeding women.

## 2.5 Iron

Iron deficiency, one of the most common nutritional disorders, affects individuals of all ages and ethnicities in the world, although, infants, children, and women of childbearing age are disproportionately affected (230). For example, based on a published 2013 analysis of 257 surveys (232 nationally representative sources) from 190

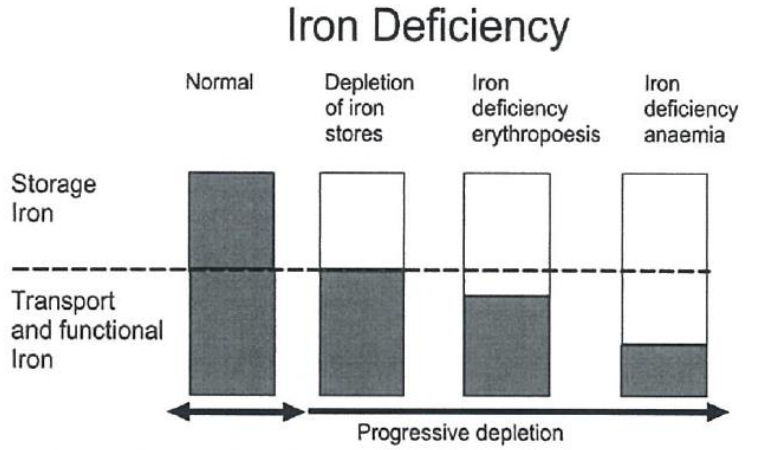
countries categorised into 11 regions, anaemia prevalence decreased in pregnant women (43% to 38%), in non-pregnant women aged 15-49 years (33% to 29%), and in children aged less than five years (47% to 43%) through the years 1995 to 2011. However, eight hundred million women and children remained affected by anaemia globally in 2011, with iron deficiency contributing to at least 50% of anaemia in women, and 42% in children (230). According to the Global Health Observatory data repository, in 2016, 40% of pregnant women, 33% women of childbearing age were anaemic (231), and 42% of children younger than 5 years (232).

Women of childbearing age are disproportionally affected by iron deficiency without anaemia (ID)/iron deficiency anaemia (IDA) (114), since low iron intake is probably the major cause. Failure to compensate for iron loss through heavy menstrual bleeding and failure to reach an increased iron requirement during pregnancy may play a part in developing ID/IDA in women (233). A cross-sectional study examined the prevalence of ID and IDA with women aged 18 -35 years living in the state of New South Wales in Australia (n = 300), where the prevalence of ID and IDA were 14% and 6% respectively (234). In New Zealand, results from the 2008/2009 Adult Nutrition Survey found the highest prevalence of ID and IDA were observed among females aged 31 to 50 years old, measured at 12% and 6% respectively (224).

### 2.5.1 Iron and health

Iron is a principal component of haemoglobin (Hb) and myoglobin (oxygen reserve in muscles) (20). The balance of the body's iron presents as iron-containing enzymes which are involved in cellular respiration, cell proliferation and differentiation, optimal immune function, and hormone synthesis (20). The primary function of iron is erythropoiesis (formation of red blood cells), and this process is well protected, even if the iron store becomes depleted. Impaired iron status presents in discrete stages, from an early stage of iron storage depletion, tissue iron deficiency, then moving on to the last stage of impaired Hb production [Figure 2.5, (235)]. People with depleted iron stores usually do not show any noticeable clinical symptoms (235). When iron

deficiency occurs, Hb production is initially compensated for by drawing on stored iron. Concurrently, other iron functional roles are compromised in order to maintain erythropoiesis. If deficiency continues, this not only affects Hb formation, but may also cause abnormal red blood cells to form. Individuals then exhibit IDA, which may present as clinical symptoms, varying from fatigue, to hair loss, or spoon-shaped fingernails in more severe cases (235).




| Iron status | Normal | Mild deficiency | Marginal deficiency | Severe deficiency |
|---|---|---|---|---|
| Haemoglobin | Normal | Normal | Normal | Low |
| Other Fe functions | Normal | Normal/low | Low | Low |
| Stores (SF) | Normal | Normal/low | Low | Low |

**Figure 2.5 Spectrum of Iron deficiency and diagnosis [Coad and Pedley, 2014 (235)]**

Iron deficiency may negatively impact on both the physical and mental health of reproductive aged women, including reduced work capacity, impaired cognitive function and adverse mental health (236). A recent cross-sectional study assessed cognitive function of women aged 18-35 years living in the state of New South Wales, Australia (n = 300), in relation to their iron status [iron replete (IR), ID, and IDA] (234). The results showed significant reduced attention in women with IDA but not observed in women with ID or IR after controlling the body mass index, inflammation and physical activity, despite the five cognition domains assessed (impulsivity, attention, information processing, memory and executive function) using the online

computer-based questionnaire. Authors suggested cognition is less likely affected at early stages of iron deficiency (234).

Several interventional studies have examined iron status in relation to physical fitness, general mental health, and cognitive functioning. A randomised placebo-controlled trial of a group of 22 non-anaemic iron deficient (SF< 16 μg/L and Hb > 120 g/L) American women aged 18-33 years has shown improved fitness levels (measured by the $VO_2$ max – the maximum rate of oxygen consumption) after correcting iron deficiency by a six-week daily supplementation (50 mg $FeSO_4$) (237). Moreover, reduced fatigue and marginal improvement of general mental health were observed from a randomised controlled study of iron deficient Australian women aged 18 to 50 years, after consuming a high iron diet (providing 2.25 mg absorbed iron daily) or iron supplements (350 mg $FeSO_4$) over a 12-week trial period (238). Another placebo-controlled stratified intervention study investigated the improved iron status on the accuracy of cognitive function (attention, memory and learning) among American women aged 18-35 years (239). According to the iron status, women were classified into three groups: one control (iron sufficient, n = 43), and two treatment groups (ID, n = 75; and IDA, n = 34). Women in each group were given either 160 mg $FeSO_4$ daily or a placebo for 16 weeks. Results showed that slower information processing was observed in women with ID when compared with others with normal iron status, and the iron supplementation led to an improvement in both cognitive performance and the timing required to complete the tasks (239). Strengths of this trial include detailed cognitive performance tasks (from Detterman's Cognitive Abilities Test) at baseline and 16 weeks after treatment, and iron status was treated as continuous data to enable detection of subtle cognitive changes. Research shows that the developing brain is sensitive to iron depletion, which explains the link between iron deficiency and impaired neuronal functioning, more specifically, with cognitive function (attention and memory) and related psychological effects (240).

### 2.5.2 Iron physiology and intake patterns

Dietary iron intake is required to meet the balance of losses from skin, urine and the gastrointestinal tract, and the loss of menstrual blood in women of childbearing age. However, iron balance is regulated by the amount of iron absorbed. In total, only 1-2 mg of iron is absorbed from the 10-20 mg of iron consumed from food daily. The amount absorbed is mostly regulated by the divalent metal ion transporter 1 (DMT-1), and transferrin (the major iron transport protein) transports iron in the blood circulation (235). In addition, iron is recycled from the breakdown of red blood cells via macrophages into the circulating iron pool. The remainder is stored in bone marrow and the liver. Iron is stored as ferritin predominantly in hepatocytes, and a small amount of ferritin is present in serum which can be detected. Therefore, serum ferritin acts as a good indicator for assessing current iron storage. Figure 2.6 provides a visual representation of iron balance in the body (235).

Most dietary iron, initially present as non-haem iron consumed in the ferric form, is reduced to ferrous iron before being absorbed and transported by DMT-1 expressed by enterocytes which line the intestine. Other metal ions, such as zinc and manganese compete for the DMT-1, which may limit iron absorption (20). The amount of iron entering the blood circulation is regulated by the secretion of hepcidin, a peptide hormone from the liver, which causes sequestration of iron by the enterocytes and macrophages. For example, when iron deficiency is present, hepcidin release is reduced to maximise iron absorption into the blood. Dietary haem iron from animal products, such as meat, fish, and poultry is more readily bioavailable for absorption (possibly by using haem carrier protein and haem oxygenase, Figure 2.7) than non-haem iron(235), as well as being less likely to be influenced by meal composition (241). However, the detailed mechanism as how haem-iron is absorbed is less understood when compared with the mechanism used in non-haem iron (242, 243).

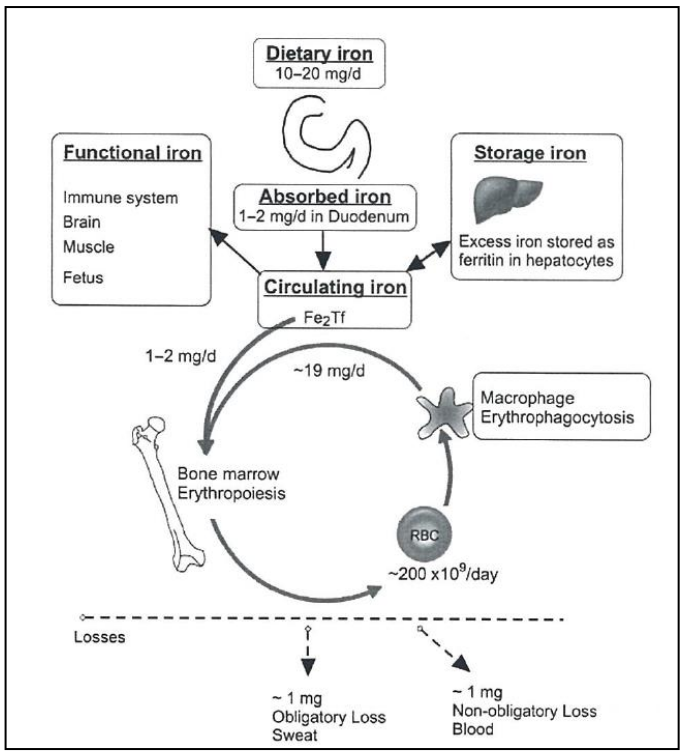



**Figure 2.6 Iron balance in the body [Coad and Pedley, 2014 (235)]**

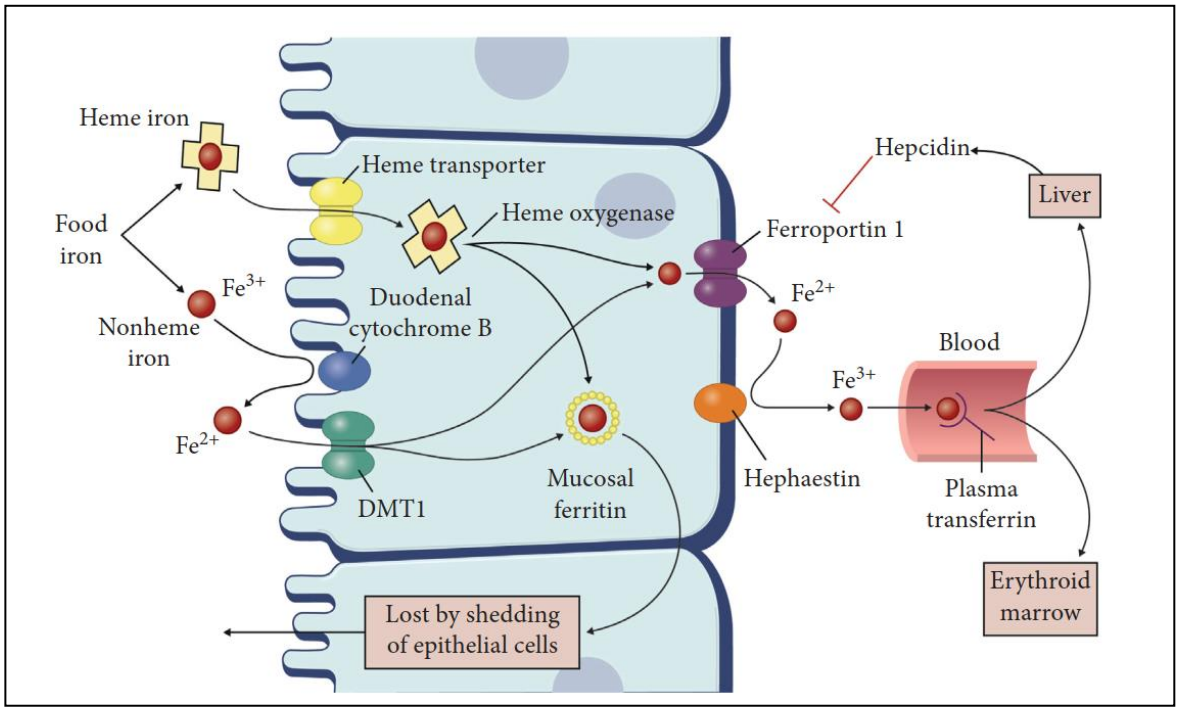



**Figure 2.7 Mechanisms of intestinal iron absorption [Milman, 2020 (243)]**

Moreover, several enhancers and inhibitors impact on non-haem dietary iron absorption. Ascorbic acid, alongside citric acid and lactic acid is one of the most recognised iron absorption enhancers, mostly evident from single meal consumptions rather than from a complete diet (244). The possible mechanism is due to its ability to increase iron solubility in the small intestine and to reverse the negative effects of tea/coffee/phosphate. In addition, the "meat-fish-poultry" factor is also responsible for an increase in iron absorption (244, 245, 246), although a single mechanism is yet to be clearly identified. In contrast, many dietary factors inhibit iron absorption, including polyphenols derived from tea or coffee, phytates found in whole grains and legumes, or oxalic acid from spinach, berries, or chocolate.

Iron balance in the human body is complex and influenced by dietary intake, metabolic processes, and physiological stages of life. When evaluating iron intake or status, dietary intakes, or enhancers and inhibitors in the diet, other non-dietary factors need to be considered. Most commonly, dietary assessment methods are used to estimate iron intake, as for example, weighed food records. Detailed selection of dietary assessment methods has been discussed in a previous section (2.3.2). However, dietary assessment of iron intake is more difficult than for other nutrients, as the complexity of a person's diet contains both enhancers and inhibitors for iron absorption. Thus, in recent years, new approaches have been developed to consider the whole diet and to include food combinations in the diet (dietary pattern analysis) to evaluate iron-related nutrition. A food frequency questionnaire has been developed and validated in New Zealand to identify dietary patterns in relation to iron status (114). Subsequently, dietary patterns such as 'meat and vegetable" or "milk and yoghurt" have been identified, which may reduce or enhance the likelihood of there being suboptimal iron status among young New Zealand women (247). In the MINI study, this validated FFQ is used to explore iron-related dietary patterns among postpartum women, and the relation of these patterns to their iron status.

### 2.5.3 Iron status and its assessment

IDA is usually confirmed by a combination of lower serum ferritin (SF) and Hb; however, the diagnosis of ID is more complicated due to a wider selection of biomarkers and the lack of standards. SF is the most effective and recommended indicator to assess the adequacy of iron stores individually. SF below 12 ug/L indicates a depleted iron store (248). Interpretation of results from SF is complicated by acute and chronic inflammation and infection. Therefore, to avoid an underestimated iron deficiency, C-reactive protein (CRP) is measured. An increased CRP (with a cut-off range of 10-30 mg/L) may indicate that the high SF result is unreliable (140). In contrast, elevated serum soluble transferrin receptors (sTfR) can confirm functional iron deficiency independently of inflammatory state. A recent meta-analysis suggested the ratio between sTfR, and the log value of SF may offer a better sensitivity and specificity to indicate IDA (249). The other commonly used biomarker is zinc protoporphyrin, where a value above 8 μg/L in red blood cells indicates ID (250). If feasible, assessing Hb and SF (provided inflammation is excluded), either zinc protoporphyrin or sTfR together is the best combination to measure iron status (140).

### 2.5.4 Iron intake and status in postpartum women

Postpartum mothers should have a lower risk of developing ID or IDA, when compared to other women at reproductive ages (248). The low risk results from a period of amenorrhoea, low levels of iron in breastmilk, and possible increased absorption of iron from the small intestine (251).

To estimate postpartum anaemia prevalence, data collected from over 46,000 German postpartum women between 1993 and 2008 were analysed and the study reported that 22% of women at the second day after delivery were anaemic (Hb < 100 g/L) (251). This figure may be slightly overestimated since water balance regulation changes between 24 to 48 hours after childbirth. However, this large sample size enabled exploration of attributable risk factors contributing to immediate postpartum anaemia, and blood loss during childbirth (> 500 mL) was identified as the most

important determinant (251). A similar dataset from the United States determined the prevalence of postpartum anaemia. Bodnar examined nearly 60,000 low-income American women four to 26 weeks postpartum and identified 27% as being anaemic (Hb < 120 g/L) (252). After assessing a range of potential determinants, the researchers identified prenatal anaemia as a key contributing factor to postnatal anaemia (252). A protective effect of breastfeeding status through the presence of amenorrhoea was also suggested. Both studies were inconclusive as to whether anaemia status was due to iron deficiency, or perhaps other nutrient deficiencies such as vitamin B12, given the absence of any further measurement of nutrient status, especially iron (251).

With regard to ID and IDA, based on the analysis of data collected for the National Health and Nutrition Examination in the United States from 1988-94, a 16% ID (SF <15 μg/L) and around 4% IDA (SF < 12 g/L, Hb < 120 g/L) among women from childbirth to six months postpartum (253). After controlling confounding variables, early postpartum women from low income groups presented a four times higher risk of IDA than their non-pregnant counterparts, and such heightened risks continued for those between 7-12, and 13-24 months postpartum (253). A cross-section study of Nepali lactating women [only 7% (33/465) < three months postpartum including exclusive and partial breastfeeding] reported that over 70% of studied women presented with inadequate iron intake (≤ 23.4 mg), but only 5% were identified as being ID (plasma ferritin < 15 μg/L) (254). There was a possibility of overestimation of inadequate intake since an EAR value of over three months postpartum was used. Low prevalence of ID and IDA was, perhaps, due to protective effects from amenorrhoea and over six-month consumption of iron supplements during pregnancy.

Supplementation during later pregnancy may lower the prevalence of postpartum anaemia (255) and enhance iron status (256). An earlier double-blinded placebo pregnancy supplementation trial (28 gestation weeks, receiving 100 mg elemental Fe daily) in Niger also found that at three months postpartum there was less prevalence of ID among women from the supplementation group (12%), compared to the placebo group (34%) assessed by serum ferritin levels (< 12 μg/L) (257). The 2016 WHO

guideline recommends oral iron supplementation may be provided to women at six to twelve weeks postpartum where a prevalence of gestational anaemia is higher than 20% and emphasised this was conditional due to low quality evidence (258).

In New Zealand, a recent study from the Massey research group (unpublished data from the Mother and Baby Study in 2011) found the median iron intake met the RDI and no individual intake was below the EAR level, confirming that iron intake was adequate in these studied postpartum women. Although iron intake was estimated from repeated 24-hour dietary recalls, iron status itself was unexamined. Iron storage and Hb levels of postpartum women in New Zealand are rarely examined routinely, unless higher than usual levels of blood loss during childbirth are recorded (259), thus, research concerning iron intake and status of postpartum women is limited.

## 2.6 Postnatal Depression (PND)

Postnatal depression (PND) is one of the major maternal health issues to arise and can exacerbate negative health effects on their newborns (poorer growth, higher risk of diarrhoea) and children (delayed motor development and behavioural problems) (257, 259). Its onset begins six weeks to six months after childbirth. Most women will recover over time from PND, though approximately one quarter of affected women report being depressed when their infant reaches their first birthday (260). It has been established that minor depressive symptoms during the postpartum stages may increase the risk of recurrence of depression throughout the reproductive years (261). Of additional concern, is that mothers with newborns are often reluctant or unable to seek help when they experience symptoms of PND (262). Such under-diagnosed and, at times, untreated mental health conditions affect both the mother and their children's ongoing cognitive, emotional, and behavioural development (263).

A meta-analysis from 59 studies globally suggested the prevalence of PND was approximately 13% among women after partition (264). Using the Edinburgh Postnatal Depression Scale (EPDS), the prevalence of PND in New Zealand was around 8% in 1994, and 16% in 2006. In the 2015 New Mothers' Mental Health Survey,

the prevalence was estimated to be 14% (262), and it is now regarded as the most common disorder for mothers in their first postpartum year (265).

Despite other instances of social and psychological aetiology of depression, potential risk factors have been extensively explored, including hormonal factors and the micronutrients status of birth mothers.

### 2.6.1 Thyroid antibodies and thyroid hormones

Endocrinological factors have been suggested to play a significant role in the development of PND, since women experience dramatic hormonal changes (such as progesterone) after parturition (264, 265, 266). In attempts to seek predictive factors, studies have measured antenatal thyroid function to evaluate risk of PND. They have found that pregnant women with low total and fT3 (267) or total T4 (268), and low fT3 and fT4 (269) may indicate increased risk of developing PND. However, other cofounding factors can also play a role in these studies, such as prepartum depression, or subjects being single mothers. Since thyroid dysfunction and depression were experienced concurrently by some postpartum women, some researchers have investigated their associations. A follow-up study from the Netherlands found that even though thyroid function was normal during pregnancy, 7% (n = 21) of women developed thyroid dysfunction after giving birth, with 38% (8/21) developing depression (270). In contrast, a Spanish observational study of pregnant women with follow-up at 1, 3, 6, 9 and 12 months postpartum, detected no cases of PND among a group of women with thyroid dysfunction, and no statistically significant depression scores (as measured by Beck Depression Inventory) between healthy women and those with thyroid dysfunction (271).

A double-blinded comparison study in Wales recruited a cohort of postpartum women with TPOAb positive (n = 145) and negative (n = 229), and found that women with positive antibodies (43%) were more likely to be depressed, when compared to similar women with negative antibodies (28%) (272). Such associations were also reported by Breese McCoy et al. (2008, n = 51) at four weeks postpartum (273) and Le

Donne et al. (2012, n = 74) at day three of postpartum (274), although only relatively smaller sample sizes were studied. In contrast, other studies of 57 Greek women (one and six weeks postpartum) and 1053 Spanish women (at eight and 32 weeks postpartum) have reported no associations between positive thyroid antibody status and risk of PND (based on EPDS scores) (267, 273). However, based on the possible effects of thyroid antibodies, only one known randomised control trial was conducted by supplementing 100 μg thyroxine daily (n = 167) or placebo (n = 174) to women with thyroid antibody positive from six weeks to six months postpartum (275). Even so, no significant effect on PND (measured by the Montgomery and Asberg Depression Rating Scale) was found to exist.

In addition, individual thyroid hormones have been examined in relation to their association with PND. In a small sample observational study (n = 51), a high plasma TSH at four weeks postpartum has been found to increase the risk of depression of American women at the same time, however, only one person in the cohort showed abnormally high TSH (273). A larger sample of Swedish women's study (n = 365) reported a high TSH (> 4 mIU/L, measured at childbirth) increased the risk of PND at six months postpartum (2). The specificity (69.4%) and sensitivity (76.2%) of using serum TSH at childbirth to predict PND at six months postpartum were examined, suggesting serum TSH can be used as a routine screening test for PND (2). However, a 2003 prospective study examined 57 breastfeeding British women and did not find any association between TSH examined at one and four weeks postpartum and the risk of PND (EPDS scores ≥ 10) at six months after birth (276). Further, a large observational Spanish study (n = 1053) reported thyroid function indicators (TPOAb, fT4, and TSH), measured immediately after childbirth, did not predict later postnatal depression risks examined at eight weeks and 32 weeks postpartum (277).

Overall, it must be stated, that inconsistent results on the relationship between thyroid function on the risks of PND were more often reported. Further studies will be required using a standardised measure of postnatal depression scales to evaluate its predictive factors.

### 2.6.2 Selenium status

Selenium is suggested to offer increased protective effects on impaired mental health. Lower dietary selenium intake has been associated with an increased risk of de novo major depressive disorder among women (278), whereas adequate dietary intake of selenium has been shown to improve the mental outlook among the general population (279), including young adults living in New Zealand (280).

Results from a longitudinal Canadian study (Alberta Pregnancy Outcomes and Nutrition, APrON) found that women who used selenium supplementation during their pregnancy presented with a lower risk of having depressive symptoms at 12 weeks postpartum (281). Micronutrient supplementation in the APrON study was measured repeatedly by a self-reporting questionnaire, though selenium status was not measured and dietary intake from food was not included in the analysis. In contrast, a randomised clinical trial of supplementing selenium (100 μg/day as selenium yeast) to Iranian women early in primigravid pregnancy (n = 83) through to childbirth provided supportive evidence that prenatal supplementation may itself prevent PND (based on the EPDS), when compared to the placebo group (n = 83) (282). The mean EPDS scores in the selenium treatment group was 8.8, which is significantly lower than 10.7 in the placebo group, at eight weeks postpartum ($P < 0.05$). Such study outcome remained after adjusting for multiple confounders, including education, job and social support scores, and excluded subjects with existing depression (Beck Depression Inventory Test scores ≥ 31) (282). These results have demonstrated that prenatal selenium supplementation can impact positively on women's postnatal mental health. However, there have been limited published studies which examine postpartum women's selenium status in relation to the risk of postnatal depressive symptoms, particularly where continuous measurements were taken from childbirth to the end of the first postpartum year.

### 2.6.3 Iron status

Research shows an association between iron status and cognitive performance among women of childbearing age. American women (n = 37) from Pennsylvania, with a low Hb (< 120 g/L, analysed by HemoCue device) at the first week of postpartum, had an increased risk of developing PND 28 days after childbirth (based on the Centre for Epidemiological Studies-Depressive Symptomatology Scale, 16.4 vs 6.9, $P$ = 0.001) (283). This American cohort observational study suggested those postpartum women with symptoms of anaemia may benefit from iron supplementation (283). Unfortunately, iron status (serum iron or ferritin levels) was not measured in this study, neither was thyroid dysfunction screened.

Two well-designed interventional studies have also investigated the relation between iron status and postpartum women's emotion and cognition. A randomised six-month double-blind placebo-controlled trial was conducted by Beard et al. in 2005 (284). Low income postpartum South African mothers were allocated into three groups: nonanemic controls, anaemic mothers receiving placebo of the combination of 10 μg folate and 25 mg vitamin C, and anaemic mothers receiving 125 mg $FeSO_4$ in conjunction with 10 μg folate and 25 mg vitamin C (284). Their iron status (Hb, mean corpuscular volume, and transferrin saturation), cognitive and behavioural variables were measured at six weeks and nine months postpartum. The researchers observed a strong relation between IDA and depression and anxiety (measured by the Raven's Progressive Matrices test) at nine months postpartum, suggesting an accumulative effect of IDA on maternal cognition. Another more recent randomised placebo-controlled trial in 2017 was implemented on 70 Iranian mothers at seven days postpartum diagnosed with PND (EPDS score ≥11 and confirmation from further psychiatric interviews) (285). This was a six-week interventional study, non-anaemic women with PND were given 50 mg elemental iron daily starting from one week after childbirth. Reduced EPDS scores from 12 to 9 ($P < 0.001$) was observed in the iron supplementation group, while non-significant changes from 13 to 12 ($P$ = 0.13) in the placebo group. The results highlighted that early iron supplementation had led to a

positive reduction in the EPDS scores, together with 43% of women from the treatment group having negative psychiatric interviews (285). It was noted also that a higher percentage of women with depletion of iron stores (serum ferritin < 15 μg/L) continuously presented with postnatal depression (27%) when compared to women with sufficient iron stores (4%, *P* = 0.02) (285). These results suggested iron may play a critical role in the aetiology of PND.

Further, similar lowering effects of EPDS scores was reported in Italian women (n = 424) at one month postpartum after daily supplementing with iron-containing multiple-mineral and vitamins (Elevit), particularly evident among women with a baseline EPDS score higher than 12. Unsurprisingly, no significant changes were found among women (n = 428) in the control group with dual supplementation of 500 mg calcium and 400 IU vitamin D3 (286). However, it was unclear the amount of iron which was supplemented in the treatment group. The researchers concluded iron mixed with vitamins played a favourable role in improving postnatal depression (286).

In respect of the studies described above, iron status at different timing and stages of postpartum was measured, also their chosen screening scales and cut-offs varied, and some appear to have used follow-up psychiatric interviews to further diagnose PND. Therefore, it is inconclusive whether, and to what degree, unfavourable iron status contributes to the risk of depression or at which defined stages of postpartum. Although some high-quality control trials revealed a positive contribution of improving iron status towards postpartum women's mental health (287), PND is a multifactorial condition. Further research is required to better identify risk factors, and to understand the role of ID and IDA in relation to depression.

## 2.7 Summary of the literature review

Postpartum thyroiditis is one of the thyroid dysfunctions occurring in the first year after parturition, and even subclinical forms may develop into permanent thyroid dysfunction. However, as far as can be ascertained, there are no current available research data regarding the prevalence of postpartum dysfunction in New Zealand.

Previous literature has suggested that iodine, selenium, and iron are important in the synthesis of thyroid hormones. However, limited research has investigated the interaction of these three nutrients simultaneously within the thyroid function.

Iodine deficiency affects all ages and stages of human life, especially postpartum women and their infants. Studies conducted to date support the importance of early gestational iodine status in infants' neurodevelopment, which enables children to reach their optimal cognitive potential and academic performance in later years. Accordingly, the first 1000 days of life remain an important window of opportunity for optimal growth and development. Further research is required to investigate maternal postpartum iodine status in relation to early infant development. Re-emerging iodine deficiency has continued be a concern in New Zealand since the 1990s, so in an attempt to overcome such a deficiency, two government initiatives were introduced: 1) mandatory fortification of bread and bread products with iodised salt from September 2009, and 2) the provision of iodine supplementation (150 μg/day) for all pregnant and lactating women in 2010. Subsequent studies have reported that most adults and children in New Zealand may now have reached adequate iodine intake/status, but further research is needed to examine more widely iodine intake and status of postpartum women and their infants in New Zealand.

Selenium plays an important role as an antioxidant involved in thyroid function, mental health, and child development. New Zealand soils continue to provide low selenium to local food supplies. Low selenium intake has been reported in women of childbearing age and postmenopausal women, however, few research studies on breastfeeding women and their infants have been published. Given recent changes in dietary habits (excluding animal-products, such as chicken), increased food product availability and changing agricultural practices, the continual monitoring and reporting of both selenium intake and status in this vulnerable postpartum population is essential.

Postpartum women are known to pose a low risk of developing ID and IDA. However, unless significant blood loss during childbirth, iron status is not routinely monitored during the postpartum period. Despite possible negative effects on maternal-infant interactions and infant development, there has been insufficient recent research concerning postpartum women's iron status in New Zealand.

Lastly, PND is one of the major maternal health issues to arise and can often exacerbate negative health effects for mothers and their newborns. Previous literature has indicated possible biological contributors to PND are suboptimal thyroid hormones, and low selenium and iron status, but results published to date are inconclusive. Further opportunities of investigating iodine (the major component of thyroid hormone), selenium and iron together may provide additional evidence on their potential influence on the risk of postnatal depression.

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# Chapter 3 Selenium intake in iodine-deficient pregnant and breastfeeding women in New Zealand

*Historically, insufficient selenium intake exists in the New Zealand population, and numerous studies have investigated selenium intake during the decade of 1980-1990. However, limited studies have monitored selenium intake/status in recent years, especially in pregnant and breastfeeding women (noted in Chapter 2).*

*This chapter reports the results of a secondary data analysis arising from the Mother and Baby pilot study over the 2009 and 2011 period (Study 1). It explores the selenium intake in a sample of iodine-deficient pregnant and breastfeeding women residing in Palmerston North, in the North Island of New Zealand. Ethics approval was obtained from the Massey University Human Ethics Committee (Southern A 08/32 and 10/54). The main findings suggest suboptimal dietary selenium intake continues to be of concern for both pregnant and breastfeeding women and their breastfed infants in New Zealand. Inadequate intake of selenium for pregnant women was not followed up, as it is not within the scope of this PhD thesis. The focus of this thesis is on a cohort of breastfeeding women; it is pivotal to measure their actual selenium status by assessing plasma selenium. As selenium has numerous roles, it is also necessary to investigate any effects of low intake on other health outcomes during the perinatal period, specifically postnatal depression. The research gaps arising from this pilot study predominantly contributed to form the basis of the Mother and Infant Nutrition Investigation (MINI) study research questions and final design.*

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## 3.1 Abstract

Background

Selenium plays a role in antioxidant status and, together with iodine, in thyroid function. Iodine deficiency exists in New Zealand during pregnancy and lactation, and selenium deficiency may further affect thyroid function.

Objective

This study investigated selenium intakes of pregnant and lactating women, in Palmerston North, in the North Island of New Zealand.

Methods

Dietary intake was estimated using three repeated 24-hour dietary recalls. Dietary intake in pregnancy was also estimated from 24-hour urinary excretion of selenium. Selenium concentrations were determined in urine and breastmilk using inductively coupled plasma mass spectrometry.

Results

Median (p25, p75) selenium intakes based on dietary data were 51 (39, 65) μg/day in pregnancy and 51 (36, 80) μg/day in lactation, with 61% and 68% below estimated average requirement (EAR). Median daily selenium intake in pregnancy based on urinary excretion was 49 (40, 60) μg/day, with 59% below EAR. Median selenium concentration in breastmilk was 11 (10, 13) μg/L and estimated median selenium intake for infants was 9 (8, 10) μg/day, with 91% below the Adequate Intake of 12 μg/day.

Conclusions

These pregnant and breastfeeding women were at risk of dietary selenium inadequacy. Further research is required to assess selenium status in relation to thyroid function and health in this group.

## 3.2 Introduction

The intake of selenium worldwide ranges from 7 to 4990 μg/day and varies greatly from deficient to toxic intakes (1). New Zealand soils contain low levels of selenium, leading to low levels in the food supply (2). The most recent New Zealand Total Diet Survey suggested dietary selenium intake was inadequate throughout the New Zealand population, putting them at risk of deficiency (3). Recent New Zealand studies have shown low selenium intakes in women of childbearing age and older women based on urinary selenium excretion (4, 5).

Selenium is essential in human health to produce selenoproteins, which have antioxidant and anti-inflammatory roles, and for production of thyroid hormones (6). Selenoproteins (iodothyronine deiodinases) are required for generating the active thyroid hormone $T_3$ (triiodothyronine) from the inactive $T_4$ (thyroxine) form (7). Selenium is also an essential cofactor for glutathione peroxidase, a potent antioxidant, which protects thyroid cells from damage due to any excessive hydrogen peroxide generated from the synthesis of thyroid hormones (8).

Selenium has been suggested to play an important role in normal brain development, although the mechanism is not clear. Two recent large cohort studies from Poland and Spain found selenium status in the first trimester was adversely associated with neuropsychological development assessed at one year and two years of age by the Bayley Scales of infants and Toddler development (9), and five years of age by the McCarthy Scales for Children's Abilities (MSCA) (10). Varsi et al. (2017) investigated the effect of maternal selenium status on neurodevelopment of infants and reported that low serum selenium concentration in pregnancy was negatively associated with infant psychomotor score at six months of age (11).

The interaction between selenium and iodine in thyroid hormone synthesis is of particular concern in New Zealand due to dietary insufficiency of both selenium and iodine. Iodine deficiency has historically been a health problem in New Zealand (12) and the mandatory fortification of all bread (except organic and unleavened) with

iodised salt was introduced in September 2009 (13). Since mandatory fortification, most adults (14) and school-aged children (15) in New Zealand have adequate iodine intakes. Despite an iodine supplement being recommended and available to all pregnant and lactating women in New Zealand, this population group still has insufficient intakes and low status (16). Selenium deficiency could potentially exacerbate the consequences of mild iodine deficiency in this vulnerable group (12).

During pregnancy and lactation, there are increased selenium requirements for the growing fetus and newborn (3). Low maternal serum selenium concentrations are associated with adverse pregnancy outcomes such as pre-eclampsia (17), other types of pregnancy-induced hypertension (18) and preterm birth (19). Human milk is critical for an exclusively breastfed infant's optimal selenium status. A study in the South Island of New Zealand (1998–1999) showed postpartum women and breastfed infants had low plasma selenium, suggesting suboptimal status (20). Since then, no data about selenium intakes have been collected for this population. Given changes in dietary habits, food product availability and agricultural practices, continual monitoring of selenium intake in this vulnerable population is essential.

This study aimed to assess current maternal selenium intake during pregnancy and lactation and estimate infant selenium intake in a sample of women in Palmerston North, North Island, New Zealand.

## 3.3 Materials and methods

### 3.3.1 Study population

Pregnant and breastfeeding women were recruited from January to July 2009 and January to September 2011 via local health professionals who work closely with pregnant and breastfeeding women, as described previously (16). Volunteers were aged 16 years and older, in their third trimester of pregnancy (greater than 26 weeks of gestation), or at least three weeks postpartum and breastfeeding. Women who had medical complications during their pregnancy were excluded. Women had to actively

volunteer for this study and no data were kept from women who did not meet the selection criteria (16). Ethical approval was obtained from the Massey University Human Ethics Committee (Southern A 08/32 and 10/54). Written consent was obtained from all participants.

### 3.3.2 Dietary data collection

A 24-hour dietary recall was conducted based on the US Department of Agriculture Automated Multiple-Pass Method but excluded the Forgotten Foods List (21). A photographic food atlas was provided to estimate portion sizes (22). Participants were also asked to include any dietary supplements taken, including the brand name and the amount. Two subsequent recalls were collected via telephone interviews over the following two weeks, ensuring a weekend day was included; food portion sizes were estimated using household measures. Previous research has found no difference in energy intakes when comparing 24-hour dietary recalls collected in person versus via the telephone (23). Dietary data were analysed using Foodworks 2009 (Xyris Software, Brisbane, Australia) based on the New Zealand food database (24). Dietary supplements used by participants were included in dietary data analysis.

The estimated average requirement (EAR) cut-point method can be used to assess population nutrient intake providing nutrient requirements are normally distributed (e.g., selenium); the percentage below the EAR approximates the proportion that is at risk of dietary inadequacy (25). For a population to have a very low prevalence of inadequate dietary intakes, the mean/median intake should be above the recommended daily intake (RDI) (25). Current intakes based on diet and urine data were compared to Australian and New Zealand recommendations; the EAR and RDI for selenium for pregnant women are 55 and 65 μg/day, and for lactating women are 65 and 75 μg/day, respectively (3).

### 3.3.3 Sample collection and selenium analysis of urine and breastmilk

All participants were asked to collect a 24-hour urine sample and provided with an insulated box containing two polythene bottles for urine storage and frozen silica pads to keep the sample cool. Lactating women were also requested to provide a breastmilk sample (around 30 mL) and provided with a breast pump if required; timing of collection of breastmilk samples was not standardized, since no significant differences have been found in selenium concentrations between hind-milk and fore-milk (26). The concentration of selenium in breastmilk varies most significantly during the first 21 days from the transition from colostrum to mature milk (26), thus breastmilk samples were collected after three weeks postpartum. All samples were brought immediately to the Human Nutrition Research Unit for processing after collection. The total volume of urine collected over 24 hours was measured for each participant. Samples were stored without preservative at –20 ◦C, prior to analysis. Urine samples were defined as inaccurate if urine volume was below 1 L and urinary creatinine below 5 mmol/day, or extreme outliers of creatinine (> 3 standard deviation) (27). However, no study samples were classified accordingly.

Selenium concentrations of urine and breastmilk samples were determined by Hill Laboratories, Hamilton, New Zealand, using inductively coupled plasma mass spectrometry (28). Quality Control procedures included analysis of blanks, analytical repeats and spiked samples in order to ensure accuracy and precision. Calibration standards and checks were undertaken on every run with the limit of detection at 0.002 mg/kg. Dietary selenium intake was estimated for pregnant women, based on a urinary excretion of 55% of selenium intake (29). However, it was not possible to estimate dietary selenium intake for lactating women via urine, as we were unable to determine the daily loss of selenium from breastmilk. Creatinine was measured using the Jaffe Method Flexor E (Vital Scientific NV, 6956 AV Spankeren/Dieren, Rheden, Gelderland, The Netherlands) at the Massey University Nutrition Laboratory.

### 3.3.4 Statistical analysis

Data were analysed using IBM SPSS (Statistics Package for the Social Sciences, IBM, Armonk, NY, USA) version 20. Data were tested for normality using Shapiro-Wilk's test. Non-parametric data were expressed as median ($25^{th}$, $75^{th}$ percentile) and parametric data expressed as mean (± standard deviation; SD). Bivariate correlations were tested using the nonparametric Spearman's rho correlation coefficient. Scatter plots were generated for suspected bivariate correlations and visually inspected for verification. Fisher's exact test was used to detect associations between dietary and biological methods in assessing dietary intake.

## 3.4 Results

Fifty-nine pregnant and 68 lactating women were recruited. The mean age was 31.6 ± 5.7 and 31.3 ± 5.0 years for pregnant and breastfeeding women, respectively (Table 3.1). The ethnicities of participants were Caucasian (80%, 81%), Maori (12%, 9%), Asian (5%, 2%) and other (3%, 8%). Participants were predominantly educated at tertiary level (86% pregnant and 68% breastfeeding), with approximately half being pregnant with or breastfeeding their first infant.

**Table 3.1 Description of pregnant and breastfeeding participants.**

| n (%) | Pregnant (n = 59) | Breastfeeding (n = 68) |
|---|---|---|
| **Age, years (Mean ± SD)** | 31.6 ± 5.7 | 31.3 ± 5.0 |
| **Tertiary Education** | 51 (86) | 46 (68) |
| **Ethnicity (Caucasian)** | 47 (80) | 56 (81) |
| **Ethnicity (Maori)** | 7 (12) | 6 (9) |
| **Ethnicity (Asian)** | 3 (5) | 1 (2) |
| **Ethnicity (Other)** | 2 (3) | 5 (7) |
| **Nulliparous** | 31 (53) | - |
| **First time lactation** | - | 36 (53) |
| **Age of infants, days (Mean ± SD)** | | 113.4 ± 96.9 |

Median selenium intake based on dietary assessment among pregnant women was 51 (39, 65) µg/day, below both the RDI (65 µg/day) and EAR (55 µg/day), with 61% below the EAR (Table 3.3). Median urinary selenium for pregnant women was 14.1 (9.1, 18.2) µg/L (Table 3.2) and median selenium intake based on urinary excretion was 49 (40, 60) µg/day (Table 3.3); below both the RDI, with 59% below the EAR. Dietary and urinary data both suggest inadequate selenium intakes among pregnant participants. Only four of the 59 pregnant and six of the 68 lactating women were taking selenium-containing supplements.

**Table 3.2 Selenium and creatinine in 24-hour urine samples and selenium in breastmilk.**

| Median (p25, p75) | Pregnant | Breastfeeding |
|---|---|---|
| **Numbers of participants (n)** | 59 | 68 |
| **Urine volume (L)** | 2.2 (1.5, 3.0) | 1.8 (1.2, 2.5) |
| **Urinary selenium concentration µg/L** | 14.1 (9.1, 18.2) | 12.1 (7.8, 19.9) |
| **Measured 24-hour urinay selenum µg/day** | 27.1 (22.0, 32.9) | 21.2 (14.5, 29.9) |
| **Urinary creatinine g/L** | 0.5 (0.4, 0.7) | 0.7 (0.5, 1.1) |
| **Urinary creatinine g/day** | 1.2 (1.0, 1.5) | 1.3 (1.2, 1.4) |
| **Selenium: creastinine µg/g** | 22.8 (17.7, 28.7) | 16.5 (12.3, 23.8) |
| **Selenium in breastmilk µg/L** | - | 11.3(10.0, 13.3) [a] |

[a] n= 64 for breastmilk samples.

Based on dietary assessment the median selenium intake for breastfeeding women was 51 (36, 80) µg/day (Table 3.3), also below both the EAR (65 µg/day) and RDI (75 µg/day), with 68% below the EAR. Median selenium concentration in breastmilk (n = 64) was 11 (10, 13) µg/L (Table 3.2). Using an estimated daily breastmilk intake of 750 ml (30), the median estimated selenium intake for infants was 9 (8, 10) µg/day; 70% (45/64) were below the daily minimum of 10 µg/day suggested by Levander (31), and 91% (58/64) below the Adequate Intake of 12 µg/day (32).

For breastfeeding women, selenium concentration in breastmilk was weakly, positively correlated with 24-hour selenium excretion in urine as µg/day (r = 0.269, *P* = 0.032, see Appendix 16.2). Pregnant participants' dietary selenium intake based on dietary assessment was not associated with selenium excretion as either µg/L (r = 0.053, *P* = 0.692) or µg/day (r = 0.230, *P* = 0.079). However, the classification of intakes

as either above or below the EAR were associated for the two methods of assessing dietary intake ($P$ = 0.016, Fisher's Exact Test).

**Table 3.3 Estimated selenium intake in pregnant and breastfeeding women, infants and comparison to recommendations.**

| Selenium Intake | Pregnant (n = 59) | Breastfeeding (n = 68) | Infant (n = 64) |
|---|---|---|---|
| **Estimated selenium intake; median (p25, p75)** | | | |
| **Based on 24-hour urine, µg/day** | 49 (40, 60) | | |
| **Based on 24 hour dietary recalls, µg/day** | 51 (39, 65) | 51 (36, 80) | - |
| **Below EAR (55 µg/day) (n, %)** | | | |
| **Based on 24-hour urine** | 35 (59) | | |
| **Based on 24-hour dietary recalls** | 36 (61) | 45 (68) | - |
| **Estimated selenium intake; median (p25, p75) Based on 750 ml breastmilk per day** | - | - | 9 (8, 10) |
| **Below (10 µg/day) (n, %)** | - | - | 45 (70) |
| **Below (12 µg/day) (n, %)** | - | - | 58 (91) |

[a] EAR = estimated average requirement, 55 µg/day for pregnant women and 65 µg/day for breastfeeding women.

## 3.5 Discussion

This study found 59–61% of pregnant and 68% breastfeeding participants had estimated selenium intakes below the EAR, suggesting this vulnerable group is at risk of an inadequate selenium intake. This supports the latest New Zealand Adult Nutrition Survey 2008/2009, which estimated that 44–72% of women aged 19–50 years had inadequate selenium intakes (33). Previous research shows that low selenium status is associated with an increased risk of thyroid enlargement, which may indicate compromised thyroid function (34). Iodine deficiency has previously been reported in both pregnant and breastfeeding women in New Zealand in the same cohort investigated in this study (16), and selenium deficiency could further compromise thyroid function.

In the present study, dietary intake was assessed by three 24-hour dietary recalls, due to its low participant burden and good compliance. Under- or over-reporting is a concern for dietary assessment. As energy expenditure was not recorded, we were

unable to determine if participants had misreported dietary intake. A large daily variation of selenium intake was reported in an earlier study of American pregnant and postpartum women using duplicate-plate food and drink composites and dietary recalls (35). Single 24-hour recalls do not consider day-to-day variation, therefore repeated 24-hour dietary recalls are frequently used to estimate usual intake (36).

In the current study, 24-hour urinary selenium excretion was used to estimate selenium intake. It is estimated that 50–60% of dietary selenium is excreted in urine (29), and selenium intake determined in this manner is suggested to be more accurate than dietary assessment data (37). However, collecting 24-hour urine samples requires motivated participants and is not practical for all populations or large studies. Urinary selenium has been shown to be a valid method to assess recent selenium intake in populations that live in selenium-deficient areas (37, 38). Research has shown that serum selenium and glomerular filtration rate increase in pregnancy, and studies have shown an increase in selenium in urine during pregnancy (39). Thus, the selenium excretion of 55% could be overestimated, so actual selenium intakes could be even lower than estimated values. A previous New Zealand study found selenium intake determined from a Food Frequency Questionnaire was associated with 24-hour urine excretion in pregnant women (39). Although the current study found no such association in pregnant women, the classification of intakes as either above or below the EAR was associated for the two methods of assessing dietary intake.

Median intake of selenium for pregnant women in the current study was 51 µg/day based on dietary intake and 49 µg/day based on urine excretion. In previous studies of New Zealand pregnant women, Watson and McDonald found median intakes ranging between 33.5 µg/day excluding dietary supplements to 67 mcg/day including dietary supplements (40), however, these data were based on dietary assessment with no verification using biomarkers. The median selenium intake of 51 µg/day for breastfeeding women was higher than previously reported (46 µg/day) in the 1998–1999 study of lactating mothers from the South Island of New Zealand (20). This was not unexpected, as selenium intake is typically lower in the South Island of New

Zealand, where bread is made from local wheat, compared to the North Island, where bread is manufactured from wheat imported from Australia, which has higher levels of soil selenium (12). It could also be due to changes occurring in diet in the last 20 years. Even though selenium intake is higher among breastfeeding women in the current study than previously reported, many current intakes are still below the EAR, thus suggesting a risk of dietary inadequacy.

Breastmilk selenium concentration is associated with maternal selenium intake and/or status. Selenium is generally higher in colostrum (26 μg/L), and then decreases to nadir levels in mature milk (1–3 months, 15 μg/L) (31). Median selenium breastmilk concentrations (11.3 μg/L) in the present study were similar to those reported in the South Island in 1992 (13.4 μg/L) (41) and also a recent study in the North Island (14 μg/L) (42). Adequate selenium concentrations in breastmilk have been observed to maintain optimum selenium status in both preterm and term infants (26). For exclusively breastfed infants, breastmilk is the only source of selenium; in the current study, 70% of infants would not have achieved the 10 μg/day suggested as adequate by extrapolation from adults (31) and 91% did not achieve the Adequate Intake of 12 μg/day (18). This suggests infants in the present study are at risk of selenium deficiency.

The inadequate selenium intakes in this vulnerable population are of concern. Studies in rats have previously shown that in utero selenium deficiency can impair neonatal lung development (43). Maternal selenium status in French women was negatively associated with risk of wheezing in children aged 1–3 years; this could potentially lead to asthma later in life (44). Low selenium status in childhood in New Zealand has also been associated with increased risk of wheeze (45), for which New Zealand has a high incidence (46). Low maternal selenium status in Norwegian women has been associated with an increased risk of neonatal infections in the first 6 weeks of life and lower psychomotor score at six months (11). Adequate dietary intake of selenium has been suggested to be beneficial in improving mental outlook among the general population (47). Lower dietary selenium intake has also been associated with an

increased risk of de novo major depressive disorder among women (48). Selenium supplementation during early pregnancy has been found to reduce postnatal depression (49), which has a 7.8% to 16% prevalence in New Zealand (50).

Determining selenium concentrations in blood (whole, plasma or erythrocyte), plasma selenoprotein P or GPx activity in blood (whole, plasma or platelet) are considered more reliable markers of selenium status (51, 52) However, urinary selenium excretion is associated with both plasma selenium and dietary intake in populations with low selenium intake (12). A limitation of the current study is not measuring selenium or GPx activity in blood, however, determining daily urinary selenium excretion serves as a proxy measure for selenium intake and indicates the need for further research.

This study included a small sample of pregnant and breastfeeding women who were predominantly well educated and more likely to be affluent, thus the sample is not representative of the New Zealand population. However, women who volunteer for health studies tend to be interested in health and motivated towards a healthy lifestyle, thus it is of concern that these women are at risk of selenium deficiency. Further, we would not expect such women to have a poorer health status than less affluent women. The age spread of infants may indicate that some infants were not exclusively breastfed, and consuming complementary food may impact on infant selenium intake.

Additionally, supplement intakes could contribute to participants' dietary selenium intake; however, only a small proportion of participants consumed selenium-containing supplements. Thus, we were not able to meaningfully investigate the potential impact of supplement intake on other measures.

## 3.6 Conclusions

This current research suggests dietary selenium intake is a concern for pregnant and breastfeeding women and their infants in New Zealand. Further research is required

to assess selenium status among these groups by measuring biomarkers such as plasma selenium or GPx activity in blood selenium. Further investigations should also include all socioeconomic groups. It is essential that we assess whether suboptimal intake of selenium adversely affects thyroid function in this already iodine deficient population. As selenium is a nutrient with numerous roles, it is also necessary to investigate any effects of low intake on other health outcomes potentially related to selenium in the perinatal period, such as postnatal depression and impaired infant neurodevelopment.

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# Chapter 4 Study Protocol: Mother and Infant Nutrition Investigation in New Zealand (MINI Project): An Observational Longitudinal Cohort Study

*Chapter 4 describes the study protocol followed in the Mother and Nutrition Investigation (MINI) study. Ethics approval was obtained from the Health and Disability Ethics Committee (15/NTA/172) in December 2015. The study was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12615001028594). This manuscript presents information on the research participant recruitment; the questionnaires used to collect data; methods used to estimate dietary intake; and maternal and infant anthropometry measurements. In addition, it details the process of biological sample collection and biomarker analysis of urine, breastmilk, blood, and nail clipping samples. It provides information on the study outcome measures, including iodine, selenium and iron intake and status; maternal thyroid function; longitudinal assessment of maternal mental health and infant neurodevelopment.*

*A large volume of data has been collected in this observational longitudinal cohort study. Only certain data analyses are reported in this thesis, including data collected from the maternal questionnaires; weighed four-day dietary diary, maternal and infants' anthropometric measures; thyroid gland volume determined via ultrasound; biological sample analysis of iodine, selenium and iron status; and thyroid hormone concentrations. Also, the thesis contains the results from assessing maternal mental health using the Edinburgh Postnatal Depression Scale.*

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## 4.1 Abstract

Background

Thyroid dysfunction is associated with cognitive impairment, mood disturbance, and postnatal depression. Sufficient thyroid hormone synthesis requires adequate intake of iodine, selenium, and iron. Iodine deficiency was historically a problem for New Zealand, and initiatives were introduced to overcome the problem: (1) mandatory fortification of all bread (except organic and unleavened) with iodized salt (2009) and (2) provision of subsidised iodine supplements for pregnant and breastfeeding women (2010). Subsequent to these initiatives, most adults and children have adequate iodine status; however, status among breastfeeding women and their infants remains unclear. This paper outlines the methodology of the Mother and Infant Nutrition Investigation (MINI) study: an observational longitudinal cohort study of breastfeeding women and their infants.

Objectives

This study will determine women's iodine intake and status between supplement users and nonusers; women's intake and status of iodine, selenium, and iron relating to thyroid function; associations between women's selenium status, thyroid function, and postnatal depression; and infants' iodine and selenium status relating to first year neurodevelopment.

Methods

Breastfeeding women aged over 16 years with a healthy term singleton infant were recruited from Manawatu, New Zealand. Participants attended study visits three, six, and twelve months postpartum. Maternal questionnaires investigated supplement use before and after birth, iodine knowledge, and demographic information. Dietary assessment and urine, blood, and breastmilk samples were taken to measure iodine, selenium, and iron intake/status. The Edinburgh Postnatal Depression Scale was used repeatedly to screen for postnatal depression. Thyroid hormones (free

triiodothyronine, free thyroxine, thyroid stimulating hormone, thyroglobulin, antithyroglobulin antibodies, and thyroid peroxidase antibodies) were measured in blood samples, and thyroid gland volume was measured by ultrasound at six months postpartum. Infant iodine and selenium concentrations were determined in urine. The Ages and Stages Questionnaire was used to assess infant development at four, eight, and twelve months.

Results

Data collection was completed. Biological samples analysis, excluding nail clippings, is complete. Data analysis and presentation of the results will be available after 2020.

Conclusions

This study will provide data on the current iodine status of breastfeeding women. It will also provide a greater understanding of the three essential minerals required for optimal thyroid function among breastfeeding women. The prospective longitudinal design allows opportunities to examine women's mental health and infant neurodevelopment throughout the first year, a crucial time for both mothers and their infants.

## 4.2 Introduction

Postpartum women experience abnormalities in thyroid function at twice the prevalence of the general population (1). Thyroid hormone is essential in maintaining the human body's metabolism, temperature (thermoregulation), and psychological mood (2). In a developing brain, thyroid hormone is responsible for adequate myelination, neuron cell maturation, and central nervous system development (3). Optimal thyroid function relies on adequate biosynthesis of thyroid hormones, which depends on three dietary minerals: iodine, selenium, and iron (4, 5). Pregnancy increases thyroid hormone turnover; women with limited thyroidal reserve or marginal iodine deficiency are at increased risk to develop thyroid dysfunction after birth (6), which is one of the most common endocrine disorders that postpartum women experience (7).

Iodine is the major component of thyroid hormones and a regulator for the synthesis and secretion of the thyroid hormones triiodothyronine ($T_3$) and thyroxine ($T_4$). Selenium, as a component of the selenocysteine-containing proteins glutathione peroxidase, protects the thyroid gland from oxidative damage (5). Selenoproteins are required to convert $T_4$ to $T_3$, the active form of thyroid hormone. Iron is required for haem-dependent thyroperoxidase activity, which is required for the synthesis of adequate thyroid hormone. Selenium deficiency and iron deficiency anemia may negatively affect thyroid hormone synthesis by impairing selenium- and iron-dependent enzyme activities, even if iodine status is adequate (5). Previous research has investigated iodine, selenium, and iron intake/status separately or a combination of any two of them among women of childbearing age (8) and postmenopausal women (9). However, further research is needed to explore all three micronutrients together, acknowledging their close relationship in thyroid hormone synthesis.

Thyroid dysfunction is a significant health issue in New Zealand, with women diagnosed at five times the prevalence in men (10, 11). Concerning adequate thyroid function, iodine, selenium, and iron play important roles. In New Zealand, soils

provide low levels of available iodine and selenium, resulting in low concentrations in the food supply (12), hence in the diet (13). Iodine deficiency in early life is associated with impaired neurodevelopment (14). Iodine deficiency was a concern in New Zealand in the early years of the 20th century, but its prevalence was mostly reduced through the introduction of iodized salt in the 1930s. However, since the 1990s, a number of studies in New Zealand have shown iodine deficiency has reemerged in adults (15), pregnant and breastfeeding women (16, 17), school children (18) and breastfed infants and toddlers (19). To improve iodine status in New Zealand, two government initiatives were introduced: mandatory iodized salt in commercially made bread and bread products from September 2009 and the provision of iodine (150 μg/day) supplementation for all pregnant and lactating women in 2010 (20). Although recent studies suggested that adults (21, 22) and children (23) in New Zealand may now have adequate iodine intake/status, both pregnant and breastfeeding women remain deficient. A pilot study of a small sample of self-selected highly educated pregnant and breastfeeding women assessed urinary iodine excretion, breastmilk iodine concentration, and blood thyroglobulin and suggested iodine deficiency (24). There is a need for a more robust investigation into the iodine status of postpartum women and their infants from a wide range of socioeconomic backgrounds.

Low selenium status in New Zealand has been partially reversed by increased consumption of imported flour from Australia (which generally has higher selenium concentrations than flour produced in New Zealand) (25, 26). In addition, both pregnant and breastfeeding women have an increased requirement for dietary selenium due to the demands from the fetus and breastfed infants. Previous research, which investigated selenium status among postpartum women and their infants in New Zealand 20 years ago (27), measured urinary selenium excretion and plasma selenium and indicated that such women were at risk of selenium deficiency. To our knowledge only one small study of breastfeeding women, by our research group, assessed dietary selenium, urinary selenium excretion, and breastmilk selenium

concentration and suggested selenium inadequacy was still a concern (28). Research investigating selenium status among postpartum women and their infants is limited.

Health professionals closely monitor the iron status of women during pregnancy. However, after birth, management of iron status can be inconsistent. Results from a UK multicentre study reported only 50% of postpartum women had hemoglobin levels checked after delivery (with 30% of those women confirmed as anemic), while the overall iron stores of participating women remained unexamined (29). Generally, postpartum women's iron status recovers as a consequence of cessation of menstrual bleeding since conception or a minimal secretion of iron via breastmilk if breastfeeding (30). However, if women have suffered iron deficiency before and/or during pregnancy and/or have experienced significant blood loss during childbirth, their iron status may not reach optimal levels even if an intervention subsequently occurs. A New Zealand study of 186 women found 77% of women were not tested for hemoglobin levels after childbirth. Further, out of those most at risk (with low iron status during late pregnancy and high blood loss, exceeding 500 mL, during childbirth), few women were then retested for their iron status after 10 days postpartum (31). Iron status of postpartum women remains largely underreported.

Low serum selenium has been identified as an independent risk factor for depression (32) and selenium supplementation has been observed to reduce postnatal depression (33). Postnatal depression is one of the main disorders women experience postnatally, its onset being timed at six weeks to six months after birth (34). Most women will recover from postpartum depression, though approximately one-quarter of affected women report being depressed when their infant reaches their first birthday (34). Using the measured criteria of postnatal depression on the Edinburgh Postnatal Depression Scale (EPDS), the prevalence of postnatal depression in New Zealand was about 8% in 1994 and 16% in 2006 (35). In the 2015 New Mothers' Mental Health Survey (36), the prevalence was 14% and is now recorded as the most common disorder for mothers in their first year after childbirth (37).

Of additional concern, mothers are often reluctant or unable to seek help when they experience symptoms of postnatal depression (36). Such underdiagnosed and untreated mental health conditions affect both the mother and their children's ongoing cognitive, emotional, and behavioral development (38). Despite other social and psychological etiology of depression, potential links between micronutrient status, thyroid hormone, and the risk of postpartum depression need to be further explored. This may help develop new preventive approaches to lower the risks of postpartum depression.

## 4.3 Study objectives

The study's primary outcomes include investigating breastfeeding women's iodine intake and status among supplement users and nonusers following the implementation of two government initiatives to improve iodine status; examining maternal iodine, selenium, and iron intake status; and exploring iodine, selenium, and iron status in maternal thyroid function.

In addition, the study provides preliminary data on possible associations between women's selenium status, thyroid function, and postnatal depression over a one-year period and infants' iodine and selenium status in relation to neurodevelopment during their first year of life. Ultimately, this research will inform a future larger study of potential variables impacting maternal thyroid function and the risk of postnatal depression, together with early infant neurodevelopment.

## 4.4 Methods

### 4.4.1 Study design and overview

The MINI study is an observational longitudinal cohort study spanning the first postpartum year. It was approved by the Health and Disability Ethics Committee (15/NTA/172) in December 2015. The study's ethics approval was registered with the

Royal New Zealand Plunket Ethics Committee in June 2016. The MidCentral District Health Board in New Zealand also approved the study.

The study is being conducted in the Human Nutrition Research Unit at Massey University, Palmerston North, New Zealand. The first study visit for participants is at approximately three months postpartum (3MPP), and follow-up assessments take place at six months (6MPP) and twelve months postpartum (12MPP) (Appendix 7).

### 4.4.2 Selection criteria

The target population for the study were healthy breastfeeding women aged over 16 years who had birthed a healthy term singleton infant three months prior. Women were excluded if they developed significant health problems, such as metabolic disease or cancer. Women were excluded if they had been diagnosed or treated at any time for hyperthyroidism or hypothyroidism. Participants were required to live within or near the local Palmerston North area and be able to attend Massey University for scheduled study visits. Women of any ethnic and socioeconomic status were eligible.

### 4.4.3 Recruitment and participation

Posters to promote the study were placed at selected sites (General Practitioner surgeries, midwifery clinics, pharmacies, antenatal classes, ultrasound clinics, maternal wards in hospitals, local community playgroups, and early childhood centres, etc.). Local newspapers and social media sites were used to publicise the study. Local midwives, childbirth educators, and lactation consultants were asked to raise awareness of the MINI study to their clients. An effort was made to recruit women from a wide range of socioeconomic backgrounds and ethnic groups, including Maori, Pacific Islanders, and Asian women. Potential participants responded by recording an expression of interest online or via telephone or email. Prospective participants were provided with a study information sheet. Interested participants then completed a screening questionnaire to ensure eligibility. Written informed consent was obtained from all participants before their enrolment in the

study. Mothers also gave written content to their infants' participation in the study. After providing informed consent, participants were assigned a unique identifier code and scheduled for their first study visit.

### 4.4.4 Sample size calculation

The main outcome measure was iodine excreted per day, and the sample size was calculated using G*Power 3.1 (Heinrich Heine University) based on data (mean and standard deviation) from a preliminary study of breastfeeding women (24). Calculation used one-way analysis of variance with two groups (95% power, $\alpha = 0.05$, two-tailed) and three repeat measures; 80 participants were needed, using expected mean daily urine iodine concentrations of 140 and 100 μg/L for iodine supplement users and nonusers, respectively, and a standard deviation of 60.

### 4.4.5 Outcome measures

#### *4.4.5.1 Questionnaires*

At the initial visit, general baseline questions were asked about salt and supplements use, nutrition knowledge of iodine, tobacco and alcohol use, breastfeeding patterns, general health, and demographic information (including age, ethnicity, educational attainment, household size, and income). Potential changeable information including tobacco and alcohol use, breastfeeding patterns, and general health was also sought at the second and third visits.

Participants were assessed about their general health and that of their infants by online questionnaire when infants reached six months and twelve months of age. During the postpartum period, stress may negatively affect immunity, and the occurrence of infection symptoms can be an estimated measurement of postpartum immune function. The Carr Infection Symptom Checklist, which has been validated for use with postpartum women (39), was used to measure the symptoms of infection experienced by the mother since the childbirth. The Infant Symptom Checklist (which

reports the frequency of symptoms of common illnesses in young infants) was used to measure the health of infants (39).

The 10-item EPDS was completed online by participating women to assess any symptoms of depression and anxiety over the previous seven days. Women recorded severity of symptoms on a 4-point scale (40). Specified anxiety disorders were evaluated using the EPDS-3A, a cluster of selected question items numbered three, four, and five from the original EPDS (41). This is a validated tool to screen for probable anxiety and depression during the postpartum period. A cut-off point of 13 or above was used to define high levels of depressive symptoms (36). Any woman whose score equalled 13 or above was advised to see her general practitioner for further evaluation as well as being provided with an information sheet containing postnatal depression services in New Zealand. Only study participants with the correct link supplied via emails could complete these questionnaires. All questions were answered in the same order. Participants could not go back to change their answers once the questionnaire was completed. Answers from incomplete questionnaires may be used for analysis.

The first year of infant neurodevelopment was assessed using a parent-completed Ages and Stages Questionnaire (ASQ) when the infant was aged four, eight, and twelve months (42). These questionnaires were self-administered and completed in hard copies. This screening tool uses parent observation to assess child development and behaviour and records results in five developmental domains: communication, gross motor, fine motor, problem solving, and personal-social. There are six questions in each domain, with answers of yes, sometimes, or not yet. A yes indicates reaching the achievement with ten points awarded, a sometimes indicates partial achievement with five points awarded, and a not yet indicates not achieved with 0 points awarded. The sum score of each domain was calculated and compared with the cut-off scores reached, which were derived empirically by subtracting two standard deviations from the mean for each area of development (42). A score below the cut-off point indicates a fail on the ASQ. The questionnaires were used to assess the relationship between

maternal and infant iodine and selenium status, maternal iron status, and recorded early child neurodevelopment.

#### *4.4.5.2 Dietary Intake*

To assess participant dietary intake including nutrients that may be associated with mental health and child development including iodine, selenium, and iron intake, participants were asked to complete a weighed four-day diet diary within two weeks of the initial study visit. All four days were consecutive and included one weekend day. Each participant was requested to record food items, brands, amount consumed, and the content of the nutritional information panel if applicable. All food and beverage items consumed were weighed and measured with a QM-7288 electronic kitchen scale (Digitech), and household measurement cups and spoons were provided. The Digitech scale can weigh up to five kilograms with an accuracy to one gram; all women were shown how to use the scale to quantify food items. All participants received both written and oral instructions on how to complete the record, which included a written example of a one-day food record. Women were also asked to include dietary supplements consumed. When eating or dining out, participants were asked to estimate the portion size of all food eaten. The food record and equipment were collected, or return posted two weeks after the initial visit.

A 69-item self-administrated semiquantitative iodine- and selenium-specific food frequency questionnaire, adapted from an Australian study of pregnant women (43), was used to estimate habitual maternal iodine and selenium intake at the first and third study visits. An iron-specific food frequency questionnaire, validated by other female population groups in New Zealand, was used to assess women's iron-related dietary patterns (44) at the second study visit. Within two weeks of this visit, participants also completed a three-day estimated food dietary record for their infants to enable assessment of infant nutrient intakes at weaning periods.

All dietary data were entered into Foodworks 9 Professional (Xyris Pty Ltd) online and analyzed using data sets from the New Zealand Foodfiles 2016 to estimate nutrient

intake. When food items were not included in Foodfiles 2016, new food items were created based on the information directly provided by participants (i.e. food packages) or from appropriate international databases from Australia and the United States. Estimates for iodine concentrations of categories of bread (e.g. white, fiber white, fruited, mixed grain) were based on data from the Ministry of Primary Industries (22), since iodine content has not been determined for all commercially made bread in New Zealand after the mandatory fortification of bread with iodized salt. It was difficult to quantify the amount of discretionary salt added to food. However, for women who reported using iodized salt, 48 μg of iodine (equivalent to one gram of salt) was added to their iodine intakes (21). Dietary supplements used by participants were entered into Foodworks as a new food item based on nutritional information obtained from the manufacturers. To ensure accuracy and completeness, a registered nutritionist (YJ) checked all dietary data and then transferred the data to SPSS Statistics (IBM Corporation) version 23 for statistical analysis.

#### *4.4.5.3 Anthropometry*

Maternal and infant anthropometry measurements were obtained at each study visit. Women's weight was measured using the same annually calibrated weighing scale with a capacity of 150 kilograms (Detecto). Before standing on the scale, participants were asked to remove their shoes and to wear minimum clothes. Body weight was recorded to the nearest 0.1 kilogram. Height was measured by using a Toledo stadiometer and recorded to the nearest millimeter (45). Maternal body composition was determined using both bioelectrical impedance analysis (InBody230, InBody Co) and air displacement plethysmography (BodPod, COSMED SRL). Measurements were completed under the following conditions: minimal clothing, wearing swimming cap, before midday, after urination, normal room temperature (20°C to 25°C), with no exercise, eating, drinking, or bathing/showering within two hours prior to measurement (preferably completing the measurement after breastfeeding the baby). On the day of the test, quality control steps for BodPod were carried out by following

the manufacturer's instructions, with acceptance criteria being volume ±100 mL of actual volume and standard deviation ≤ 75 mL.

Infant recumbent length was measured crown to heel using an infant length board and recorded to the nearest millimeter. Infant weight (without clothing and diapers) was measured using a baby weighing scale (Nagata Scale Co Ltd) and recorded to the nearest 10 grams. Infant head circumference was measured over the most prominent part on the back of the head (occiput) and just above the eyebrows (supraorbital ridges) by using a flexible non-stretch tape (45) and recorded to the nearest even millimeter.

#### *4.4.5.4 Ultrasound measurement of thyroid volume*

A portable ultrasound (uSmart 3200T Ultrasound System, Teratech Corp) equipped with a linear transducer (7 to 15 MHz) was used for the thyroid measurement. Women were examined in a supine position (an adequate neck extension was achieved by placing pillows under the shoulders). Longitudinal and transverse scans were performed. Measurements of anteroposterior diameter and width (mediolateral diameter) were obtained with electronic calipers on a transverse image. The maximum lobe length was measured on a longitudinal width. The total volume of each thyroid gland was the sum of the volumes of left and right lobes, excluding the volume of the isthmus but including any nodules and/or cystic areas. The formula used to calculate the volume for each lobe is anteroposterior diameter × width × length × 0.479 (46). A total volume greater than 18 mL was defined as thyroid enlargement based on the normative thyroid volume in iodine sufficient populations (47). Any participant with observed abnormalities was referred to clinical health professionals for further assessment.

#### *4.4.5.5 Biomarker analysis*

During each study visit, spot urine samples from each participating woman and her infant were collected to assess iodine, selenium, and creatinine excretion. All maternal

spot urine samples were collected in the morning and immediately frozen and stored at –20°C. Infant urine was collected using a 100 mL pediatric urine bag placed inside the diaper and checked every 10 minutes. The collected urine was frozen and stored at –20°C for later analysis. Spot urine samples can be used to estimate iodine status of a population but not for individual iodine deficiency diagnosis (48). As creatinine output is relatively constant, the adjusted iodine/creatinine ratio (μg iodine per gram creatinine) can be used as a proxy measure of iodine excretion (49). However, the total dietary iodine and selenium intake for lactating women cannot be estimated from urinary output because some of the iodine and selenium is diverted to breastmilk.

Lactating women were asked to provide a breastmilk sample (approximately 30 to 50 mL) at each visit using an Allegro electric breast pump (Unimom NZ) if required. All breastmilk samples were collected before noon on the study visit day, and timing of breastmilk collection was not standardized. Breastmilk samples were analyzed for iodine and selenium concentration, allowing for estimations of infant intake of iodine and selenium based on 750 mL/d of milk production (50).

Iodine and selenium concentration in both urine and breastmilk samples were determined by an accredited commercial laboratory (Hill Laboratories) using inductively coupled plasma mass spectrometry (ICP-MS) (51). Quality control procedures included analysis of blanks, analytical repeats, and certified reference material to ensure accuracy and precision. The Massey University Nutrition Laboratory measured creatinine using the Jaffe method in a Flexor E (Vital Scientific) biochemistry analyser.

To assess further selenium status, toenail clippings from women and nail clippings from infants were collected. Toenail clippings have been used to determine selenium concentrations in large cohort or epidemiological studies, such as for the preeclampsia risk in pregnant women (52). The instruction for sample collection was explained to participants during each study visit and nail clippings were self-collected by participating women at home, with the collected samples brought back by the

participants at the following study visit. All toenail clippings were stored at room temperature prior to analysis. Nail clipping samples will be prepared by using the method adapted from nail zinc analysis (53). This involves washing all nail clipping samples by using five minutes contact with 25 mL portions in the order of acetone, water, acetone, water, and water (54). Selenium concentration will be measured by ICP-MS.

During the second study visit, to assess blood hemoglobin concentrations, the handheld Hemocue Hb 201+ device (HemoCue America) was used, a standard in hemoglobin point-of-care testing (55, 56). It requires a finger prick and wicking of capillary blood into a pretreated microcuvette for analysis. Quality tests using external, liquid controls were necessary for each day of instrument use prior to sample analysis.

A qualified and experienced phlebotomist collected non-fasting maternal venous blood samples (22 mL) at the second study visit. Samples were centrifuged and aliquoted into microcentrifuge tubes prelabeled with participant unique sample identification number and then stored at –80°C. In conjunction with the hemoglobin results, collected maternal venous blood samples were used to determine iron status by measuring soluble transferrin receptors and serum ferritin [using the chemiluminescent microparticle immunoassay (CMIA) method], which reflects iron storage, but if serum ferritin levels are increased during infection or inflammation, it may mask any iron deficiency results (57). Therefore, an inflammatory marker, C-reactive protein, was measured [tested by an immunoturbidometric method analyzed on an Abbott C Series analyzer (Abbott Labs)].

Venous blood samples were assayed for hormonal biomarkers: free $T_3$, free $T_4$, and thyroid stimulating hormone via CMIA method; thyroglobulin (Tg, Beckman Coulter Access method); and antithyroglobulin antibodies (anti-Tg, CMIA method) at Canterbury Health Laboratories. Serum thyroglobulin has been suggested as an alternative method to assess individual iodine status reflecting a period of months

(58); to avoid potential underestimation of thyroglobulin, anti-Tg and thyroid peroxidase antibodies (TPOAb) were measured. Selenium status was assessed by determining the biomarker plasma selenium via ICP-MS method (59).

Details of data and biological samples collected from both mothers and infants throughout the study period are summarized in Tables 4.1 and 4.2.

**Table 4.1 Summary of outcomes collected from participating women and their infants.**

| Outcome | Visit 1 | Visit 2 | Visit 3 |
|---|---|---|---|
| **Dietary intake** | | | |
| Maternal 4-day dietary diary | x | | |
| Maternal food frequency questionnaire–iodine/selenium | x | | x |
| Maternal food frequency questionnaire–iron | | x | |
| Infant 3-day dietary diary | | x | |
| **Anthropometry** | | | |
| Maternal weight and height | x | x | x |
| Maternal body composition via BodPod and BIA | x | x | x |
| Infant weight, height, and head circumference | x | x | x |
| **Biochemistry** | | | |
| Maternal spot urine samples | x | x | x |
| Maternal breastmilk samples | x | x | x |
| Maternal toenail clipping samples | x | x | x |
| Maternal venous blood samples | | x | |
| Maternal capillary blood samples | | x | |
| Infant spot urine samples | x | x | x |
| Infant nail clipping samples | x | x | x |
| **Others** | | | |
| Maternal thyroid gland volume via ultrasound | | x | |
| Maternal Edinburgh Postnatal Depression Scale results | x | x | x |
| Maternal self-reported health questionnaire | x | x | x |
| Maternal iodine nutritional knowledge questionnaire | x | | |
| Infant health questionnaire reported by mothers | x | x | x |

**Table 4.2 Analysis from biological data collected at each study visit**

| Samples | Visit 1 | | Visit 2 | | Visit 3 | |
|---|---|---|---|---|---|---|
| | Mothers | Infants | Mothers | Infants | Mothers | Infants |
| **Spot urine** | | | | | | |
| Iodine | x | x | x | x | x | x |
| Selenium | x | x | x | x | x | x |
| Creatinine | x | | x | | x | |
| **Breastmilk (if available)** | | | | | | |
| Iodine | x | | x | | x | |
| Selenium | x | | x | | x | |
| **Blood** | | | | | | |
| Iodine status[a] | | | x | | | |
| Selenium status[b] | | | x | | | |
| Iron status[c] | | | x | | | |
| Thyroid function[d] | | | x | | | |
| **Nail clippings for selenium** | | | | | | |
| Toenails | x | x | x | x | x | x |
| Fingernails | | x | | x | | x |

[a]Iodine status: testing thyroglobulin and antithyroglobulin.

[b]Selenium status: testing plasma selenium.

[c]Iron status: testing hemoglobin, serum ferritin, soluble transferrin receptors, and C-reactive protein.

[d]Thyroid function: testing serum free triiodothyronine, free thyroxine, thyroid stimulating hormone, and antithyroid peroxidase.

### 4.4.6 Statistical analysis

Statistical analysis will be performed using SPSS Statistics version 23. The Shapiro-Wilk test will be used to test for data normality. Nonparametric data will be expressed as median ($25^{th}$, $75^{th}$ percentile), and parametric data will be expressed as mean and standard deviation. Bivariate correlations will be tested using the nonparametric Spearman $\rho$ correlation coefficient. Repeated-measures analysis of variance will be used to calculate continuous variables between groups. Nonparametric Mann-Whitney U test (2-tailed) will be used to examine iodine intake and status between

supplement users and nonusers. Multiple regression model analysis will be used to determine the associations between iodine, selenium, iron status, and thyroid function, as well as considering confounding factors. Multivariate analysis will be used to examine possible associations between women's selenium status, thyroid function, and postnatal depression and infant first year neurodevelopment.

## 4.5 Results

Recruitment traversed the 19-month period between June 2016 and December 2017, and a sample of 91 women-infant pairs was enrolled (Figure 4.1). Data collection has been completed. Biological samples analysis, excluding nail clippings, is complete. Data analysis and presentation of the results will be available after 2020.

## 4.6 Discussion

A unique aspect of this study is that it will investigate all three micronutrients responsible for adequate thyroid hormone synthesis concurrently, rather than each separately in isolation. This observational longitudinal cohort study will measure the iodine and selenium status of women repeatedly in their first year after childbirth, which provides an evaluation of their nutritional status. Iodine status among supplement users and nonusers will provide up-to-date data on this postpartum group in New Zealand around eight years after government interventions. Results will explore whether maternal iodine and selenium status could be used as a proxy measure of infant status. It provides an opportunity to examine the association of maternal iodine and selenium with infant neurodevelopment during their first year. This study explores selenium status using both short-term and long-term measures in relation to neurodevelopment at six months and twelve months of age, which has not been reported previously. Furthermore, the study results will add preliminary data on iron status of women at six months postpartum.

Importantly, the study will investigate overall thyroid function of women at six months postpartum with respect to the risk of postnatal depression. Measurement of

thyroid hormones, thyroid stimulating hormone, TPOAb, Tg, and anti-Tg in serum as well as measuring thyroid gland volume via ultrasound will provide an overall picture of maternal thyroid function after childbirth. This is an opportune time to check thyroid status, especially as women with limited thyroidal reserve or iodine deficiency in pregnancy may develop postpartum thyroid dysfunction, one of the most common endocrine disorders women experience (6, 60).

Additionally, there will be longitudinal assessment of mothers' mental health via repeated screening by using the EPDS. The results may add to the literature in postpartum mental health status. Their offspring's growth and neurodevelopment will be followed during the first postpartum year. The findings from this study have the potential to inform future public health policy and practice regarding postpartum women's nutritional status and mental health together with infant health outcomes.

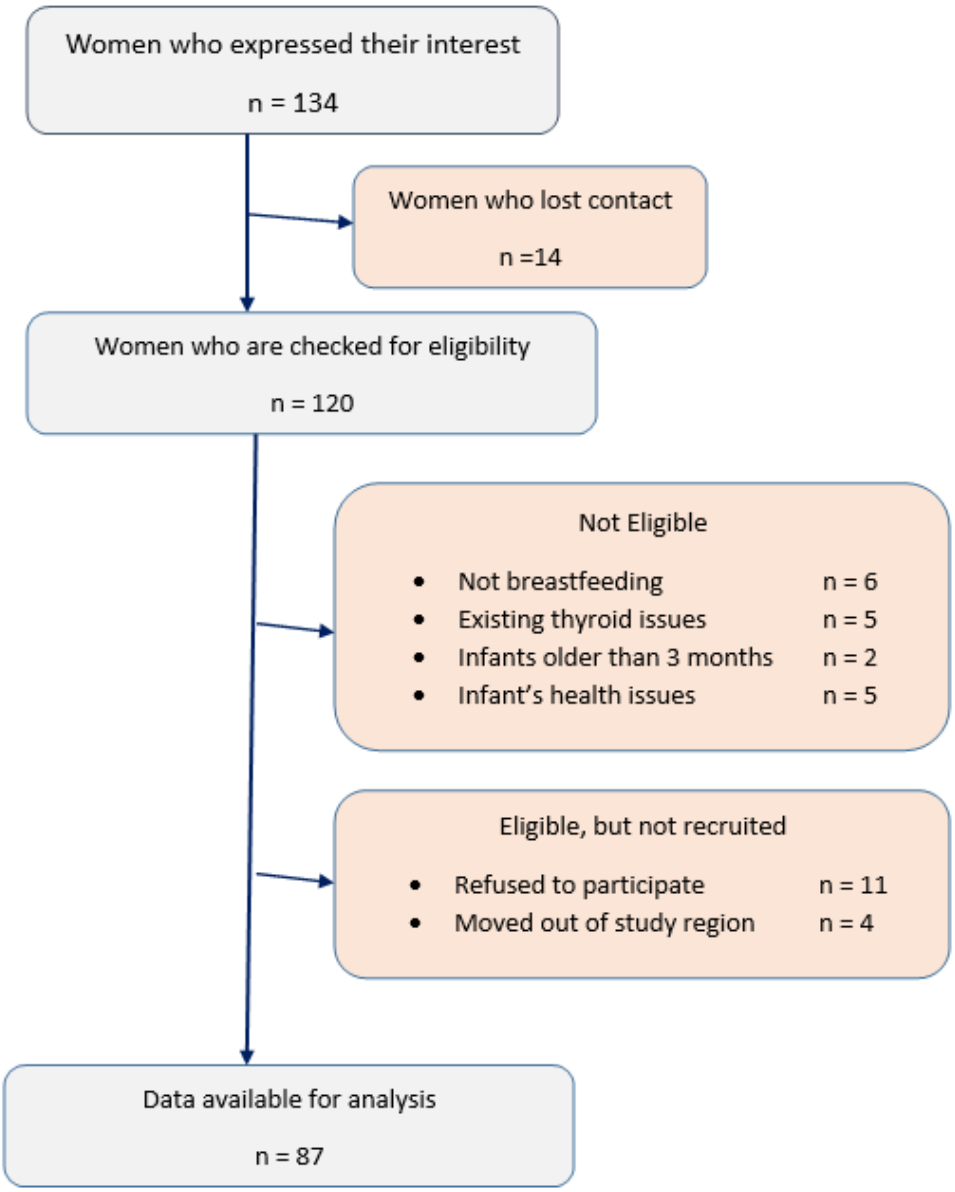


**Figure 4.1 MINI Study Recruitment Flowchart**

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# Chapter 5 Prevalence of thyroid dysfunction in postpartum women with suboptimal iodine and selenium and adequate iron status

*This chapter is the first of four articles (published and forthcoming) presenting the research findings from the MINI study, each, in turn, describing different features obtained from the analysis of data collected from the participating postpartum women and their infants. Despite the critical roles of iodine, selenium, and iron in synthesising thyroid hormones, limited research has investigated the interaction of these three nutrients simultaneously within the thyroid function (noted in Chapter 2). This forthcoming article focuses on research objectives which investigate the roles of iodine, selenium, and iron status in maternal thyroid function, and explore maternal iron status at six months postpartum. It reports on postpartum women's thyroid function including the prevalence of thyroid dysfunction; and maternal thyroid volumes which were compared to data from other countries. In addition, maternal status of all three nutrients are reported. This article presents further examination of the extent to which iodine status (urinary iodine concentration, breastmilk iodine concentration, serum thyroglobulin), selenium status (plasma selenium), and iron status (serum ferritin and soluble transferrin receptors) were likely to relate to maternal dysfunction among a cohort of women at six months postpartum.*

*This forthcoming publication addresses* ***hypothesis 1****: Suboptimal iodine, selenium or iron status will impede maternal thyroid function at six months postpartum; and* ***hypothesis 7****: High prevalence of iron deficiency and iron deficiency anaemia exist in women at six months postpartum.*

***This chapter has been submitted for publication to the Clinical Endocrinology and is currently under review****.*

## 5.1 Abstract

Background

Postpartum women experience thyroid dysfunction at twice the prevalence of the general population. Optimal thyroid function relies on adequate biosynthesis of thyroid hormones, which depends on three trace elements: iodine, selenium, and iron.

Objective

This study aimed to investigate thyroid dysfunction within a cohort of women at six months postpartum in relation to iodine, selenium, and iron status.

Methods

This cross-sectional study was part of an observational longitudinal cohort Mother and Infant Nutrition Investigation; data obtained at six months postpartum are reported. Thyroid hormones [free triiodothyronine, free thyroxine, thyroid stimulating hormone (TSH)] and thyroid peroxidase antibodies were measured. Urinary iodine concentration, breastmilk iodine concentration, serum thyroglobulin, plasma selenium, serum ferritin, and serum soluble transferrin receptors were determined.

Results

Mother-infant pairs (n = 87) were recruited at three months postpartum and followed up at six months postpartum (n = 78). Overall, 18% of women had thyroid dysfunction, and 4% of women had iron deficiency without anaemia. Median urinary iodine concentration was 85 (43, 134) µg/L, median breastmilk iodine concentration was 59 (39, 109) µg/L, and median serum thyroglobulin at 11.4 (8.6, 18.6) µg/L, indicating iodine deficiency. Women with marginally lower plasma selenium were 1.14 % times more likely to have abnormal TSH concentrations ($P$ = 0.001).

Conclusions

There was a high prevalence of thyroid dysfunction. Plasma selenium was the only significant predictor of the likelihood that women had thyroid dysfunction within this cohort, who were iodine deficient and mostly had adequate iron status. Future research should investigate how selenium interacts with other micronutrients in thyroid function.

## 5.2 Introduction

Thyroid dysfunction has been associated with anxiety, depression, cognitive deficit (1), and adverse effects on reproductive health (2). Thyroid dysfunction occurring in the first year after parturition is defined as postpartum thyroiditis (PPT). In 2017, the American Thyroid Association reported from different cohorts of studies the prevalence of PPT ranged from 1.1% to 16.7% (3). Most women initially diagnosed with PPT have normal thyroid hormones concentrations by the end of their first postpartum year (3). However, longitudinal follow-up studies have reported that between 12% and 30% of women developed permanent hypothyroidism three to five years after the original episode of PPT (4). Maternal thyroid dysfunction has been linked to the development of postnatal depression (5, 6), is weakly associated with reduced breastmilk production and milk-ejection reflex ("let-down") (3) and may impact on infant neurodevelopment (7).

Iodine, selenium and iron are essential for the synthesis of thyroid hormones (8, 9). Iodine is an integral component of thyroid hormones, thyroxine ($T_4$) and triiodothyronine ($T_3$). Selenium is a component of the selenocysteine-containing protein, glutathione peroxidase (GPx), which protects cells from damage by neutralising the excessive hydrogen peroxide generated during thyroid hormone synthesis (10,11). The deiodinases, which convert biologically inactive $T_4$ into active $T_3$, are selenoproteins (10). Furthermore, the activity of thyroid peroxidase, a haem-dependent enzyme required for adequate synthesis of thyroid hormones, is impaired in iron deficiency (11).

The interaction between selenium and iodine in synthesising thyroid hormones is of particular concern within New Zealand due to dietary insufficiency of both nutrients (12). Two New Zealand government initiatives aim to improve iodine status: the mandatory fortification of bread with iodised salt, introduced in 2009, and the provision of iodine supplements for all pregnant and lactating women introduced in 2010 (13). Despite these initiatives, this population group continues have suboptimal

iodine intake and status (14). Selenium deficiency could potentially aggravate the negative consequences of mild iodine deficiency in this vulnerable group (15). Further, the iron status of postpartum women in New Zealand is rarely clinically determined, except in women who had significant blood loss during childbirth (16).

This study aimed to investigate thyroid dysfunction in a cohort of women at six months postpartum in relation to maternal iodine, selenium, and iron status.

## 5.3 Methods

The Mother and Infant Nutrition Investigation (MINI) study was approved by the Health and Disability Ethics Committee, New Zealand (15/NTA/172) in December 2015 (ACTRN12615001028594). Prior written consent was obtained from all participants. Infants' participation was consented to by their mothers.

### 5.3.1 Study design and participants

The MINI study was an observational longitudinal cohort study spanning the first postpartum year in Palmerston North within the North Island of New Zealand. Data from six months postpartum are reported here, since biomarkers for iodine, selenium and iron status, and thyroid hormone concentrations were only determined at this time point. Women aged 16 years and older, who had given birth to a healthy term singleton infant aged less than three months of age, were invited to join the study. Women were excluded: 1) if they had pre-existing or developed significant health problems, such as metabolic disease and cancer; 2) if they had been diagnosed or treated at any time for hyperthyroidism or hypothyroidism. The full study protocol is published (17).

### 5.3.2 Data collection

#### *5.3.2.1 Assessment of thyroid hormones and thyroid volume*

Thyroid hormone biomarkers [serum free $T_3$, free $T_4$, and thyroid stimulating hormone (TSH)] were measured using the chemiluminescent microparticle

immunoassay (CMIA) method at Canterbury Health Laboratories, New Zealand which is accredited with International Accreditation New Zealand (IANZ). The adult reference ranges of these biomarkers were used due to the absence of reference ranges specifically for lactating women. Thyroid peroxidase antibody (TPOAb) concentration above 10 IU/mL was regarded as indicative of a potential autoimmune disorder. Reference ranges for euthyroid are: TSH, 0.40 - 4.00 mIU/L; free T4, 10 - 24 pmol/L; and free T3, 2.5 - 6.0 pmol/L. These reference ranges were used to calculate the prevalence of thyroid dysfunction including subclinical hypothyroidism (TSH > 4.00 mIU/L and normal free T4), overt hypothyroidism (TSH > 4.00 mIU/L and free T4 < 10 pmol/L), subclinical hyperthyroidism (TSH < 0.40 mIU/L and normal free T4), and overt hyperthyroidism (TSH < 0.40 mIU/L and free T4 > 24 pmol/L) (18).

Thyroid volume was measured by a portable ultrasound (Terason uSmart3200TTM, Terason Corporation, USA) equipped with a linear transducer (7 - 15 MHz). The measurements of anteroposterior diameter and width (mediolateral diameter) were obtained with electronic calipers on a transverse image. The total volume of the thyroid gland was the sum of the volumes of left and right lobes, excluding the volume of the isthmus, but including any nodules and/or cystic areas. The formula used to calculate the volume for each lobe was: Anteroposterior diameter x Width x Length x 0.479 (19). A total volume greater than 18 mL was defined as thyroid enlargement, based on the normative thyroid volume in an iodine sufficient population (20,21). Any participant with observed abnormalities was referred to clinical health professionals for further assessment.

#### *5.3.2.2 Assessment of iodine, selenium, and iron status*

Maternal non-fasting spot urine samples (approximately 120 mL) were collected to measure maternal urinary iodine concentration (UIC, µg/L) and creatinine concentration to determine maternal urinary iodine creatinine ratio (µg/g). Women were asked to provide a breastmilk sample (approximately 30-50 mL) using an electric breast pump, if needed, and breastmilk iodine concentration (BMIC, µg/L) was

determined. The timing of breastmilk collection was not standardised, although all samples were collected before 12 noon on the study visit day. All samples were stored without preservative at -20°C prior to analysis. Non-fasting maternal venous blood samples (22 mL) were collected by a phlebotomist, and separated into plasma and serum samples before storage at -80°C.

Iodine concentrations in urine and breastmilk samples were determined by Hill Laboratories, Hamilton, New Zealand, using inductively coupled plasma mass spectrometry (22). Quality control procedures included analysis of blanks, analytical repeats, and spiked samples in order to ensure accuracy and precision. Calibration standards and checks were undertaken on every run with the limit of detection at 0.002 mg/kg. Each batch (25 samples) of urinary samples was analysed together with an external reference standard (Seronorm Trace Elements Urine, L-2, Norway) giving a mean ± SD iodine concentration of 286 ± 12 µg/L, with a coefficient of variance (CV) of 4.2% (n = 14). Creatinine was measured in maternal urine using the Jaffe Method Flexor (Randox Assayed Multisera levels 2&3) at Massey University Nutrition Laboratory in Palmerston North. Each batch of breastmilk samples was analysed together with an external reference standard (Skimmed milk powder, Elements in organic matrix, European) giving a mean ± SD iodine concentration of 1.603 ± 0.029 mg/kg, with a coefficient of variance (CV) of 4.9% (n = 6).

Serum thyroglobulin (Tg) was measured by the Beckman Coulter Access method and anti-thyroglobulin antibodies were determined by the CMIA method, at Canterbury Health Laboratories, Christchurch, New Zealand. Serum Tg has been suggested as a biomarker to assess individual iodine status reflecting a period of weeks or months (23, 24); if anti-thyroglobulin antibodies were detected positive (≥ 10 IU/mL), Tg concentrations were disregarded.

Plasma selenium concentration was assessed by the inductively coupled plasma spectrometry method at Canterbury Health Laboratories, New Zealand (25). A plasma

selenium concentration of 95 μg/L has been suggested to saturate GPx activity (26), this was used as a cut off for the current study.

Hemoglobulin (Hb), serum ferritin (SF), and soluble transferrin receptor (sTfR) concentrations were determined to evaluate maternal iron status. Hb concentrations were measured using a handheld Hemocue Hb 201+ device (HemoCue® Hb 201+, Sweden) (27). sTfR (using the Nephelometry method) was measured to determine iron demand versus iron supply. Serum ferritin (using the CMIA method) and C-reactive protein (CRP) were determined (using an immunoturbidimetric method analysed on an Abbott c series analyser), and, if CRP ≥ 8 mg/L (indicating inflammation), the serum ferritin concentration was disregarded (28). The iron status of participants was defined using the following definitions: sufficient iron stores: SF ≥ 12 μg/L and Hb ≥ 120 g/L; anaemia without iron deficiency: SF ≥ 12 μg/L but Hb < 120 g/L; iron deficiency without anaemia (ID): SF < 12 μg/L and Hb ≥ 120 g/L; iron deficiency anaemia (IDA): SF < 12 μg/L and Hb < 120 g/L (29).

### 5.3.3 Data analysis

All data were analysed using IBM SPSS (Statistics Package for the Social Sciences, IBM, Armonk, NY, USA) version 20 and R statistical programme (Vienna, Austria. https://www.R-project.org/) (30).

Data were tested for normality using the Shapiro-Wilk test. Non-parametric data were expressed as median ($25^{th}$, $75^{th}$ percentile), and parametric data expressed as mean (± standard deviation; SD). Bivariate correlations were tested using either the parametric Pearson correlations or the nonparametric Spearman's rho correlation coefficient, as appropriate. Differences in categorical variables were tested by Fisher's exact t-test. Plasma selenium concentrations were split into two categories (≥ 95 μg/L or < 95 μg/L) for comparison with the biomarkers; independent t-test was used for parametric data after natural log transformation (including UIC, urinary iodine creatinine, BMIC, Hb, SF, free T3:T4), biomarkers which were unable to be transformed into parametric data (including serum Tg, sTfR and TSH) were tested by Mann Whitney U test. A logistic

regression model was employed to consider factors likely to influence abnormal TSH concentrations. The dependent variable was binary, with the value of one being abnormal TSH concentration and the value of zero being normal TSH concentration. Age, parity and iodine status have been suggested as risk factors for maternal thyroid disease (31). Selenium is involved in thyroid autoimmunity (32) and iron deficiency impairs the synthesis of thyroid hormones (9). Therefore, age of participants; parity (categorical variable); UIC; BMIC; plasma selenium; and sTfR were selected as covariates to enter the logistic regression model simultaneously (Table 5.1). Average marginal effects are less sensitive to changes in the specification of the logistic regression model when compared to odds ratios (33). Therefore, average marginal effects were calculated to illustrate the effect of small changes in covariates on the binary dependent variable (TSH concentration).

**Table 5.1 Description of the variables entered the logistic regression model.**

| Variables | Description | Definition |
|---|---|---|
| **Dependent variable** | | |
| TSH | 0 - Normal (0.4-4 mIU/L)<br>1- Abnormal (< 0.4 mIU/L or > 4.0 mIU/l) | Abnormal TSH concentrations indicate thyroid dysfunction. |
| **Covariates** | | |
| Age of participants | Continuous (Years) | Age of participants in years |
| Parity | 1 – Primiparity<br>0 - Multiparity | The number of childbirths; single or multiple |
| UIC | Continuous (µg/L) | Indicator for iodine status |
| BMIC | Continuous (µg/L) | Indicator for iodine status |
| Plasma selenium | Continuous (µg/L) | Indicator for selenium status |
| sTfR | Continuous (mg/L) | Reflection of functional iron status |

## 5.4 Results

A total of 87 breastfeeding mother-infant pairs were recruited at three months postpartum, nine participating pairs dropped out from the study at six months

postpartum (n = 78). A description of characteristics of breastfeeding women and their infants at enrolment are shown in Table 5.2. At six months postpartum, 31% (24/78) were exclusively breastfeeding, 4% (3/78) had stopped breastfeeding, with the remainder providing partial breastfeeding. Iodine, selenium, and iron containing dietary supplements were used by nine, one and six women, respectively.

**Table 5.2 Description of breastfeeding participants and their infants at recruitment.**

| **Maternal characteristics** | n | % |
|---|---|---|
| **Maternal age, years (Mean ± SD)** | 31.5 ± 4.2 | |
| **Tertiary Education** | 67 | 77 |
| **Ethnicity (Maori)** | 9 | 10 |
| **Ethnicity (Caucasian)** | 66 | 76 |
| **Ethnicity (Asian)** | 9 | 10 |
| **Ethnicity (Other)** | 3 | 4 |
| **Annual household income (Above median)*** | 54 | 62 |
| **Primiparity** | 38 | 44 |
| **Caesarean delivery** | 19 | 22 |
| **Gestational ages at birth, weeks (Mean ± SD)** | 39.4 ± 1.5 | |
| **Age of infants at recruitment, days (Mean ± SD)** | 88.5 ± 14.8 | |
| **Infants birth weight, kilograms (Mean ± SD)** | 3.6 ± 0.6 | |

* Median annual household income based on Statistics New Zealand is 75,995 New Zealand dollars for the year ended June 2016 (36)

### 5.4.1 Thyroid hormones and thyroid volume

The median (p25, p75) maternal total thyroid volume was 6.1 (4.4, 8.4) mL, ranging from 2.2 to 15.2 mL, with none above 18 mL which is the suggested cut-off of thyroid enlargement for women (21). Based on maternal thyroid function markers, 18% (13/74) had thyroid dysfunction, with 8% (6/74) having positive TPOAb indicating autoimmune thyroid disorders (Table 5.3). Positive TPOAb was correlated with abnormal TSH (r = 0.261, $P$ = 0.025). Total thyroid volume was weakly, positively associated with serum free T4 (r = 0.314, $P$ = 0.006).

**Table 5.3 Thyroid function biomarkers and thyroid dysfunction prevalence (n = 74).**

| Thyroid markers | Median (p25, p75) |
|---|---|
| **TSH, mIU/L** | 0.96 (0.69, 1.33) |
| **free $T_4$, pmol/L** | 12 (11, 12) |
| **free $T_3$, pmol/L** | 3.8 (3.6, 4) |
| **free $T_3$:$T_4$ ratio** | 0.33 (0.31, 0.36) |
| | **n (%)** |
| **TPOAb (+)[a]** | 6 (8) |
| **Subclinical hypothyroidism[b]** | 0 (0) |
| **Overt hypothyroidism[c]** | 2 (3) |
| **Subclinical hyperthyroidism[d]** | 11 (15) |
| **Overt hyperthyroidism[e]** | 0 (0) |

[a] TPOAb ≥ 10 IU/mL indicates positive.

[b] Subclinical hypothyroidism: TSH > 4.0 mIU/L and 10 < free T4 < 24 pmol/L.

[c] Overt hypothyroidism: TSH > 4.0 mIU/L and free T4 < 10 pmol/L

[d] Subclinical hyperthyroidism: TSH < 0.4 mIU/L and 10 < free T4 < 24 pmol/L

[e] Overt hyperthyroidism: TSH < 0.4 mIU/L and free T4 > 24 pmol/L.

### 5.4.2 Iodine, selenium, and iron status

The WHO (2007) defines iodine deficiency in a population by using the median UIC and suggests a cut-off for deficiency in non-pregnant women below 100 μg/L (34). Maternal median UIC was 85 (43, 134) μg/L, indicating deficiency (35). Median BMIC was 59 (39, 109) μg/L, below 75 μg/L which has been suggested as the index of sufficient infant iodine intake (36). After excluding nine women with positive Tg antibodies, median Tg was 11.4 (8.6, 18.6) μg/L, above the suggested cut-off of 10 μg/L, and suggests iodine deficiency (37, 38, 39). All three biomarkers indicated iodine deficiency in this cohort of postpartum women. Median maternal plasma selenium was 105.8 (95.6, 115.3) μg/L; with 23% (17/74) below 95 μg/L, suggesting inadequate status as this concentration is required to saturate GPx activity (26).

During childbirth, 17% (13/78) of women experienced severe blood loss (> 500 mL), 46% (6/13) received an iron transfusion and 15% (2/13) had a blood transfusion. Six women reported taking iron-containing supplements of either 60 mg (5/6) or 5 mg (1/6) daily. Mean Hb was 132.5 ± 9.00 g/L. Median SF was 41 (27, 78) µg/L after excluding three women with elevated CRP. Median sTfR was 1.12 (1.00, 1.26) mg/L, with 4% having elevated values suggesting iron-deficient erythropoiesis (40). In total, 90% (64/71) of women were classified as having sufficient iron stores. Four participants were classified as having anaemia without iron deficiency. Only three women were classified as being ID, and no participants were classified as having IDA.

Women who had a plasma selenium concentration below 95 µg/L had a significantly lower urinary iodine creatinine ratio, SF and TSH, and a higher serum Tg, compared to women with an adequate plasma selenium concentration (≥ 95 µg/L) (Table 5.4).

**Table 5.4 Iodine and iron status based on plasma selenium cut-off at 95 µg/L.**

| **Median ($25^{th}$,$75^{th}$ percentile)** | **Plasma Se (< 95 µg/L) n = 17** | **Plasma Se (≥ 95 µg/L) n = 57** | **Independent t-Test *P* value** |
|---|---|---|---|
| UIC µg/L[a] | 58 (38,116) | 89 (42, 159) | 0.318 |
| Urinary iodine creatinine µg/g[a] | 99 (63, 159) | 145 (93, 208) | 0.024 |
| BMIC µg/L[a] | 56 (38, 90) | 62 (39, 114) | 0.769 |
| Hb g/L[a] | 133 (123, 139) | 132 (126, 140) | 0.865 |
| SF µg/L[a] | 29 (17, 50) | 48 (30,81) | 0.024 |
| free $T_3$:$T_4$ [a] | 0.33 (0.32, 0.35) | 0.33 (0.30, 0.36) | 0.996 |
| | | | **Mann-Whitney U test *P* value** |
| Serum Tg µg/L[b] | 23.6 (10.6,34.0) | 10.2 (8.6, 15.8) | 0.017 |
| Serum sTfR mg/L[b] | 1.18 (1.11, 1.35) | 1.10 (0.97, 1.25) | 0.063 |
| TSH mIU/L[b] | 0.62 (0.04, 1.17) | 1.02 (0.77, 1.50) | 0.005 |

[a] Data become normally distributed after natural log transformation

[b] Data remans non-parametric after natural log transformation

### 5.4.3 Iodine, selenium and iron status and thyroid hormone concentrations

All women using iodine-containing supplements (9/74) had normal TSH concentrations, while 20% (13/65) who did not use iodine-containing supplements had abnormal TSH concentrations. The proportion of women with thyroid dysfunction was higher in those who had plasma selenium concentrations below 95 μg/L compared with women who had plasma selenium concentrations of 95 μg/L or above (41% vs. 10.5%, $P = 0.008$).

The logistic regression showed a negative but statistically significant coefficient of plasma selenium, and the marginal effect suggests women with lower plasma selenium were 1.14 % times more likely to have abnormal TSH concentrations. However, none of other covariates had a significant effect when entered the model (Table 5.5). Although UIC was weakly correlated with BMIC, the variance impact factor was close to one, thus no correction was needed for the multicollinearity.

No significant correlations were observed between biomarkers for iron status (Hb, SF and sTfR) and thyroid hormone concentrations. There were no statistically significant differences in iron status observed between women with abnormal and normal TSH concentrations.

**Table 5.5 Logistic model of factors affecting abnormal TSH concentrations (n = 69)**

| | Coefficients | P > [z] | Marginal effect |
|---|---|---|---|
| **Age of Participants** | 0.117 | 0.185 | 0.01356 |
| **UIC ug/L** | 0.004 | 0.181 | 0.00048 |
| **BMIC ug/L** | -0.000 | 0.973 | -0.00003 |
| **Plasma Se ug/L** | -0.103 | 0.001 | -0.01144 |
| **Parity** | -0.323 | 0.761 | -0.02516 |
| **Serum sTfR mg/L** | -1.222 | 0.300 | -0.16920 |

## 5.5 Discussion

This cross-sectional study of 78 women at six months postpartum found thyroid dysfunction in 18%, including 3% with overt hypothyroidism, and 15% with subclinical hyperthyroidism. This was higher than a previous study of Australian women (n = 748) which detected 11.5% with thyroid dysfunction at six months postpartum (41). In New Zealand (2006/2007), 4.8% of adults aged from 18 to 80 years old registered with General Practices had thyroid dysfunction (42), however, the prevalence in women of childbearing age was not determined. Median thyroid volume (6.1 mL) in the MINI study was similar to that estimated in a study of Cuban women (6.4 mL) aged 18-50 years who lived in iodine sufficient areas (43). There was no obvious thyroid enlargement observed in the current study, however, ultrasound echogenicity of the thyroid gland was not examined, which has been suggested as a predictor in thyroid dysfunction development (44). A follow-up study in the United Kingdom has suggested that women experiencing PPT may present hypoechogenicity (structural changes revealed in ultrasound images) during the first postpartum year, but most were improved by the 77-81 month-follow-up (45).

In the MINI study, low selenium status was the only significant predictor of the likelihood that women had thyroid dysfunction. Women with a plasma selenium concentration below 95 μg/L had a higher likelihood of experiencing thyroid dysfunction (41.2%), compared to only 10.5% in women with a plasma selenium concentration above 95 μg/L. This aligns with the results of a large Chinese observational study (n = 6152) which reported a higher prevalence of thyroid disease in people living in a low selenium region compared to those living in an adequate selenium region (31% vs 18%, $P < 0.001$) (46). The authors suggested low selenium may result in inadequate expression of selenoproteins, hence less protection of the thyroid gland from oxidative damage. In the current study, women with a plasma selenium concentration below 95 μg/L had a significantly lower TSH and SF, but higher serum Tg, compared to women with adequate plasma selenium concentrations. Although, median TSH and SF concentrations in the low versus high

selenium groups were significantly different, these were still within the normal range. However, median serum Tg in women with low selenium status was double the suggested normal concentration of 10 μg/L, indicative of iodine deficiency. Similar dietary sources contribute to the intake of iodine and selenium. Low selenium may exacerbate the consequences of iodine deficiency in this group of postpartum women, due to the requirement for both selenium and iodine for the synthesis of thyroid hormones (12).

Iodine status (UIC or BMIC) was not a predictor of thyroid dysfunction in the current study, which was not entirely unexpected as UIC has high intraindividual variation (10 -15 urine samples are suggested for assessing habitual status) (47). This could also be due to a small number of women who had abnormal thyroid hormones, and that thyroid hormones may remain in the normal range even in a mild-to-moderate iodine deficient population (35). In contrast, a cross-sectional study of Australian women at six months postpartum (n = 149) found a lower median UIC was significantly associated with TSH above normal concentrations (48). The current study found women using iodine-containing supplements (9/74) presented with normal TSH concentrations, while 20% (13/65) of women not using iodine-containing supplements were found to have abnormal TSH concentrations. This suggests there is a beneficial effect from iodine supplementation on thyroid function. However, it is inconclusive since the numbers using iodine-containing supplements were too small.

It was unexpected that most women at six months postpartum achieved adequate iron status, with only 4% being iron deficient without anaemia (ID). The low rate of ID in the current study could be due to the protective effects of six-months' lactational amenorrhoea. However, a previous study of American women up to six months postpartum (n = 220) reported that 12.7% were ID, and women up to six months postpartum from low income groups had a fourfold increased risk of ID compared to than their non-pregnant counterparts (49). In the current study, 62% reported a household income above the median New Zealand household income (50). Although iron status in the current cohort was mostly adequate, further research is required to

ascertain the iron status of low-income postpartum women in New Zealand, who are known to consume a diet having lower iron bioavailability (51).

Iron status in the current study was not associated with thyroid hormone concentrations, which may be due to the predominantly adequate iron status of the women. However, a nationally representative study of pregnant Swedish women, who lived in an area of iodine deficiency (median UIC at 139 μg/L), reported that lower iron status (sTfR to SF ratio) predicted elevated levels of TSH (≥ 4.0 mU/L) and lower concentrations of T4 (< 100 nmol/L). The authors suggested that a low iron status blunts the activity of the haem-dependent enzyme (thyroid peroxidase) which results in a reduction of thyroid hormone synthesis (52).

To the best of our knowledge, the MINI study was the first study to examine iodine, selenium and iron concurrently in relation to thyroid function of postpartum women. It is one of a few studies to investigate the prevalence of thyroid dysfunction in a New Zealand postpartum cohort. One of the strengths of this study is the comprehensive range of biomarkers used to measure iron, iodine, and selenium status. Measuring SF is a well-established method to assess iron storage (53), but it does not indicate the severity of iron depletion. Increased serum sTfR would indicate functional iron deficiency without being confounded by inflammation. Changes in the reciprocal relationship between these measures would provide a better understanding of iron status and allow early detection of ID before the occurrence of IDA (40). Iodine status was determined by measuring UIC and BMIC to reflect recent iodine intake and serum Tg which is a sensitive biomarker (23). Plasma selenium reflects short-term status and was used because it is the most used biomarker in other published research which would allow comparison. A potential limitation of this study is not determining functional selenium status by measuring plasma or platelet GPx (54) to assess nutritional deficiency. Measuring selenium in nail clippings may be another useful assessment of selenium status over a long-term exposure time (up to 52 weeks) (55). Other limitations of the study include the small sample size, and the participants were predominantly well-educated and may not have been representative of the wider New

Zealand population. Less educated women are under-represented, thus their thyroid function, and their iodine, selenium, and iron status may require further investigation. Few participants reported the use of selenium-containing supplements; therefore, this study has insufficient statistical power to examine the effects of such supplementation on thyroid function.

## 5.6 Conclusion

A high prevalence of thyroid dysfunction (especially subclinical hyperthyroidism) was observed, and low plasma selenium significantly increased the risk of thyroid dysfunction within this group of iodine deficient postpartum women who predominantly had adequate iron status. Future studies are required to provide a greater understanding of the role of selenium status, when combined with other micronutrients, in optimal thyroid function. Measuring long-term selenium status via nail clippings, could identify the risk of selenium deficiency and its impacts on thyroid function. Strategies may be required to improve both iodine and selenium status, which may support optimal thyroid function for women during perinatal period.

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# Chapter 6 Use of iodine supplements by breastfeeding mothers is associated with better maternal and infant iodine status

*Although maternal iodine status did not predict thyroid dysfunction, the use of iodine-containing supplements showed some beneficial effect (noted in Chapter 5). Despite the establishment of two government initiatives, continually monitoring iodine intake and status is essential (see Chapter 2).*

*Chapter 6 is the second article (published) derived from the analysis of the MINI study data. It concentrates on the research objective to compare breastfeeding women's iodine intake and status between iodine-containing supplement users and non-users. In addition, this article records the participant's levels of iodine knowledge, including the importance of iodine in health; good dietary sources of iodine; the two New Zealand government initiatives to improve iodine status; and the behaviour related to iodine. Finally, the effects of maternal use of iodine-containing supplements on the iodine status of mothers and infants are discussed and implications for future research and practice are considered.*

*This published article addresses **hypothesis 2**: Breastfeeding women who used iodine-containing supplements will achieve better iodine status for themselves and their breastfed infants.*

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## 6.1 Abstract

Background

Adequate iodine status during conception, pregnancy and lactation is essential for supporting infant neurodevelopment. Iodine status in adults and children was improved after two New Zealand government initiatives, but the status of breastfeeding women is unknown.

Objectives

This study aimed to investigate the iodine intake and status of lactating mother-infant pairs at three months postpartum, and to assess maternal iodine knowledge and practice.

Methods

Iodine intake was estimated by a weighed four-day diet diary (4DDD). Maternal urinary iodine concentrations (UIC) in spot urine, breastmilk iodine concentrations (BMIC), infant UIC were measured. Questions about iodine specific knowledge and practice were asked.

Results

In 87 breastfeeding mother-infant pairs, maternal iodine intake was 151 (99, 285) μg/day, 58% had an intake below the Estimated Average Requirement (EAR) of 190 μg/day. Maternal median UIC (MUIC) was 82 (46, 157) μg/L indicating iodine deficiency (i.e. < 100 μg/L). Women who used iodine-containing supplements had a significantly higher MUIC (111 vs 68 μg/L, $P$ = 0.023), and BMIC (84 vs 62 μg/L, $P$ < 0.001) than non-users. Infants fed by women using iodine-containing supplements had a higher MUIC (150 vs 86 μg/L, $P$ = 0.036) than those of non-users. 66% (57/87) of women had no or low iodine knowledge. The iodine knowledge score was a statistically significant predictor of consuming iodine-containing supplements [(beta = 1.321, $P$ = 0.008)].

Conclusions

Despite a decade of initiatives to increase iodine intakes in New Zealand, iodine knowledge was low, iodine intake and status of these lactating women were suboptimal, but women who used iodine-containing supplement were more likely to achieve adequate status.

## 6.2 Introduction

Iodine is an essential trace element for thyroid hormone synthesis. Adequate thyroid hormone is required for the myelination of the central nervous system during the early postnatal period (1). Severe iodine deficiency may result in iodine deficiency disorders, such as goitre, and may negatively affect infant growth and mental development (2, 3), even at mild to moderate iodine deficiency (4).

During lactation, an adequate iodine intake is required for maternal thyroid function, and iodine is also secreted into breastmilk (5). Breastfed infants depend on the iodine content in breastmilk to ensure their optimal thyroid function during the first six months of life (3, 6). Therefore, it is essential that lactating women have adequate iodine status for their own health and that of their newborns. In countries with low iodine intake from dietary sources, supplementation is recommended for breastfeeding mothers (5).

In New Zealand, soils provide low levels of available iodine, resulting in low concentrations in the food supply (7), and hence the diet (8). Iodine deficiency was a concern in New Zealand in the early 20th century, but its prevalence was mostly reduced through the introduction of iodised salt after the 1930s (9). However, mild iodine deficiency re-emerged in the 1990s (9, 10, 11, 12, 13). To improve iodine status, two New Zealand government initiatives were introduced: mandatory fortification of bread with iodised salt in 2009, and the provision of a subsidised iodine supplement (150 µg) for all pregnant and lactating women in 2010 (14). Subsequent studies have demonstrated that most adults (15, 16) and children (17) in New Zealand have achieved adequate iodine intake and status, but the status of pregnant and breastfeeding women is unknown. A pilot study assessed a small sample of self-selected highly educated breastfeeding women before (n = 25 and 32; 2009) and after (n = 34 and 36; 2011) these initiatives, by testing their urinary iodine excretion, breastmilk iodine concentration and blood thyroglobulin; the results suggested iodine deficiency (18).

Globally, personal knowledge empowers people to make optimal decisions in dietary practices, including choice of food and use of supplements. Good general nutrition knowledge by women during pregnancy has been reported in the United Kingdom (19). However, researchers remained concerned about very low awareness of iodine-specific recommendations for pregnant women (19). Poor knowledge of important food sources of iodine for women of reproductive age living in the UK/Ireland has been reported (20). Breastfeeding women from Norway and Australia had low knowledge of the importance of iodine in normal child growth and development, or sources of iodine, which may have contributed to their iodine deficiency (21, 22, 23). Overall, very few studies investigating iodine knowledge, practice, intake and status of breastfeeding women have eventuated.

The aims of this study were: to evaluate iodine intake and status of breastfeeding women at three months postpartum; and to assess current iodine knowledge and practice of breastfeeding women in New Zealand.

## 6.3 Materials and methods

### 6.3.1 Participants and recruitment

The Mother and infant Nutrition Investigation (MINI) followed a cohort of women from three months postpartum over the first year postpartum (24). This paper reports on data from three months postpartum. Healthy breastfeeding women were recruited from June 2016 to December 2017. Participants were required to live within or near the local Palmerston North area and be able to attend scheduled study visits. Women aged 16 years and older, who had given birth to a healthy term singleton infant at around three months old were invited to join the study. Women were excluded: 1) if they had pre-existing or developed significant health problems, such as metabolic disease including uncontrolled diabetes and cancer; 2) if they had been diagnosed or treated at any time for hyperthyroidism or hypothyroidism. Written consent was obtained from all participants, mothers provided consent for their infants.

### 6.3.2 Data collection at three months postpartum

#### *6.3.2.1 Assessment of iodine status*

Non-fasting maternal (approximately 120 mL) and infants' spot urine samples (approximately 20 mL) were collected to measure urinary iodine concentrations. Women were asked to provide a breastmilk sample (approximately 30-50 mL) expressed by hand or with an electric breast pump if needed. Timing of breastmilk collection was not standardised. All biological samples were collected between 0900 and 1200 on the study visit day. Samples were stored without preservative at –20°C prior to analysis.

Iodine concentrations in maternal and infant urine and breastmilk samples were determined by Hill Laboratories, Hamilton, New Zealand, using inductively coupled plasma mass spectrometry (25). Quality Control procedures included analysis of blanks, analytical repeats and spiked samples in order to ensure accuracy and precision. Calibration standards and checks were undertaken on every run with the limit of detection at 0.002 mg/kg. Each batch (25 samples) of urinary samples was analysed together with an external reference standard (Seronorm Trace Elements Urine, L-2, Norway) giving a mean ± SD iodine concentration of 286 ± 12 µg/L, with a coefficient of variance (CV) of 4.2% (n = 14). Creatinine was measured in maternal urine using the Jaffe Method Flexor (Randox Assayed Multisera levels 2&3) at Massey University Nutrition Laboratory in Palmerston North. Each batch of breastmilk samples was analysed together with an external reference standard (Skimmed milk powder, Elements in organic matrix, European) giving a mean ± SD iodine concentration of 1.603 ± 0.029) mg/kg, with a coefficient of variance (CV) of 4.9% (n = 6).

#### *6.3.2.2 Assessment of iodine intake and practice*

Iodine intake was estimated from a weighed four-day diet diary (4DDD). Participants were asked to complete the food record within two weeks of the initial study visit. The

four days were consecutive and included one weekend day. Detailed food items, brands, amount consumed, and the content of the nutritional information panel if applicable were recorded. All food and beverage items consumed were weighed with an electronic kitchen scale (Digitech, QM-7288), or measured using household measurement cups and spoons, which were provided. All participants received both oral and written instructions as to how to complete the record, which included a written example for one day. Women were also asked to include any dietary supplements consumed each time, including the brand and dose. When eating or dining out, participants were asked to estimate the portion size of all food eaten. All dietary data were entered in Foodworks 9 Professional (Xyris software, Brisbane, Australia), and analysed using data sets from the New Zealand Foodfiles 2016 to estimate nutrient intake. Where food items were not included in Foodfiles 2016, new food items were created based on the information directly provided by participants (i.e. food packages), or from appropriate international databases from Australia and the United States. To ensure accuracy and completeness, a registered nutritionist (YJ) checked all dietary data. Iodine-containing supplement use 24 hours prior to the biological sample collection was also collected via a separate question at the study visit day. This was also used in statistical analysis of biological variables to compare supplement users with non-users.

Iodine related practice was investigated via a self-administered online questionnaire. Participants reported on the type of salt they usually used individually at the table or in cooking, and on their habitual use of iodine-containing supplements in pregnancy and lactation together with the brand, dose, and duration of consumption. Women were asked to give reasons if they chose not to take any supplements at any stage. Iodine contribution from discretionary iodised salt was added as 48 μg/day to individual's dietary intake (15), for those who had reported regular use of iodised salt at the table or in cooking.

For a population to have a very low prevalence of inadequate dietary intake, the mean/median intake should be above the Recommended Daily Intake (RDI); while

the percentage below the Estimated Average Requirement (EAR) approximates the proportion that is at risk of dietary inadequacy, according to the EAR cut-point method (26, 27). Current intakes based on the 4DDD were compared to Australian and New Zealand recommendations; the EAR and RDI for iodine for lactating women,190 and 270 μg/day, respectively (28).

#### *6.3.2.3 Assessment of iodine knowledge*

Iodine knowledge was assessed in two parts. Firstly, four questions examined iodine knowledge: Q1 and Q3 (each has only one correct answer); Q2 (four correct answers) and Q4 (three correct answers) (Appendix 9). One point was awarded to each correct answer given, and a maximum of nine points were generated (Table 6.1). Secondly, participants were asked to identify good or poor food sources for iodine from a list of 12 food items (Appendix 9). Two points were awarded to each correctly identified good food source (high contribution of iodine to a normal New Zealand diet, e.g. fish and milk); one point was awarded to each correctly identified poor food source (19). If all parts were answered correctly, a maximum 17 points were generated. Those who gained more than 14 points from the second part of the questionnaire were awarded 2 points towards their final knowledge score, while those gaining between 10-13 points were awarded one point towards their final knowledge score. Total iodine knowledge scores were determined from the two parts ranging from 0 to 11 and categorised as follows: no knowledge (0-2 points), low knowledge (3-5 points), medium knowledge (6-8 points), and high knowledge (9-11 points) (Table 6.4).

#### *6.3.2.4 Other assessments*

Sociodemographic characteristics of the mothers including age, ethnicity, educational attainment, smoking status, household size and income were collected on the initial study visit. Information on infants' birth was collected from the "Well Child Tamariki Ora" book (New Zealand child health record) provided by participants, including gestational age at birth, birth weight, and date of birth which was used to calculate the ages of infants at the day of study visit.

**Table 6.1 Iodine knowledge score calculation and number (%) of participants.**

| Questions | Correct choices | Points | n (%) |
|---|---|---|---|
| **1) Why iodine is important** | Need iodine for thyroid gland | 1 | 64 (74) |
| **2) Health issues associated with iodine deficiency** | Goitre | 1 | 42 (48) |
| | Birth defects | 1 | 33 (38) |
| | Mental retardation | 1 | 23 (26) |
| | Impaired physical development during childhood | 1 | 28 (32) |
| **3) Awareness of iodine deficiency in NZ** | Yes | 1 | 42 (48) |
| **4) Awareness of NZ government initiatives** | Mandatory iodised salt fortification of bread | 1 | 18 (21) |
| | Recommended routine taking iodine supplements – pregnant | 1 | 78 (90) |
| | Recommended routine taking iodine supplements – breastfeeding | 1 | 49 (56) |
| **5) Identify good food source for iodine** | | | |
| | Poor | 0 | 68 (77) |
| | Some | 1 | 16 (18) |
| | Good | 2 | 3 (3) |
| | **Maximum Total** | **11** | |

### 6.3.3 Data Analysis

All data were analysed using IBM SPSS (Statistics Package for the Social Sciences, IBM, Armonk, NY, USA) version 20. Data were tested for normality using the Shapiro-Wilk test. Non-parametric data were expressed as median with interquartile range ($25^{th}$,$75^{th}$ percentile) and parametric data expressed as mean (± standard deviation; SD). Bivariate correlations were tested using the nonparametric Spearman's rho correlation

coefficient. Mann-Whitney U test was used to examine iodine status between exclusively breastfeeding and mixed feeding (providing breastmilk and infant formula) women and their infants. Differences in iodine status between iodine-containing supplement users and non-users were tested with a nonpaired t-test after log transformation of these variables. Logistic regression was used to predict the likelihood of using iodine-containing supplements based on all variable(s) entered simultaneously: education attainment, parity, household income, and iodine knowledge scores. Logistic regression was fitted to examine the likelihood of a higher iodine knowledge score (> 5), based on all variable(s) entered simultaneously: educational attainment, habitual use of iodised salt, habitual use of iodine-containing supplement during this pregnancy. Chi-square tests were used to examine whether women with higher education (tertiary or lower than tertiary) or higher income (above or below the median income of New Zealand population) were more likely to habitually use iodine-containing supplements during this pregnancy and lactation (iodine practice).

## 6.4 Results

In total, 87 breastfeeding mother-infant pairs were recruited at three months postpartum. The mean age of the women was 32 years, and 83% were exclusively breastfeeding (no other food or drink, not even water, except breastmilk) at three months after childbirth (Table 6.2). Compared with the 2013 New Zealand Census data among women aged 30-34 years, this sample had a lower proportion of Māori (10% vs. 13%), a higher percentage of Caucasians (76% vs 60%), and a similar proportion of Asians (10% vs. 8%) (29), a higher proportion of women who achieved a tertiary qualification (77% vs. 61%) (30), and 38% reported an annual household income below the national median for 2016 ($NZ75995) (31). In addition, 23 women reported that they had been smoking but most stopped after becoming pregnant, apart from one woman who continued. Further, three women stopped smoking during this pregnancy, but started again after the birth.

**Table 6.2 Description of breastfeeding participants and their infants.**

| **Maternal characteristics** | n | % |
|---|---|---|
| **Maternal age, years (Mean ± SD)** | 31.5 ± 4.2 | |
| **Tertiary Education** | 67 | 77 |
| **Ethnicity (Māori)** | 9 | 10 |
| **Ethnicity (Caucasian)** | 66 | 76 |
| **Ethnicity (Asian)** | 9 | 10 |
| **Ethnicity (Other)** | 3 | 4 |
| **Annual household income (Above median)*** | 54 | 62 |
| **Primiparity** | 38 | 44 |
| **Caesarean birth** | 19 | 22 |
| **Gestational ages at birth, weeks (Mean ± SD)** | 39.4 ± 1.5 | |
| **Age of infants, days (Mean ± SD)** | 88.5 ± 14.8 | |
| **Infants birth weight, kilograms (Mean ± SD)** | 3.6 ± 0.6 | |

* Median annual household income based on Statistics New Zealand is 75,995 New Zealand dollars for the year ended June 2016 (31).

### 6.4.1 Iodine status

Iodine deficiency in a population is defined by the median urinary iodine concentration (MUIC); the WHO guidelines (2007) currently recommend the cut-off as 100 μg/L for lactating women (32). Maternal MUIC ($25^{th}$, $75^{th}$ percentile) in the current cohort was 82 (46, 157) μg/L below 100 μg/L (32), which indicated iodine deficiency in this population of breastfeeding women (Table 6.3). Infant MUIC of 115 μg/L was lower than the suggested cut off for infant MUIC of 125 μg/L based on the Estimated Average Requirement of 72 μg/day of infants aged two to five months derived from a Swiss study of infants (33). The MUIC of women who were exclusively breastfeeding was not significantly different from those who were mixed feeding (78 vs. 117 μg/L, $P$ = 0.511); the MUIC of infants who were exclusively breastfed was not significantly different from those who were mixed breastfed (111 vs. 134 μg/L, $P$ = 0.280). Median breastmilk iodine concentration (BMIC) of 69 μg/L was below 75 μg/L

which has been suggested as an index of sufficient iodine intake (34). Women who used iodine-containing supplements achieved significantly higher MUIC (111 vs 68 µg/L), maternal urinary iodine creatinine ratio (197 vs 96 µg/g) and BMIC (84 vs 62 µg/L) than non-supplement users ($P < 0.001$). Infants from women who used iodine-containing supplement attained a higher infant MUIC of 150 (97, 193) µg/L, above the recommended cut-off of 125 µg/L, while infant MUIC from infants of non-users was 86 (53, 171) µg/L ($P = 0.036$).

The iodine knowledge score was statistically significant predictor of consuming iodine-containing supplements [(beta = 1.321, $P = 0.008$)], while educational attainment, parity, household income, breastfeeding patterns were not predictors. Although educational attainment and household income was weakly correlated, the variance impact factor equals to one, thus no correction was required for the multicollinearity.

**Table 6.3 Maternal and infant iodine status**

| Median (p25, p75) | Total | Iodine-containing Supplement users | Iodine-containing Supplement non-users | *P* Value[1] |
|---|---|---|---|---|
| **Numbers of participants (n)** | 87 | 35 | 52 | - |
| [a] **Maternal MUIC µg/L** | 82 (46, 157) | 111(46, 240) | 68 (44, 123) | 0.023 |
| **Maternal urinary creatinine g/L** | 0.6 (0.3, 1.1) | 0.6 (0.2, 0.9) | 0.7 (0.3, 1.3) | 0.131 |
| **Maternal urinary iodine creatinine ratio µg/g** | 124 (77, 231) | 197 (129, 335) | 96 (69, 169) | <0.001 |
| [b]**Infant MUIC µg/L** | 115 (69,182) | 150 (97, 193) | 86 (53, 171) | 0.036 |
| [c]**BMIC µg/L** | 69 (52,119) | 84 (68, 167) | 62 (47, 81) | <0.001 |

[a] Maternal MUIC cut-off is 100 µg/L (32); [b] Infant MUIC cut-off is 125 µg/L (33); [c] BMIC cut-off is 75 µg/L (34).

[1] As determined by unpaired t-test

### 6.4.2 Iodine intake and practice

Median estimated iodine intake including iodine supplements based on 4DDD was 151 (99, 285) μg/day below the RDI of 270 μg/L, with 58% below the EAR of 190 μg/day (Table 6.4). Median iodine intake for women using iodine-containing supplements was 289 μg/day, significantly higher than for non-users (120 μg/day, $P < 0.001$). For women using iodine-containing supplements, median iodine intake was above the RDI with only 10% (3/30) below the EAR, whereas median intakes for non-users were below the RDI with 89% (42/47) below the EAR.

**Table 6.4 Maternal iodine intake based on 4DDD.**

| Estimated iodine intake, μg/day | Total | Iodine-containing Supplement users | Iodine-containing Supplement non-users |
|---|---|---|---|
| **Numbers of participants (n)** | 77 | 30 | 47 |
| **Median (p25, p75)** | 151 (99, 285) | 289 (250, 356) | 120 (984, 145) |
| **Below the EAR of 190 μg/day n (%)** | 45 (58) | 3 (10) | 42 (89) |

Despite iodised salt being available in New Zealand for over 80 years, only 51% (44/87) reported using iodised salt at the table or in cooking. Median consumption of fortified bread with iodised salt was 56 (36, 105) g/day, which is about 1.5 slices (based on 38g per slice of bread) (15), and iodine obtained from fortified bread was 23 (15, 41) μg/day. Median contribution of fortified bread to the total iodine intake was 15% (7%, 27%). Eighty-six percent (75/87) of women took iodine-containing supplements (ranging from 150 to 270 μg iodine per day, with 79% (59/75) consuming the government subsidised iodine of 150 μg/day) during pregnancy but this reduced to 46% (40/87) during lactation (ranging from 100 to 250 μg iodine per day, with only one person consuming a supplement containing less than 150 μg/day iodine, and 58% (23/40) consuming the government subsidised iodine). Habitual iodine-containing

supplement use during pregnancy was moderately correlated with education attainment (r = 0.434, $P < 0.001$), but not household incomes. Further Chi-square test showed during pregnancy, women with a tertiary education were more likely to use iodine-containing supplements than those who has not obtained tertiary education (94% vs 58%, $P < 0.001$). During lactation, habitual iodine-containing supplement use was not associated with either educational attainment or household incomes. The reasons for not taking iodine supplement during lactation included: 1) never being advised to do so by health professionals (31%, 16/87); 2) believing adequate nutrient intake was achieved from their overall diet (24%, 12/87); and 3) not remembering to take supplements daily (22%, 11/87).

### 6.4.3 Iodine knowledge and its related iodine intake/status

The median iodine knowledge score was 5 (3, 6), and 66% (57/87) of women had no or low iodine knowledge (Table 6.5). Forty-eight percent (42/87) of women identified that goitre was related to iodine deficiency (Table 6.1). When considering two government initiatives to combat iodine deficiency, only 21% (18/87) of women reported being aware of iodine fortification of bread. Although 90% (78/87) knew that iodine supplementation is recommended during pregnancy, the figure dropped to 56% (49/87) for awareness that iodine supplementation is also recommended for breastfeeding women. Overall, the iodine knowledge score was moderately correlated with the estimated iodine intake (r = 0.304, $P = 0.007$). The iodine knowledge score was weakly correlated with iodine status determined by the maternal urinary iodine creatinine ratio (r = 0.250, $P = .026$), but not associated with maternal UIC (r = -0.026, $P = 0.811$) or BMIC (r = - 0.009, $P = 0.931$). The habitual use of iodised salt was a statistically significant predictor of a higher iodine knowledge score [(beta = 0.975, $P = 0.042$)], while educational attainment and habitual iodine-containing supplement use during pregnancy were not.

**Table 6.5 Categorised total iodine knowledge scores (n = 87).**

| Categories of scores (points) | Indicators | n (%) |
|---|---|---|
| 0-2 | No knowledge | 18 (21) |
| 3-5 | Low knowledge | 39 (45) |
| 6-8 | Medium knowledge | 24 (27) |
| 9-11 | High knowledge | 6 (7) |

## 6.5 Discussion

### 6.5.1 Iodine intake/status of iodine-containing supplement users and non-users

The MINI study found iodine deficiency was present in this sample of New Zealand breastfeeding women, despite the implementation of two government initiatives to improve iodine status. The maternal MUIC for current study participants (82 μg/L), was below the WHO cut-off suggesting deficiency, although it was similar to that reported among lactating women (74 μg/L) in the pilot study carried out in the same region one year after the government initiatives had been implemented (18). Although lower than reported among Australian breastfeeding women (125 μg/L) from a post-fortification study (35), this was not unexpected due to a relatively higher iodine content in the Australian food supply in comparison to that in New Zealand (36). In the current study, there was not a significant difference in maternal and infant MUIC between women who were exclusively breastfeeding and those who were mixed feeding (providing breastmilk and infant formula) (78 vs 117 μg/L, $P$ = 0.511; 111 vs 134 μg/L, $P$ = 0.280), although both maternal and infant MUIC from women who were mixed feeding were higher than the suggested cut-offs of 100 μg/L (32) and 125 μg/L (33) respectively for iodine adequacy. Careful interpretation of this result is needed as the lack of statistical power from the low numbers of mixed feeding women (n = 11) in this study. Potentially supplementing breastfeeding with infant formula may increase UIC for both mothers and their infants in this study cohort. The overall iodine status of breastfeeding women remained deficient, which raises concern regarding the long-

term effectiveness of the present strategies in establishing iodine sufficiency. Further studies on the long-term effects on infants' neurodevelopment are required to determine definitions of the levels of iodine deficiency, as there for other population groups.

In the present study, maternal and infant MUIC, BMIC, and maternal urinary iodine creatinine ratio were all significantly higher for women who used iodine-containing supplements compared to non-users. Median values for women who used iodine-containing supplements were higher than recommended cut-offs suggesting adequacy; this was not observed for non-users. Similarly, an Australian post-fortification study of 60 breastfeeding women reported a significantly higher MUIC from iodine supplement users (206 μg/l) than non-users (97 μg/L) and identified iodine deficiency among breastfeeding women who did not consume iodine supplements (23). This finding was consistent with the results from a supplementation trial in an iodine deficient area, in Morocco, where maternal supplementation of one dose of 400 mg iodine in early lactation effectively provided adequate iodine for their breastfed infants up until the age of six months (37). Our results suggest that appropriate supplementation of iodine during breastfeeding will effectively improve iodine status for both women and their breastfed infants.

The estimated median iodine intake of lactating women in the current study based on the 4DDD suggested an inadequate intake. However, the median intake in women who used iodine-containing supplements was 289 μg/day above the RDI, with only 10% below the EAR compared to median intake of 120 μg/day in the women who did not take supplements with 89% below the EAR. The inadequate iodine intake in this vulnerable population is of concern. Significant differences of the median iodine intake between iodine-containing supplements users (139 μg/day) and non-users (126μg/day) was reported in a Danish adult study (DanThyr), where only 34% of the participants used iodine supplements ranging from 50-150μg/day (38). However, the median iodine intake in both groups were below the RDI for adults at 150 μg, which may due to low levels of iodine supplementation. An observational study of

Norwegian pregnant women also reported an improvement of median iodine intake among supplement users [122 μg/day with 28% below the Nordic Nutrition Recommendations (NNR) of 175 μg/day], compared to non-users [116 μg/day with 80% below the NNR], however, the percentage of those using iodine-containing supplements was not reported (39).

### 6.5.2 Iodine knowledge and iodine intake/status

In the current study only 56% of breastfeeding women were aware of the recommendation of iodine supplementation during lactation, although 90% were aware of the need during pregnancy. This was reflected in 86% of current study participants reporting using iodine-containing supplements during pregnancy, which is compatible to 81% reported in a large pregnancy cohort study (n = 783) in Australia (40), but only 46% during breastfeeding which is lower than 57% of lactating women (41) who reported using iodine-containing supplements in an Australian study. A previous study in 2011 in Palmerston North, New Zealand indicated that there was a low awareness of the need for iodine supplements for breastfeeding women, with only 35% reporting use of any iodine-containing supplements (18). Recently, low use of iodine-containing supplements among both pregnant and lactating women was also found in the United States (42). Currently, iodine-only supplements (government subsidised) and a suitable range of dietary supplements containing iodine are known to be available within New Zealand. Consumers, however, may become confused as to which may be the most desirable for their own personal circumstances. Women on a tight budget cannot afford the cost of government subsidised iodine-only supplements outside the free antenatal care period (six weeks after birth) from their registered general practitioners (GPs). A New Zealand study reported that 13% of women who received prescriptions for iodine supplements did not fill their prescription (43). In the current study, iodine knowledge score was the largest predictor for the use of iodine containing supplements by breastfeeding women, but educational attainment and household income were not predictors. Whereas, a previous large multicentred study of pregnant women in New Zealand (n = 968)

suggested low use of iodine-containing supplements among women with low education and income, despite the New Zealand Government subsiding the cost (44). The present study also found women with a tertiary education were more likely to consume iodine-containing supplements during pregnancy, but household income did not play a significant role in iodine-containing supplement use. This finding could be useful for targeting future educational interventions around iodine.

The lack of awareness of the need for iodine supplementation during lactation is concerning. In New Zealand pregnant women are most reliant on midwives and GPs for their postnatal health advice. A 2014 Australian study investigating antenatal care (45), reported that even GPs could not correctly identify the best dietary food sources of iodine, and had expressed limited knowledge of the existing recommendations of iodine supplementation in Australia. The research found that a majority of Australian GPs questioned confirmed that they did not openly recommend iodine supplements to their patients (45). More recently an Australian-wide survey examined health professionals' knowledge about iodine supplementation for pregnant and breastfeeding women, and results showed a clear lack of awareness of the appropriate dose and duration (46). Both Australian studies highlighted a need for iodine specific nutritional education initiatives being disclosed to all primary health care providers.

Despite the current recommendation for taking iodine supplements during pregnancy and breastfeeding in New Zealand (14), the time at which supplement use should commence is currently under debate, To maintain adequate iodine status throughout pregnancy and lactation, adequate iodine stores in the thyroid prior to conception are essential. The time required for iodine to be absorbed then incorporated into thyroid hormones may be several weeks. A recent study of women from Tasmania, Australia has reported a higher MUIC from women who were supplemented with iodine at 150 μg/day prior to conception than those who started supplementation during pregnancy (196 vs 140 μg/L, $P$ = 0.032) (47). Starting iodine supplementation after a confirmed pregnancy could be too late for the iodine to have maximal effect (48). Thus, inadequate iodine stores prior to pregnancy may result in sub-optimal

iodine nutrition in mothers, their foetuses and infants, even if iodine supplementation commences after conception and continues throughout lactation.

In the current study the iodine knowledge score was moderately correlated with the estimated iodine intake based on 4DDD. A previous study in the United Kingdom/and Ireland also had found a positive association between iodine knowledge and dietary iodine intake in women of childbearing age (20). However, in this study, iodine knowledge scores were not associated with maternal UIC, this aligns with a Norwegian lactating women's study (21). The lack of association could be due to the variation in UIC due to hydration status (49). Further, a weak association was recorded with the maternal urinary iodine creatinine ratio (µg/g), which has been suggested to be a more reliable biomarker for iodine status (50).

The current study was not able to compare iodine intake and status by ethnicity. The 2014/2015 New Zealand Health Survey found both Maori and Asian women achieved the recommended MUIC cut-off of 100 µg/L for iodine adequacy (108 µg/L and 121 µg/L, respectively), when compared to European women of 86 µg/L (51). A significantly higher BMIC was also reported in non-Caucasian South Australian mothers (n = 944), when compared to that of Caucasians, suggesting that different dietary behaviour or supplement use during lactation may contribute to these differences (40).

### 6.5.3 Strengths and limitations

The strength of this study was that it examined iodine intake and status in breastfeeding women coupled with knowledge and practice in New Zealand. It is the first study to report both breastfeeding women and their infants' iodine status following the introduction of mandatory iodine fortification. Dietary intake was assessed using weighed 4DDD which is considered the gold standard of dietary assessment (52). To enable an overall understanding of iodine practice, iodine-containing supplement use was assessed in three ways. Firstly, it was reported at the time of urine and breastmilk sample collection to allow accurate reflection on the

impacts of using supplements on biochemical measures. Secondly, women were also asked to include any dietary supplements consumed (including iodine-containing supplements) in their 4DDD to ensure correct reflection of their dietary iodine intake. Finally, retrospective data on their habitual use of iodine-containing supplements during this pregnancy and lactating period were collected to evaluate iodine practice among these breastfeeding women, as both pregnant and lactating period are critical in fetus and infant neurodevelopment.

A limitation of this study is bias, women with pre-existing iodine knowledge may have been attracted to participate. Women in this study were better educated than the New Zealand population, typically, women who volunteer to participate in such studies are often more interested and motivated about health than the general population. However, if the iodine status of these women is inadequate, it is likely that women who are less well-educated and under-represented will have even poorer iodine status. Although this is a cohort study, dietary intakes and knowledge data were only assessed at three months postpartum, because this is a critical period of infant motor and neurodevelopment. A limitation of this study is its small sample size; therefore, the results need to be interpreted with caution.

The use of a single spot urine samples can be affected by maternal hydration status; however, urinary creatinine values were determined to reduce the variation in maternal hydration. Due to the small sample of infant urine we were not able to measure creatinine in these samples, however there is less variation in infant hydration status. There is a high level of intra-individual variation in iodine intake research shows ten samples are required to determine habitual intake (53), thus using repeated measuring could have better predicted iodine status. (54). Iodised salt consumption was difficult to estimate accurately since the frequency and quantities recorded in the 4DDD can never be completely reliable. Therefore, 48 µg iodine per day was included for those women who self-reported regular usage of iodised salt at the table and/or in cooking (15) This method of estimation may still reflect a possible under or overestimate of iodine intake in this cohort.

## 6.6 Implications for research and practice

Iodine deficiency of breastfeeding women after initiatives to improve iodine status is concerning, especially for those who do not use iodine-containing supplements. Only 56% of participants were aware of the need for iodine supplements during lactation. There was also low awareness of the importance of iodine in health and good dietary sources of iodine. Further public health initiatives are important to ensure that both health professionals and women become aware of the need for iodine supplementation during lactation. It is essential that health professionals providing postnatal healthcare should promote iodine specific nutrition education to protect both maternal and infant short-term and long-term health.

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# Chapter 7 Iodine status of postpartum women and their infants aged 3, 6 and 12 months - Mother and Infant Nutrition Investigation (MINI)

*This chapter continues to report the research findings from the MINI study regarding iodine. One of the most important findings (noted in Chapter 6) was that breastfeeding women remain iodine deficient at three months postpartum, and only women who followed the recommendation of taking iodine-containing supplements achieved adequate status. This then led on to further investigation of iodine status in mothers and infants over the first postpartum year. This forthcoming publication centres on the research objective to examine iodine intake and status of postpartum women and their infants at three, six and twelve months postpartum. It describes the iodine status of mothers and their infants at all three time points by assessing urinary iodine concentrations; urinary iodine creatinine ratios; and breastmilk iodine concentrations.*

*An increased expression of sodium iodide symporter in mammary glands increases iodine secretion in breastmilk (noted in Chapter 2). However, it is not clear how maternal iodine excretion is partitioned between urine and breastmilk during lactation. To address this research gap, this article will present the results from the analysis of data collected from exclusive breastfeeding mothers at three months postpartum.*

*This article addresses* ***hypothesis 3****: Postpartum women and their infants will remain iodine deficient, despite two New Zealand government initiatives to improve iodine status.*

***This chapter has been submitted for publication to the British Journal of Nutrition and is currently under review.***

## 7.1 Abstract

Background

Iodine is essential for adequately synthesising thyroid hormones. These hormones play a key role in the neurodevelopment of infants and children during the first three years of age. Few studies have investigated mother and infant iodine status during the first postpartum year.

Objectives

This study aims to describe iodine status of supplement users and non-users (for both mothers and infants) at three, six, twelve months postpartum (3MPP,6MPP,12MPP). Partitioning of iodine excretion, between urine and breastmilk, of exclusive breastfeeding women at 3MPP was determined.

Methods

Maternal spot urinary iodine concentration (UIC), breastmilk iodine concentration (BMIC) and infant UIC were determined.

Results

In total 87 mother-infant pairs were recruited at 3MPP, followed at 6MPP (n = 78) and 12MPP (n = 71). Maternal median UIC (MUIC) (p25, p75) at 3MPP [82 (46, 157) μg/L], 6MPP [85 (43, 134) μg/L] and 12MPP [95 (51, 169) μg/L] were < 100μg/L. The use of iodine-containing supplements increased MUIC only at 3MPP. Median BMIC (p25, p75) at three time points [69 (52, 119) μg/L], [59 (39, 108 μg/L], and [35 (26, 54) μg/L] were below 75 μg/L. Infant MUIC (p25, p75) at 3MPP [115 (69,182) μg/L] and 6MPP [120 (60, 196) μg/L] were below 125 μg/L. Among exclusive breastfeeding women at 3MPP, an increased partitioning of BMIC (highest proportion 60%) was shown at lower iodine intakes, along with a reduced fractional iodine excretion in urine (lowest proportion 40%), indicating a protective mechanism for breastfed infants' iodine status.

Conclusions

In conclusion, this cohort of postpartum women was iodine deficient. Iodine status of their breastfed infants aged three and six months were suboptimal. Further research of the association between iodine status, infant thyroid function and neurodevelopment outcomes are necessary.

## 7.2 Introduction

Iodine is an essential micronutrient for adequate production of thyroid hormones, including triiodothyronine ($T_3$) and thyroxine ($T_4$). Insufficient synthesis of thyroid hormones may impair the neurodevelopment of infants and children, particularly, in the first three years of life (1, 2). During lactation, maternal iodine requirement increases to allow the secretion of iodine into breastmilk and to maintain maternal thyroid hormone concentrations. Studies have suggested that during lactation mammary glands are able to concentrate 20-50 times more iodine than maternal blood due to the active sodium-iodide symporter (NIS) (3). The World Health Organization (WHO) recommends exclusive breastfeeding during the first six months of life, and to continue to breastfeed for up to two years and beyond (4). Exclusively breastfed infants rely fully on their mothers for an adequate iodine supply to synthesise sufficient thyroid hormones during the first six months, thereafter infants obtain additional iodine from appropriate complementary foods (5).

The first postpartum year is important for women to re-adjust and meet their changing iodine requirements. Few studies have investigated iodine status at different time points from early lactation to the end of the first postpartum year (6, 7, 8, 9, 10). One small study of New Zealand women (n = 35) prior to government interventions reported median urinary iodine concentrations (MUIC) of 37 µg/L, 25 µg/L, and 47 µg/L at three, six and twelve months postpartum, suggesting iodine deficiency (7). There continues to be a need for a more robust investigation into the iodine status of postpartum women and their infants.

New Zealand soil typically provides low levels of available iodine, which results in low iodine concentrations in local food supply (11). Endemic goitre was prevalent in New Zealand in the early 20$^{th}$ Century, due to iodine deficiency. A voluntary salt iodisation programme was established in 1938 which dramatically reduced goitre rates by the 1950s (12). However, public health messages have led to reduced salt consumption to prevent high blood pressure and reduce the risks of other chronic medical conditions.

The recent shifting from traditional home cooking with iodised salt to commercially prepared food with non-iodised salt has contributed to low use of iodised salt (13). Other possible reasons which may reduce population iodine intake include the cessation of using iodophors as cleaning agents in the New Zealand dairy industries which resulted in a drop of iodine content in milk (14), and the current dietary preferences of non-dairy milks made from soy, almond, rice and oat which contain low iodine < 0.02 mg/kg (15). Re-emerging iodine deficiency was reported in the 1990s for adults (16) and school children (12), also breastfed infants and toddlers (17).

To combat iodine deficiency, mandatory fortification of bread with iodised salt (25-65 mg iodine/kg salt) was introduced in New Zealand in September 2009, applicable to all commercial bread and bread products other than organic and unleavened (18). Recent studies in New Zealand have suggested that fortification has led to adequate intakes and status in the majority of school children (19, 20) and adults (21, 22). However, fortification was predicted to be inadequate for pregnant and breastfeeding women due to their increased requirements. Thus, in 2010, the use of government subsidised iodine-only supplements (150 μg/day) was recommended to all pregnant and breastfeeding women in New Zealand (23). A pilot study (n = 36) suggested breastfeeding women remain deficient (24), and reported mean breastmilk iodine concentrations (BMIC, 63 μg/L) (24) lower than suggested for adequacy (75 μg/L) (25); although iodine status of the breastfed infants was not investigated.

The aim of this Mother and Infant Nutrition Investigation (MINI) was to describe iodine status of supplement users and non-users (for both mothers and infants) at three, six, twelve months postpartum. Further, it investigated partitioning of iodine excretion, between urine and breastmilk, of exclusive breastfeeding women at three months postpartum.

## 7.3 Methods

### 7.3.1 Study population

MINI was an observational longitudinal cohort study spanning the first postpartum year in Palmerston North, in the North Island of New Zealand. Breastfeeding women aged 16 years and older were recruited, who had given birth to a healthy term singleton three months prior. Women were excluded: 1) if they had pre-existing or developed complicated health problems, such as metabolic disorders and cancer; 2) if they had been diagnosed or treated at any time for hyperthyroidism or hypothyroidism. Recruitment spanned the 19-month period between June 2016 and December 2017. Posters to promote the study were placed at selected sites (General Practitioner surgeries, midwifery clinics, pharmacies, antenatal classes, ultrasound clinics, maternal wards in hospitals, local community playgroups, and early childhood centres, etc.). Local newspapers and social media sites were used to publicise the study. Local midwives, childbirth educators, and lactation consultants were asked to raise awareness of the MINI study to their clients. Potential participants responded by recording an expression of interest online or via telephone/email. Prospective participants were provided with a study information sheet. Interested participants then completed a screening questionnaire to ensure eligibility. Details of the study methods have been published previously (26). The first study visit for each mother-infant pair was at approximately three months postpartum, and follow-up assessments took place at six months (2$^{nd}$ study visit) and 12 months (3$^{rd}$ study visit) postpartum. Written informed consent was obtained from all participants before their enrolments in the study. Mothers also gave the written consent to their infants' participation in the study.

### 7.3.2 Ethics approval

All procedures performed in the MINI study involving human participants were in accordance with the ethical standards of the Health and Disability Ethics committee.

The MINI study was approved by the Health and Disability Ethics Committee (reference:15/NTA/172) in December 2015.

### 7.3.3 Sample size

The main outcome measure was urinary iodine concentration (UIC) and the sample size was calculated using G*Power 3.1 (Heinrich Heine University, Dusseldorf) (27) based on data [mean and standard deviation (SD)] from a preliminary study of breastfeeding women (24). The analysis utilised one–way ANOVA with two groups (95% power, alpha = 0.05, two tailed) and three repeat measures. Eighty participants were sought, using expected mean daily UIC of 140 and 100 µg/L for iodine supplement users and non-users, respectively, with a standard deviation of 60.

### 7.3.4 Assessment of iodine status

During each study visit, non-fasting spot urine samples were collected from each participating woman and her infant to assess iodine and creatinine excretion. A paediatric urine bag was placed inside the diaper and checked every 10 minutes during the study visit to collect infant urine samples. Women were asked to provide a breastmilk sample (approximately 30-50 mL) using an electric breast pump if needed. All samples were collected before 12 noon on the study visit day. Samples were stored without preservative at –20°C. Breastmilk samples were analysed for iodine concentration, allowing for estimations of daily excretion and infant iodine intake (28). Use of iodine-containing supplements was determined within 24 hours of the time of biological sample collections at each visit.

Iodine concentrations of urine and breastmilk samples were determined by Hill Laboratories, Hamilton, New Zealand, using inductively coupled plasma mass spectrometry (29). Quality Control procedures included analysis of blanks, analytical repeats and spiked samples in order to ensure accuracy and precision. Calibration standards and checks were undertaken on every run with the limit of detection at 0.002 mg/kg. Each batch (25 samples) of urinary samples was analysed together with

an external reference standard (Seronorm Trace Elements Urine, L-2, Norway) giving a mean ± SD iodine concentration of 286 ± 12 μg/L, with a coefficient of variance (CV) of 4.2% (n = 14). Creatinine was measured in maternal urine using the Jaffe Method Flexor (Randox Assayed Multisera levels 2&3) at Massey University Nutrition Laboratory in Palmerston North. Each batch of breastmilk samples was analysed together with an external reference standard (Skimmed milk powder, Elements in organic matrix, European) giving a mean ± SD iodine concentration of 1.603 ± 0.029 mg/kg, with a coefficient of variance (CV) of 4.9% (n = 6).

Iodine deficiency in a population is defined by a MUIC below 100 μg/L for lactating and non-lactating non-pregnant women, and children younger than two years (1). The WHO (2007) also recommends that for a population to be iodine sufficient no more than 20% should have a UIC less than 50 μg/L (1). There is no universal consensus on the optimal concentration for BMIC, however greater than 75 μg/L has been suggested to be sufficient for adequate infant iodine intake (25).

### 7.3.5 Infant anthropometry

At the initial visit, infant recumbent length was measured crown to heel using an infant length board and recorded to the nearest mm. Infant weight (without clothing and diapers) was measured, using a baby weighing scale (Nagata Scale Co Ltd) and recorded to the nearest 10 g. Infant's weight-for-age Z-score (WAZ) and height-for-age Z-score (HAZ) were calculated by entering the data into WHO-Anthro software (https://www.who.int/childgrowth/software/en/)(30).

### 7.3.6 General demographic and health information

At the initial visit, mode of infant feeding, maternal general health, and demographic information (including age, ethnicity, educational attainment, household size and income) were collected. Potential changeable information including mode of infant feeding and general health was also sought at the second and third visits. Infants' birth information including gestational age at birth, date of delivery, method of

delivery, birth weight, and gender was collected at the first visit, from the "Well Child Tamariki Ora" book (New Zealand child health record). Recorded date of delivery was used to calculate the age of infants on the day of study visit.

### 7.3.7 Statistical analysis

Data were analysed using IBM SPSS (Statistics Package for the Social Sciences, IBM, Armonk, NY, USA) version 20. Data were tested for normality using Shapiro-Wilk's test. Non-parametric data were expressed as median with interquartile range ($25^{th}$, $75^{th}$ percentile) and parametric data expressed as mean (± standard deviation; SD). Bivariate correlations were tested using the nonparametric Spearman's rho correlation coefficient. Nonparametric data was natural log transformed for further analysis. A one-way mixed ANOVA with two groups was used to compare the mean differences between UIC of iodine-containing supplement users and non-users (based on iodine-containing supplement use at three months postpartum) with a repeated measure at three, six and twelve months postpartum. Differences in iodine status for women who were breastfeeding at all three timepoints, determined by UIC, urinary iodine creatinine, and breastmilk iodine concentration (BMIC) between three measurement points were tested by one-way repeated measures ANOVA.

For exclusive breastfeeding women (EBF), their daily maternal iodine excretion in urine was estimated based on 1.5 L of urine per day (31). Total estimated daily iodine excretion was the sum of urinary and breastmilk iodine excretions. The fractional excretions of iodine in urine and breastmilk were calculated as percentages of total daily iodine excretions (32). Assuming 92% of iodine consumed is excreted into urine and breastmilk together (31), total estimated maternal iodine intake was calculated. The estimated total daily iodine intake of their exclusively breastfed infants was calculated based on daily urine volume assumed at 0.5 L, with an assumption of 87% iodine consumed is excreted in infants' urine (33). Independent t-test was used to compare natural log transformed maternal UIC, maternal urinary iodine creatinine

ratio, and BMIC between exclusive breastfeeding women who used iodine-containing supplements and non-users.

## 7.4 Results

### 7.4.1 Characteristics of mothers and their infants

In total, 87 mother and infant pairs were recruited at three months postpartum (3MPP) and followed up at six months (n = 78, 6MPP) and twelve months (n = 71, 12MPP). A description of characteristics of breastfeeding women and infants at enrolment are shown in Table 7.1. All women were breastfeeding at 3MPP, and 96% of women continued to breastfeed their infants at 6MPP, but only 46% continued breastfeeding at 12MPP.

**Table 7.1 Description of breastfeeding participants and their infants at 3MPP**

| **Maternal characteristics at 3MPP** | **Total (n = 87)** | **Iodine-containing supplement users (n = 35)** | **Iodine-containing supplement non-users (n = 52)** |
|---|---|---|---|
| Maternal age, years (Mean ± SD) | 31.5 ± 4.2 | 32.3 ± 3.3 | 30.9 ± 4.6 |
| Tertiary Education (n, %) | 67, 77 | 31, 89 | 37, 71 |
| Ethnicity – Maori (n, %) | 9, 10 | 4, 11 | 5, 10 |
| Ethnicity – Caucasian (n, %) | 66, 76 | 26, 74 | 40, 77 |
| Ethnicity – Asian (n, %) | 9, 10 | 5, 14 | 4. 8 |
| Annual household income (> median, n, %)* | 54, 62 | 23, 66 | 31, 60 |
| Primiparity (n, %) | 38, 44 | 15, 43 | 23, 44 |
| Caesarean delivery (n, %) | 19, 22 | 8, 23 | 11, 21 |
| **Infant characteristics** | | | |
| Gestational age at birth, weeks (Mean ± SD) | 39.4 ± 1.5 | 39.3±1.7 | 39.4±1.46 |
| Age of infants, days (Mean ± SD) | 88.5 ± 14.8 | 90.1 ± 15.6 | 87.4 ± 14.3 |
| Male (n, %) | 52, 60 | 19, 54 | 33, 63 |
| Birth weight, kilograms (Mean ± SD) | 3.6 ± 0.6 | 3.5 ± 0.5 | 3.7 ± 0.7 |
| Weight-for-age Z-score (Mean ± SD) | -0.049 ± 1.050 | -0.777 ± 0.989 | -0.029 ± 1.098 |
| Height-for-age Z-score (Mean ± SD) | 0.066 ± 1.386 | 0.025 ± 1.318 | 0.092 ± 1.442 |

* Median annual household income based on Statistics New Zealand was 75,995 New Zealand dollars for the year ended June 2016(34).

### 7.4.2 Maternal iodine status at three, six and twelve months postpartum

Maternal MUIC ($25^{th}$, $75^{th}$ percentile) at 3MPP [82 (46, 157) µg/L], 6MPP [85 (43, 134) µg/L], and 12MPP [95 (51, 169) µg/L] were < 100 µg/L, suggesting iodine deficiency. Further, greater than 20% of women had a UIC below 50 µg/L: 29% at 3MPP; 27% at 6MPP; and 23% at 12MPP. Median ($25^{th}$, $75^{th}$ percentile) BMIC was below the suggested concentration at all three time points [69 (52, 119) µg/L], [59 (39, 108 µg/L], and [35 (26, 54) µg/L]. BMIC was moderately correlated with urinary iodine creatinine ratio at all three timepoints (r = 0.441, $P < 0.001$ at 3MPP; r = 0.552, $P < 0.001$ at 6MPP; r = 0.577, $P = 0.001$ at 12MPP; Appendix 17). No significant differences between maternal iodine status measures were found among exclusive breastfeeding (EBF), partial breastfeeding (PBF) or non-breastfeeding (NBF) women (Table 7.2).

**Table 7.2 Iodine status during the first postpartum year by mode of infant feeding**

| | 3MPP | | 6MPP | | 12MPP | |
|---|---|---|---|---|---|---|
| **Median (p25, p75)** | **EBF (n=72)** | **PBF (n=15)** | **EBF (n=24)** | **PBF (n=51)** | **PBF (n=33)** | **NBF (n=38)** |
| **Number samples** | 72 | 15 | 24 | 51 | 33 | 38 |
| **UIC µg/L** | 78 (45, 150) | 117 (46, 174) | 81 (47, 148) | 93 (42, 134) | 91 (58, 156) | 104 (43, 171) |
| **Number samples** | 65 | 14 | 24 | 47 | 29 | 33 |
| **Maternal urinary iodine creatinine ratio µg/g** | 125 (76, 219) | 107 (91, 270) | 130 (99, 214) | 133 (75, 190) | 127 (90, 173) | 111 (88, 206) |
| **Number samples** | 72 | 15 | 23 | 49 | 33 | |
| **BMIC µg/L** | 68 (52, 108) | 80 (50, 139) | 78 (32, 113) | 59 (41, 94) | 35 (26, 54) | n/a |

3MPP: 3 months postpartum; 6MPP: 6 months postpartum; 12MPP: 12 months postpartum; BMIC: breastmilk iodine concentration; EBF: exclusive breastfeeding; PBF: partial breastfeeding; NBF: none-breastfeeding; UIC: urinary iodine concentration.

Overall, among women who completed the study, 46% (33/71) took iodine-containing supplements at 3MPP, this reduced markedly to 11% (8/71) at 6MPP, and 6% (4/71) at 12MPP. Defining women as supplement users or non-users at 3MPP, showed there was no significant main effect of time points on natural log UIC [F (2, 138) = 0.500, $P = 0.607$, partial $\eta 2 = 0.007$], with participants showing similar mean natural log UIC at 3MPP (4.36), 6MPP (4.97), and 12MPP (4.49). However, there was significant

interaction between iodine supplement use and time points on the mean of natural log UIC [F (2,138) = 3.550, *P* = 0.031, partial η2 = 0.049] (Figure 7.1). At 3MPP, iodine-containing supplement users showed a higher MUIC (111 µg/L) when compared to non-users (66 µg/L). The subgroup of women who were breastfeeding at the three timepoints showed a significant reduction in BMIC from 3MPP to 6MPP to 12MPP (Table 7.3). However, there were no significant variations in UIC or maternal urinary iodine creatinine ratio over the three time points.

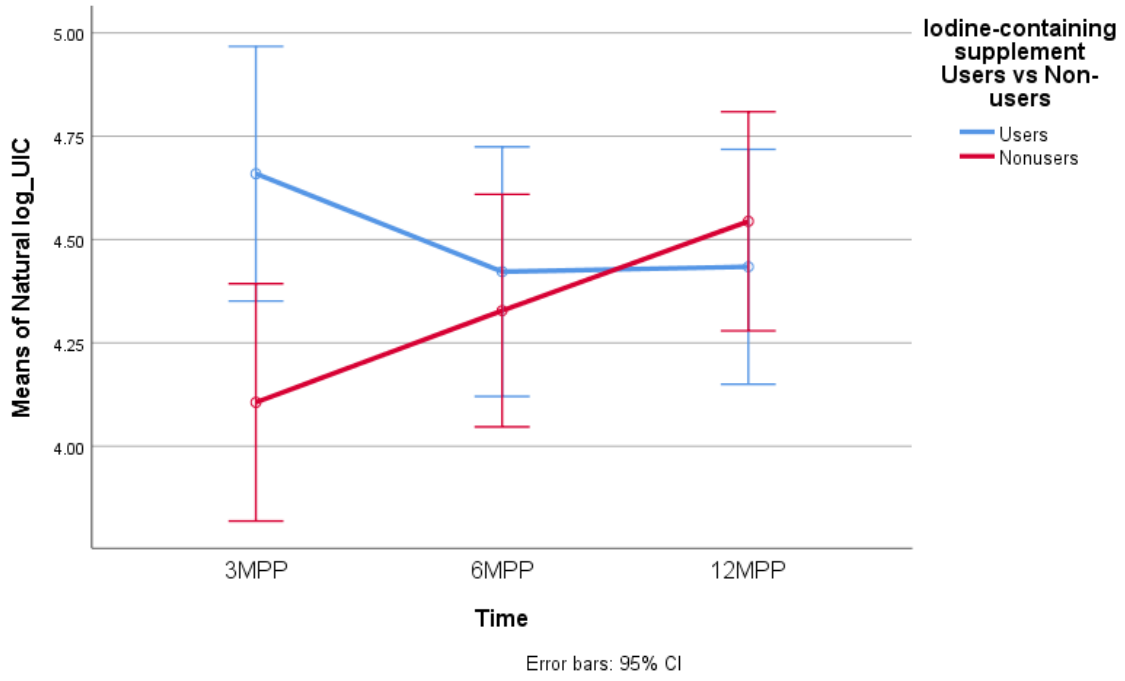


**Figure 7.1 UIC of women who completed the follow-ups at all three time points (n = 71).**

**Table 7.3 Maternal iodine status of continuous breastfeeding women (n = 33).**

| **Median (p25, p75)** | **3MPP** | **6MPP** | **12 MPP** | ***P-value**** |
|---|---|---|---|---|
| **UIC µg/L** | 69 (46, 106) | 80 (41, 133) | 91 (58, 156) | 0.259 |
| **Maternal urinary Iodine: creatinine ratio µg/g** | 132 (82, 243) | 148 (98, 199) | 127 (90, 173) | 0.420 |
| **BMIC µg/L** | 71 (52, 102) | 61 (36, 100) | 35 (26, 54) | 0.001 |

3MPP: 3 months postpartum; 6MPP: 6 months postpartum; 12MPP: 12 months postpartum; BMIC: breastmilk iodine concentration; UIC: urinary iodine concentration.

* One-way ANOVA

### 7.4.3 Iodine status of infants aged three, six and twelve months

In the current study, infant MUIC at the age of three, six and twelve months were all above 100 μg/L with fewer than 20% below 50 μg/L (Table 7.4), suggesting adequate iodine status. However, at six months, infants who were EBF had an MUIC (80 μg/L, n = 13) which was below the 100 μg/L cut-off and significantly lower than infants who were mixed-fed with complementary food (147 μg/L, n = 29, $P$ = 0.033). Exclusively breastfed infants at three months of age had a lower infant MUIC than those who were PBF, but this was not statistically significant (Table 7.4). Infant UIC was significantly, moderately correlated with BMIC at 3MPP, 6MPP and 12MPP (r = 0.598, $P$ < 0.001; r = 0.602, $P$ < 0.001; r = 0.656, $P$ = 0.011, respectively; Appendix 17).

**Table 7.4 Infant UIC from infants aged three, six and twelve months.**

| Median (p25, p75) | Urine samples (n) | 3 months | Urine samples (n) | 6 months | Urine samples (n) | 12 months |
|---|---|---|---|---|---|---|
| **UIC Total μg/L** | 67 | 115 (69, 182) | 43 | 120 (60, 196) | 33 | 118 (62, 220) |
| **UIC < 50 μg/L (n, %)** | | 10, 15 | | 5, 12 | | 4, 12 |
| **UIC EBF μg/L** | 55 | 111 (61, 182) | 13 | 80 (36, 128) | - | - |
| **UIC PBF μg/L** | 12 | 127 (104, 245) | 29 | 147 (78, 215) | 14 | 129 (70, 300) |
| **UIC NBF μg/L** | - | - | - | - | 19 | 106 (54, 210) |
| ***P-value**** | - | 0.280 | - | 0.033* | - | 0.872 |

EBF: exclusive breastfeeding; PBF: partial breastfeeding; NBF: none-breastfeeding; UIC: urinary iodine concentration.

*Mann-Wihitney U test

### 7.4.4 Iodine status of EBF women and their infants at 3MPP

The MUIC for EBF women was 78 μg/L, below 100 μg/L, suggesting iodine deficiency (Table 7.2). Median BMIC was 68 μg/L also below the suggested 75 μg/L. Median estimated maternal iodine intake was 212 (138, 331) μg/day (Table 7.5), below the Recommended Dietary Intake (RDI) of 270 μg/day and 46% (33/72) had intakes below the Estimated Average Requirement (EAR) of 190 μg/day(35). Only 44% (32/72) of EBF

women took iodine-containing supplements (range 150-250 μg iodine/day). Women who used iodine-containing supplements showed significantly higher MUIC (105 vs 66 μg/L, $P = 0.027$), urinary iodine creatinine ratio (173 vs 94 μg/L, $P < 0.001$) and BMIC (84 vs 61 μg/L, $P < 0.001$), when compared to those women who did not use iodine-containing supplements.

**Table 7.5 Daily iodine excretions and intakes in mothers and their infants at 3MPP**

| Median (p25, p75) | Mothers (EBF) (n= 72) | Infants (EBF) (n = 55) |
|---|---|---|
| **Estimated daily iodine excretion** | | |
| **Based on maternal UIC μg/d** | 116 (68, 225) | - |
| **Based on BMIC μg/d** | 53 (41, 84) | - |
| **Total μg/d** | 195 (127, 305) | - |
| **From urine %total** | 69 (53, 76) | - |
| **From breastmilk % total** | 31 (24, 47) | - |
| **Estimated daily iodine intake Total μg/d** | 212 (138, 331) | |
| **Estimated iodine intake, based on infant UIC μg/d** | | 64 (35, 105) |
| **Estimated iodine intake, based on BMIC μg/d** | | 53 (41, 84) |

BMIC: breastmilk iodine concentration. EBF: exclusive breastfeeding; UIC: urinary iodine concentration.

Based upon a visual inspection of the scatter plot of the partitioning of maternal UIC and BMIC in total daily iodine excretion (Figure 7.2), it demonstrates when total daily iodine excretion below 300 μg, an increased partitioning of BMIC (the highest proportion of 60%) was observed, together with a reduced fractional iodine excretion in urine (the lowest proportion of 40%). When total daily iodine excretion was higher than 300 μg, a constant proportion of excretion was observed in urine (80%) and breastmilk (20%).

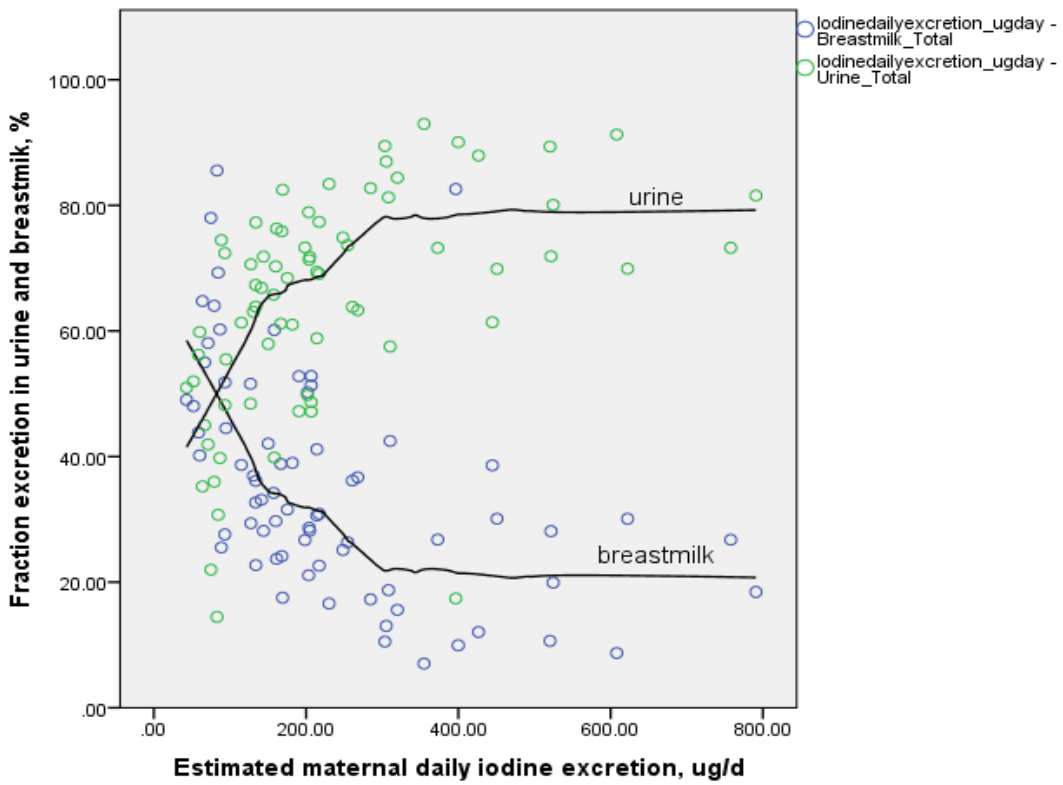


**Figure 7.2 Fractional iodine excretion in urine and breastmilk in relation to total estimated daily iodine excretion (n=72, exclusive breastfeeding).**

## 7.5 Discussion

### 7.5.1 Iodine sufficiency and deficiency during the first postpartum year

The MINI study found women who used iodine-containing supplements at 3MPP had a significantly higher MUIC than non-users (111 vs 68 µg/L). A post-fortification study of Australian breastfeeding women (n = 60) at around three months after parturition showed a similar significant positive effect of taking iodine-containing supplements on maternal MUIC (206 vs 97 µg/L) (36). However, in the current study, there was no difference in MUIC at six and twelve months postpartum based on definition of supplement use at 3MPP. This is unexpected, although could be due to the low proportion of women using iodine-containing supplements in later lactation 11% (8/71) at 6MPP, and 6% (4/71) at 12 MPP. The low use is unsurprising and the lack of awareness of the need for iodine supplementation during lactation in this population

has been reported (37). The low use of iodine-containing supplements during later breastfeeding is especially concerning for women who do not consume iodine rich foods and those who become pregnant again. In Morocco, a randomised double-blinded placebo-controlled trial (n = 241 mother-infant pairs) compared the effectiveness of using maternal supplementation, either with a single dose of 400 mg iodised oil or supplementing infants (aged ≤ 8 weeks) directly with a single dose of 150 mg. This study found that maternal supplementation is more effective in ensuring adequate infant iodine status and maintaining BMIC levels until at least six months postpartum (38). Achieving adequate maternal iodine status throughout lactation, together during pre-conception or pregnant period is critical for infants' growth (39) and contributes to optimal fetal neurodevelopment (40).

Overall iodine deficiency was present in a group of women during the first postpartum year based on the WHO epidemiology criteria (1). Our results showed an improvement in maternal iodine status, when compared to the results reported in a pre-fortification study of postpartum women in the South Island of New Zealand in 2001 (37 μg/L, 25 μg/L, 47 μg/L at three, six and twelve months postpartum) (7). This indicates that the government initiatives have improved iodine status of breastfeeding women but more needs to be implemented to strengthen these strategies.

The trend for increase in MUIC across three time points in the MINI study was also found in a study of Sudanese breastfeeding women (n = 47) living in an area with 17.5% goitre rate, where MUIC increased from three (51 μg/L) to nine (63 μg/L) months postpartum (6). Similarly, a recent large Norwegian longitudinal study of postpartum women (n = 915) reported the lowest MUIC at six weeks postpartum (57 μg/L), which increased through six, twelve and eighteen months postpartum (70 μg/L, 79 μg/L, 87 μg/L) (9). The Norwegian authors suggested the increase was possibly due to an assumed decrease of iodine excretion in breastmilk during the postpartum period. However, these studies on Norwegian and Sudanese women did not measure BMIC.

In the current study, among women who continued to breastfeed, iodine secreted into breastmilk significantly decreased from the highest concentration of 71 μg/L at three months, to 61 μg/L at six months, and with the lowest concentration of 35 μg/L at 12 months postpartum. This pattern suggests reduced transport of iodine to breastmilk. Similar observations were made in a cohort of New Zealand iodine deficient lactating women without iodine supplementation during the first six months postpartum in 2004-2005 decreasing from 43 μg/L in week one to 25 μg/L in week 24 (41). A reduction of BMIC from three months (60 μg/L) to nine months (26 μg/L) was also reported in Moroccan mothers who were supplemented with one oral dose (400 mg) of iodine after delivery (38). Research has reported a sharp decrease of iodine concentration from colostrum to mature milk, which could be due to the low volume of colostrum (42). The further reduction of BMIC from six to twelve months postpartum observed in the current study must be interpreted with caution due to the small number of samples at twelve months. However, for breastfed infants, breastmilk is still an important iodine source. Therefore, it is important for lactating women to achieve adequate iodine status.

Adequate infant iodine status was observed at three time points of this current study, using the WHO epidemiological criteria (1). However, the calculated iodine intake from a UIC of 100 μg/L is 55 μg/day (based on approximately 0.5 L daily urine volume and 92% of dietary iodine excreted into urine) (33). This is much lower than the Institute of Medicine suggested adequate intake (AI) of 110 μg/day for infants up to age of six months (43). Infant MUIC between 180 and 225 μg/L (44) are necessary to achieve the WHO recommended iodine intake of 90 μg/day (1). Further, results from a recent dose-response crossover iodine balance study of euthyroid term Swiss infants with iodine sufficiency suggested 125 μg/L as a cut-off for infant MUIC (based on the estimated average requirement of 72 μg/day for infants aged two to five months)(33). Using this cut-off, the MUIC for infants aged three (115 μg/L) and six months (120 μg/L) in the current study would indicate iodine deficiency, which was consistent with the estimated suboptimal intake from BMIC. Further, infants aged six months who were

exclusively breastfed had an MUIC (80 μg/L) lower than 125 μg/L suggesting iodine insufficiency, which was significantly lower than those who were partially breastfed (147 μg/L). This shows the importance of adequate maternal iodine status for those infants who are exclusively breastfed at six months of age.

In the present study, despite differing amounts of breastfeeding and ages of infants, BMIC was moderately correlated with infant UIC. This finding is supported by a systematic review of 14 eligible studies (42), which suggested BMIC was the primary indicator of iodine status in breastfed infant iodine status due to the positive association between BMIC and infant UIC. In the current study, median BMIC at three and six months postpartum (69 μg/L and 59 μg/L) suggested inadequate infant iodine intake. Further analysis from a South Australia post-fortification study in 2017 found that infants from mothers with lower BMIC (< 100μg/L) were less likely to achieve adequate iodine status (infant UIC > 100 μg/L), when compared to those with higher BMIC (≥ 100 μg/L) (45). The Australian authors suggest that achieving adequate iodine status for lactating women is essential to ensure sufficient iodine supply to their breastfed infants.

### 7.5.2 Iodine status of exclusive breastfeeding women and their infants at three months postpartum

This current MINI study found iodine deficiency was present in exclusive breastfeeding mothers at three months postpartum. The current MUIC remained similar to the value (74 μg/L in 2011) previously reported from the same region in New Zealand (24), although it showed a marked improvement from 34 μg/L in 2009 (prior to government interventions) (24). Women who reported consuming iodine-containing supplements at 3MPP were more likely to achieve adequate iodine status when compared to those who did not use such supplements, this has been discussed in detail previously (37). Exclusively breastfed infants fully rely on maternal breastmilk intake for thyroid function. The MUIC of exclusively breastfed infants at three months of age was 111 μg/L, below the suggested cut-off of 125 μg/L (33) indicating iodine

deficiency. Lack of iodine intake may interrupt the motor and neurodevelopment of these infants at this crucial time (46).

The present study suggests that among this cohort of exclusive breastfeeding women who were iodine deficient, increased partitioning of iodine secretion into breastmilk (the highest proportion of 60%) occurred when total daily iodine excretion was low (< 300 μg/day). This partitioning potentially provides a protective effect to ensure iodine supply to their breastfed infants. In comparison, a similar partitioning pattern (increased fraction of iodine into breastmilk but decreased iodine in urine when total daily iodine excretion < 300 μg/day) was observed from a large multi-centre study of lactating women (iodine sufficient based on median BMIC) in China (n = 386), Philippines (n = 371) and Croatia (n = 109) (32). This pattern may be due to an enhanced capacity of sodium-iodide symporter transportation by mammary glands during early lactation (47), which may ensure adequate iodine supply to exclusively breastfed newborn infants (32). However, in the MINI study, when total daily iodine excretion was higher than 300 μg, a constant partitioning of excretion was observed in urine (80%) and breastmilk (20%), which differed from the previous multi-centre study of EBF women from iodine deficient areas showing a continuous decreased excretion in breastmilk but a measurable increase in urine (32). This may be due to the smaller number of participants in the current study whose total iodine excretion was above 300 μg/day, thus the current results need to be interpreted with caution.

### 7.5.3 Strengths and limitations

This study is the first longitudinal cohort study in New Zealand to assess the iodine status simultaneously of both mothers and infants, subsequent to the introduction of mandatory fortification of bread with iodised salt (2009) and the provision of iodine-containing supplements (150 μg/day) to breastfeeding mothers (2010).

The study examined the iodine status of mother-infant pairs during the transitional period from exclusive, to partial, and in some cases, cessation of breastfeeding throughout the first postpartum year, which enhances the existing available

knowledge of iodine status throughout postpartum period. Maternal iodine status was measured in both urine and breastmilk to provide a thorough measure of each participant's iodine status. The study also contributes to the limited knowledge on the fractional uptake of iodine from the mammary gland in response to variations in iodine intake while exclusive breastfeeding. Since the study sample size was small, the results need to be interpreted with caution.

In order to determine iodine status throughout the postpartum period, participants were defined as iodine-containing supplement users or non-users at 3MPP, this could have diluted the effect, as many participants stopped using iodine supplements. Other limitations of this study also include that the self-selected participants were predominantly well-educated with a relatively high household income, thus the sample may not be representative of the overall New Zealand population. If these women were iodine deficient, iodine status of women who are less educated and of low income may be of even greater concern. In addition, a potentially high level of intra-individual variability in the iodine level of urine samples due to the variation in maternal hydration status and substantial variation in daily iodine intake, a single spot sample may not be indicative of usual iodine status. This may weaken or mask associations of interest. Urinary iodine creatinine ratios were used to reduce the variation due to maternal hydration. To determine habitual intake, using repeated measuring could have better predicted iodine status (48). Newborn TSH can be used as another indicator of young infants' iodine status by following a standardized protocol (49). However, potential confounding factors including maternal iodine status, mode of delivery, sampling time, and maternal exposure to iodine-containing antiseptics may limit the use of newborn TSH measurement (49).

## 7.6 Conclusions

In conclusion, after two New Zealand government interventions in 2009 and 2010, this cohort of women throughout the first postpartum year was iodine deficient irrespective of the amount or duration of breastfeeding. Iodine status of their

breastfed infants aged three and six months may be inadequate. Achieving optimal maternal iodine status is essential in maintaining adequate iodine intake for breastfeed infants. Importantly, when exclusive breastfeeding mothers had a low iodine intake, an increased fractional iodine excretion into breastmilk was observed, this is possibly a protective mechanism for maintaining their breastfed infants' iodine status over the mothers. Further studies to assess maternal and infants' iodine status with their thyroid function, and infant neurodevelopment outcomes are needed, to ensure optimal health of both mothers and their future offspring.

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# Chapter 8 Selenium intake and status of postpartum women and postnatal depression during the first year after childbirth in New Zealand – Mother and Infant Nutrition Investigation (MINI) Study

*Chapter 8, which focuses on selenium, is the last of four articles presenting research findings from the MINI study. A previous pilot study found suboptimal dietary selenium intake continued to be an issue for New Zealand breastfeeding women and their breastfed infants at three months postpartum (noted in Chapter 3). Although previous literature has suggested low dietary selenium intake is associated with negative mental health outcomes, there have been limited published studies which examine postpartum women's selenium status in relation to the risk of postnatal depressive symptoms, particularly where continuous measurements were taken from birth to one year after (see Chapter 2). This article investigates the selenium intake and status of mothers and infants and aims to determine the relationship between maternal selenium status and the risk of postnatal depression during the 12 months postpartum.*

*This article addresses **hypothesis 4**: Suboptimal selenium intake and status exist in New Zealand postpartum women; **hypothesis 5**: Selenium intakes of breastfed infants aged three and six months are suboptimal; and **hypothesis 6**: Low plasma selenium will increase the risk of postnatal depression at three, six and twelve months postpartum.*

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## 8.1 Abstract

Background

Selenium (Se) plays an important role in selenoproteins as an antioxidant, and is involved in thyroid function, mental health and child development. Selenium is low in the local food supplies in New Zealand. Low selenium intake has been reported in women of childbearing age and postmenopausal women, however, there is little research relating to breastfeeding women and their infants.

Objectives

The study investigates maternal and infant selenium intake and status during the first year postpartum, and possible relationships to postnatal depression and anxiety.

Methods

The Mother and Infant Nutrition Investigation (MINI) study is an observational longitudinal cohort study. In total 87 breastfeeding mother-infant pairs were recruited and followed up at three, six and twelve months postpartum. Maternal selenium intake was estimated from a weighed four-day diet diary (4DDD). Selenium concentrations were measured in maternal spot urine, breastmilk and plasma, and infant spot urine samples. Postnatal depression was screened by the Edinburgh Postnatal Depression Scale (EPDS) questionnaire.

Results

Median maternal selenium intake was 62 (50,84) μg/day, with 56% below the Estimated Average Requirement (EAR) of 65 μg/day. At three, six, and twelve months postpartum, median (p25, p75) maternal urinary selenium creatinine ratios were 29.0 (22.4, 42.0), 29.5 (23.1, 28.4), and 30.9 (24.3, 35.3) μg/g; median (p25, p75) infant urinary selenium concentrations (IUSC) were 8 (6,13), 11 (6, 15), and 24 (10, 40) μg/L; median (p25, p75) breastmilk selenium concentrations (BMSC) were 13 (11, 14), 11 (9, 11) and 12 (11, 13) μg/L; 18%, 11% and 14% of women reported probable minor depression based on the EPDS

scores equal or above 10. Estimated median (p25, p75) infant selenium intake at three and six months were 9 (8,11) and 8 (7, 10) μg/day with 85% and 93% below the Adequate Intake of 12 μg/day. Median maternal plasma selenium was 105.8 μg/L at six months postpartum. Minor depression at three months postpartum was significantly different across tertiles of plasma selenium concentrations ($P$ = 0.041), with the greatest at the medium tertile of 106 μg/L.

Conclusions

Suboptimal selenium intake was observed among breastfeeding mothers and their infants in the MINI study. Potentially, some women had insufficient selenium status. The relationship between selenium status and risk of postnatal depression and anxiety was inconclusive. Further research is required to explore effects on maternal thyroid function and infant neurodevelopment among women with inadequate selenium intake and status.

## 8.2 Introduction

Selenium (Se) is essential in human health because selenoproteins play antioxidant and anti-inflammatory roles and contribute to optimal levels of thyroid hormones (1). Iodothyronine deiodinases are required for conversion of the inactive form of thyroid hormone (thyroxine, T4) to the active form (triiodothyronine, T3) (2). Selenium as a component of the selenocysteine-containing proteins, glutathione peroxidase (GPx), protects the thyroid from oxidative damage (3). Extremely low selenium intake has been associated with Keshan disease (4). Selenium is thought to play a key role in optimal brain functioning (5). Adequate dietary selenium intake has been found to improve mood among the general population, while low selenium intake is associated with low mood and an increased risk of de novo major depressive disorder in women (6, 7). This suggests that pregnant and postpartum women with low selenium intake may be more at risk of experiencing postnatal depression, which affects 13% of postpartum women globally (8).

In New Zealand, low levels of selenium in the food supply, and low selenium intakes have been reported in women of childbearing age (9) and postmenopausal women (10). Low plasma selenium concentration among postpartum women and their infants residing in the South Island of New Zealand (1998-1999) suggested inadequate status (11). More recently, inadequate dietary selenium intake was reported in breastfeeding women three months after birth; however, selenium status from blood was not measured (12). There are no current data describing selenium status among breastfeeding women in New Zealand. Given changes in dietary habits, food product availability and agricultural practices, continual monitoring of both selenium intake and status in this vulnerable postpartum population is essential. This study aims to investigate maternal and infant selenium intake and status during the first year postpartum. Furthermore, the relationship between selenium status and postnatal depression and anxiety will be investigated.

## 8.3 Materials and Methods

### 8.3.1 Study Population

The Mother and Infant Nutrition Investigation (MINI) study is an observational longitudinal cohort study spanning the first year postpartum. Healthy breastfeeding women were recruited, in Palmerston North, New Zealand, from June 2016 to December 2017. Women aged 16 years and older, who had given birth to a healthy term singleton infant aged less than three months were invited to join the study. Women were excluded: 1) if they developed significant health problems, such as metabolic disease or cancer; 2) if they had been diagnosed or treated at any time for thyroid disorders.

The MINI study was approved by the Health and Disability Ethics Committee (15/NTA/172) in December 2015. The study was registered with the Australia and New Zealand Clinical Trials Registry (ACTRN12615001028594) in October 2015. Written consent was obtained from all participants.

### 8.3.2 Questionnaires

Sociodemographic information including age, ethnicity, educational attainment, household size and income was collected at the initial study visit. Potential changeable information including tobacco and alcohol use, breastfeeding patterns and general health was also sought at the second and third visits.

The 10-item Edinburgh Postnatal Depression Scale (EPDS) was completed online by participating women at three, six and twelve months postpartum, to assess any symptoms of depression over the previous seven days. Questions of the EPDS include "Things have been getting on top of me," "I feel so unhappy that I have had difficulty sleeping", and "I have felt sad and miserable." Women recorded severity of symptoms on a 4-point scale (13). A cut-off point of 10 or above was noted, which may indicate at least probable minor depression (14). A cut-off point of 13 or above was used to define high levels of depressive symptoms (13), which indicates probable major depression. Any woman whose EPDS score equalled 13 or above was advised to see her General

Practitioner (GP) for further evaluation and provided with an information sheet about postnatal depression services in New Zealand. Probable postnatal anxiety including generalised anxiety and panic disorder were evaluated by using EPDS-3A – a cluster of selected question items numbered 3, 4 and 5 from the original EPDS, as "I have been anxious or worried for no good reason", "I have felt scared or panicky for no very good reason", and "I have blamed myself unnecessarily when things went wrong". A score of six or more on the EPDS-3A was used to indicate probable anxiety (15). The EPDS is a validated tool to screen for probable minor or major depression during the postpartum period (13), and the EPDS-3A cluster for probable anxiety (16).

### 8.3.3 Dietary data collection

Participants were asked to complete a weighed four-day diet diary (4DDD) within two weeks of the initial study visit. The four days were consecutive and included one weekend day. Each participant was requested to record food items, brands, amount consumed, and provide the nutritional information panel if possible. All food and beverage items consumed were weighed with an electronic kitchen scale (Digitech, QM-7288), or measured using household measurement cups and spoons, which were provided. All participants received both written and oral instructions as to how to complete the record, which included a written example for one day. Women were also asked to include any dietary supplements consumed. When eating or dining out, participants were asked to estimate the portion size of all food eaten. All dietary data were entered to Foodworks 9 Professional (Xyris software, Brisbane, Australia), analysed using data set from the New Zealand Foodfiles 2016 (17) to estimate nutrient intake. Dietary supplements used by participants were included in dietary data analysis. To ensure accuracy and completeness, a registered nutritionist (YJ) checked all dietary data and then transferred the data to IBM SPSS for statistical analysis. The estimated average requirement (EAR) cut-point method can be used to assess population nutrient intake providing nutrient requirements are normally distributed, such as selenium; the percentage below the EAR approximates the proportion that is at risk of dietary

inadequacy (18). Current intakes based on diet and urine data were compared to Australian and New Zealand recommendations; the EAR and Recommended Dietary Intake (RDI) for selenium for lactating women are 65 and 75 µg/day, respectively (19).

### 8.3.4 Biological sample collection and analysis

Spot urine samples from each woman and her infant were collected at each study visit, to assess selenium and creatinine excretion. Infants' urine was collected using a paediatric urine bag (EMURIC, 100 mL), placed inside the diaper and checked every 10 minutes. The collected urine was frozen and stored at -20°C for later analysis, without preservative. Women provided a breastmilk sample (approximately 30-50 mL) at each visit, using an electric Breast Pump (Unimom Allegro, Korea) if needed. All breastmilk samples were collected before 12 noon on the study day, and timing of breastmilk collection was not standardised, since no significant differences have been found in selenium concentrations between hind-milk and fore-milk (20). Samples were stored without preservative at – 20°C, prior to analysis. At the second study visit, non-fasting maternal venous blood samples (22 mL) were collected by a phlebotomist. Plasma samples were stored at -80°C for later analysis.

Selenium concentrations of urine and breastmilk samples were determined by Hill Laboratories, Hamilton, New Zealand, using inductively coupled plasma mass spectrometry (ICP-MS) (21). Quality Control procedures included analysis of blanks, analytical repeats and spiked samples in order to ensure accuracy and precision. Calibration standards and checks were undertaken on every run with the limit of detection at 0.002 mg/kg. Each batch (25 samples) of urine samples was analysed together with an external reference standard (Seronorm Trace Elements Urine, L-2, Norway) giving a mean ± SD selenium concentration of 75 (5) µg/L (published value and 95% confidence error: 71.7 ± 14.4 µg/L) with a coefficient of variance (CV) of 6.5% (n = 14). Each batch of breastmilk samples was analysed together with an external reference standard (Skimmed milk powder, Elements in organic matrix, European) giving a mean ± SD selenium concentration of 0.188 ± 0.009 mg/kg (published value

and 95% confidence error: 0.188 ± 0.014 mg/kg) with a coefficient of variance (CV) of 4.9% (n = 6). Creatinine was measured using the Jaffe method Flexor E (Vital Scientific NV, 6956 AV Spankeren/Dieren, Rheden, Gelderland, The Netherlands) at Massey University Nutrition Laboratory. Plasma selenium was measured using ICP-MS at Canterbury Health Laboratories, New Zealand.

### 8.3.5 Statistical analysis

Data were analysed using IBM SPSS (Statistics Package for the Social Sciences, IBM, Armonk, NY, USA) version 20. Data were tested for normality using the Shapiro-Wilk's test. Non-parametric data were expressed as median ($25^{th}$, $75^{th}$ percentile); and parametric data expressed as mean (± standard deviation; SD). Bivariate correlations were tested using the nonparametric Spearman's rho correlation coefficient. Plasma selenium concentrations were categorised into tertiles, with one being the lowest and three being the highest. Independent sample Kruskal-Wallis tests were used to compare medians of EPDS and EPDS-3A scores across plasma selenium tertiles. Pearson chi-square tests were used to compare prevalence of depression and anxiety across the tertiles of plasma selenium concentration.

## 8.4 Results

In total, 87 breastfeeding women were recruited at three months postpartum, and most were followed up at six months (n = 78) and twelve months (n = 71). The mean age was 32 years, and 83% were exclusively breastfeeding at three months after birth (Table 8.1). Most participants were Caucasian and had completed tertiary education. The majority had a vaginal delivery (78%) and for 44% this was their first child. Approximately a quarter (23/87) of the women reported being smokers, with all but one indicating they had ceased during pregnancy. Three women resumed smoking following their infants' birth.

**Table 8.1 Description of breastfeeding participants and their infants (n = 87).**

| Maternal characteristics | n | % |
|---|---|---|
| **Maternal age, years (Mean ± SD)** | 31.5 ± 4.2 | |
| **Tertiary Education** | 67 | 77 |
| **Ethnicity (Maori)** | 9 | 10 |
| **Ethnicity (Caucasian)** | 66 | 76 |
| **Ethnicity (Asian)** | 9 | 10 |
| **Ethnicity (Other)** | 3 | 4 |
| **Annual household income (above average household income) *** | 33 | 38 |
| **Smokers** | 3 | 3 |
| **Primiparity** | 38 | 44 |
| **Caesarean delivery** | 19 | 22 |
| **Gestational ages, weeks (mean ± SD)** | 39.4 ± 1.5 | |
| **Age of infants, days (mean ± SD)** | 86.5 ± 15.1 | |
| **Infants birth weight, kilograms (mean ± SD)** | 3.6 ± 0.6 | |

*Average annual household income based on Statistics New Zealand is 100,103 New Zealand dollars for the year ended June 2017

Median maternal urinary selenium concentrations (MUSC) were 22, 22, and 26 µg/L at three, six, and twelve months postpartum, respectively (Table 8.2). Maternal urinary creatinine concentrations were used to correct spot urinary selenium concentrations expressed as µg/g. MUSCs were strongly correlated with urinary creatinine at three, six, and twelve months respectively (r = 0.868; r = 0.910; and r = 0.790, all Spearman's $P < 0.001$). Median infant urinary selenium concentration (IUSC) were 8, 11 and 24 µg/L at three, six and twelve months, respectively. IUSC at six months showed moderate correlations with the IUSC values measured at three months (r = 0.494, $P = 0.003$), and twelve months (r = 0.644, $P = 0.002$).

Median maternal plasma selenium was 105.8 (95.6, 115.3) µg/L at six months; 23% (17/74) were below 95 µg/L which indicates saturation of GPx activity (22); and 41% (30/74) of women met the criteria for maximum expression of selenoprotein P (> 110 µg/L), suggested by Hurst et al. (23). However, when compared to cut-offs suggested by Thomson (24), all the women in the current study showed adequate plasma selenium concentrations needed to achieve optimal activity of iodothyronine 5' deiodinases (> 65

μg/L) and to maximize plasma GPx (> 80 μg/L). Supplement users had a significantly higher plasma selenium concentration (121.6 μg/L) than non-users (105.8 μg/L, $P$ < 0.001). Maternal plasma selenium was weakly but significantly correlated with breastmilk selenium concentrations (BMSC) at three months (r = 0.397, $P$ < 0.001), and at six months (r = 0.373, $P$ = 0.002), but not at twelve months (r = 0.112, $P$ = 0.553); and with selenium: creatinine ratios at three months (r = 0.347, $P$ = 0.004) and six months (r=0.452, $P$ < 0.001), but not at twelve months (r = 0.110, $P$ = 0.407). Assuming an intake of 750 mL breastmilk per day (25), median estimated infant intakes were 9 and 8 μg/day at three and six months. In total, 70% (49/72) would not have achieved an intake of 10 μg/day based on the suggested adequate value extrapolated from adults (26) and 83% (60/72) did not achieve the Adequate Intake (AI) of 12 μg/day (27). BMSCs were associated with infant urinary selenium excretion at three and six months (r = 0.326, $P$ = 0.007; r= 0.433, $P$ = 0.005).

**Table 8.2 Selenium and creatinine in spot urine samples from women at 3, 6 and 12 months after giving birth and urinary selenium from their infants.**

| Median (p25, p75) | 3 months | 6 months | 12 months |
|---|---|---|---|
| **Number of participants (n)** | 87 | 78 | 71 |
| **Maternal Urinary Se concentration μg/L** | 22 (9, 34) | 22 (8, 37) | 26 (13,42) |
| **Maternal Urinary creatinine g/L** | 0.6 (0.3, 1.1) | 0.8 (0.3, 1.2) | 0.9 (0.4, 1.3) |
| **Maternal Urinary Se: Creatinine μg/g** | 29.0 (22.4, 42.0) | 29.5 (23.1, 28.4) | 30.9 (24.3, 35.3) |
| **Se in breastmilk μg/L** | 13 (11, 14) | 11 (9, 11)[a] | 12 (11, 13)[b] |
| **Infant Urinary Se concentration μg/L** | 8 (6, 13)[c] | 11 (6, 15)[d] | 24 (10, 40)[e] |

[a] n = 72 and [b] n = 33 for breastmilk samples

[c] n = 67 , [d] n = 43 and [e] n= 33 for infant urinary samples

Only five out of 87 women were taking selenium-containing supplements ranging from 25 to 65 μg/day. Median maternal selenium intake (including supplements) estimated from 4DDD was 62 (51, 85) μg/day, below the Recommended Dietary Intake (RDI) of 75 μg/day and 56% had intakes below the EAR (Table 8.3). This suggests inadequate intake for over half of the population. Selenium intake was weakly but significantly correlated with urinary selenium: creatinine ratio (r = 0.324, $P$ = 0.007). Using a proposed formula

to calculate selenium intake daily from blood plasma values (log Y = 1.623 log X +3.433; X = plasma Se, mg/L; Y = Se daily intake, µg/day) (28), estimated median selenium intake was 71 (60, 81) µg/day with 35% (26/74) below the EAR cut off.

**Table 8.3 Estimated maternal and infant selenium intakes**

| Estimated Selenium Intake | Median (p25, p75) µg/day | Below EAR (65 µg/day) n (%) | Below AI (10 µg/day)[a] n (%) | Below AI (12 µg/day)[b] n (%) |
|---|---|---|---|---|
| **Mothers, w/o supplements, Based on 4 DDD (n = 77)** | 58 (50, 80) | 46 (60) | | |
| **Mothers, w/supplements, based on 4 DDD (n = 77)** | 62 (51, 85) | 43 (56) | - | - |
| **Mothers, based on plasma Se (n = 74)** | 71 (60, 81) | 26 (35) | - | - |
| **Infants, aged 3 months, based on breastmilk (n = 87)** | 9 (8, 11) | - | 59 (68) | 58 (91) |
| **Infants, aged 6 months, Based on breastmilk (n = 72)** | 8 (7,10) | - | 55 (76) | 65 (90) |

[a] A daily intake of 10 µg of selenium is sufficient to meet nutritional requirement (25).

[b] The estimated daily selenium intake of infants up to six months old is 12 µg (26).

**Table 8.4 Maternal EPDS and EPDS-3A scores at 3, 6 and 12 months postpartum**

| Postpartum | EPDS score Median (p25, p75) | EPDS-3A score Median (p25, p75) | EPDS score equal to or above 10 Probable minor depression[a] n (%) | EPDS score equal to or above 13 Probable major depression[a] (n/%) | EPDS-3A score equal to or above 6 Probable anxiety[b] n (%) |
|---|---|---|---|---|---|
| **3 months (n=86)** | 5 (2, 8) | 2 (1, 4) | 16 (18) | 8 (9) | 10 (12) |
| **6 months (n=79)** | 5 (2, 7) | 2 (0, 4) | 9 (11) | 4 (5) | 3 (4) |
| **12 months (n=71)** | 4 (2, 7) | 2 (1, 4) | 10 (14) | 3 (4) | 9 (13) |

[a] Suggested cut-off based on Matthey et al 2006 (14) and Leung et al 2013.(29). .

[b] EPDS-3A – a cluster of selected question items numbered 3, 4 and 5 from the original EPDS, with a cut-off score six or more indicating probable anxiety (15).

At three months postpartum, 18% of women had an EPDS score equal to or greater than 10, indicating probable minor depression (Table 8.4), and 9% had an equal or higher score than 13 suggesting probable major depression (14). At 12 MPP, of the 8 women with EPDS scores above 13 (i.e. with probable major depression) at 3MPP, 5 had withdrawn from the study and the remaining 3 continued to have scores above 13. EPDS scores at three months postpartum were moderately and significantly correlated to the EPDS scores at six months (r = 0.642, $P < 0.001$), and at twelve months (r = 0.392, $P = 0.001$). Similarly, EPDS-3A scores at three months were significantly correlated to the EPDS-3A scores at six months (r = 0.558, $P < 0.001$), and at twelve months (r = 0.476, $P < 0.001$).

Table 8.5 presents median EPDS scores at three, six and twelve months postpartum categorised by tertile of plasma selenium concentration. Only prevalence of major, minor depression and anxiety of women at three months postpartum is shown, owing to the small number of affected women at six and twelve months postpartum. The median of EPDS scores across three plasma groups were displayed, and no statistically significant differences were found using the Independent-Samples Kruskal-Wallis test. Prevalence of major depression and anxiety across plasma selenium tertiles was examined using Pearson Chi-Square test and no significant differences were found. However, minor depression at three months postpartum was significantly different across the tertiles of plasm selenium concentrations ($P = 0.041$). The highest prevalence of minor depression was observed among those women with mean plasma selenium at tertile 2 (106 µg/L).

**Table 8.5 Median EPDS scores at 3, 6 and 12 months postpartum and prevalence of depression and anxiety at 3 months postpartum categorised by plasma selenium tertiles.**

| | Tertiles of plasma selenium concentration* | | | |
|---|---|---|---|---|
| | **1 (n = 25)** | **2 (n = 24)** | **3 (n = 25)** | |
| **Selenium, µg/L, mean±SD** | 92.8±3.7 | 106±3.5 | 123.7±14.5 | |
| **Age, years** | 30.6±4.0 | 31.4±4.3 | 32.7±4.1 | |
| | | | | Kruskal-Wallis |
| **EPDS score at 3 months, median** | 4 | 5 | 4 | 0.437 |
| **EPDS score at 6 months, median** | 3 | 7 | 4 | 0.247 |
| **EPDS score at 12 months, median** | 3 | 5 | 4 | 0.374 |
| | | | | Pearson Chi-Square |
| **Probable major depression 3 months (n= 5)** | 2 | 2 | 1 | 0.795 |
| **Probable minor depression 3 months (n =11)** | 3 | 7 | 1 | 0.041* |
| **Probable anxiety 3 months (n=5)** | 2 | 3 | 0 | 0.209 |

*1 is the lowest and 3 is the highest tertile.

## 8.5 Discussion

### 8.5.1 Sufficiency and deficiency

Suboptimal levels of dietary selenium intake were observed in our study participants. Maternal estimated selenium intake from the 4DDD was higher than previously reported from breastfeeding women (51 µg/day) from Palmerston North in 2009 and 2011 (12), and from Dunedin (South Island) between 2012 and 2013 (30). The increase could be due to different dietary methods used in these studies. Weighed food records are suggested to be the most precise dietary method in measuring usual nutrient intakes of individuals (31). A 4DDD used in the current study, does not rely on respondents' memory; dietary information was recorded at the time of food preparation and consumption. However, 24-hour dietary recalls, used in the other studies, depend on subjects' memories and are less reliable. Typically, lower selenium was reported in the South Island of New Zealand where bread is made from local wheat, compared to North Island where bread is manufactured from wheat imported from Australia which

contains higher levels of selenium (32). Further, an increase in selenium intake among females aged over 25 years from 2009 (56 μg/day) to 2016 (68 μg/day) was observed in the 2016 New Zealand Total Diet Survey compared to previous dietary surveys. This survey suggested that the increase in selenium intake was due to increased consumption of selenium rich food, such as chicken as there were no changes of selenium concentrations from key food contributors (33).

Over 20% of current study participants potentially have selenium deficiency by not achieving the suggested 95 μg/L required to saturate GPx activity (21). Mean plasma selenium concentrations found in the current study results were much higher than those reported in postpartum women in South Island of New Zealand (72 μg/L), as locally grown wheat (low concentrations of selenium) was used in South Island women (11). A recent Chinese study reported plasma selenium concentrations of women (42 days after birth) who were living in selenium deficient (78 μg/L), sufficient (112 μg/L) and toxic (184 μg/L) areas (28). Results of our study of women 180 days after birth were similar to those found for women living in selenium sufficient areas, considering that the level of plasma selenium concentration was not affected by the stage of lactation (34). Based on the suggested plasma selenium cut-off value of Robinson et al. (22), 23% of women in the current study would have insufficient selenium status. It is understood that more selenium is required to saturate selenoprotein P than optimize GPx (35), 59% did not achieve the suggested plasma selenium concentration to fully express selenoprotein P (23), but all women achieved adequate functional selenium status for GPx activities and selenoproteins, in relation to Thomson's suggested value. Overall, there remains no consensus on the established cut-off levels for plasma selenium at present, and therefore careful interpretation of such results is needed to assess the adequacy of selenium status.

### 8.5.2 Relationship between dietary and plasma selenium

In this MINI study, plasma selenium was not associated with dietary selenium intake. This finding is consistent with results from a study of Brazilian lactating women (36)

and a South Island New Zealand study (11), although in an earlier New Zealand study (37) significant correlations were found between dietary intakes and status in a mixed population group. Lack of an association between dietary and plasma selenium does not mean that dietary selenium is not the main factor determining selenium status. Rather, the result is likely to reflect reasonably rapid redistribution of recently absorbed selenium to various body tissues for which a range of biological half-lives exist (38). Another point to consider is that plasma selenium was more influenced by urinary excretion rather than dietary intake (39). During lactation, it has been suggested 3-6 μg/day of absorbed dietary selenium is secreted into breastmilk, with the remainder contributing to maternal blood plasma concentrations and selenoproteins (40). The current study shows plasma selenium concentrations were associated with selenium concentrations in breastmilk. Coupled with the above finding this might suggest that plasma (and breastmilk) selenium may be more influenced by internal homoeostatic processes than by recent dietary intake.

### 8.5.3 Selenium in breastmilk and urine

In the present study, breastmilk selenium concentration was associated with maternal selenium intake and status. Selenium is generally found to be higher in colostrum (26 μg/L), which then decreases to nadir levels in mature milk (1–3 months, 15 μg/L) (26). Median breastmilk selenium concentrations in the present study were similar to those reported in South Island of New Zealand in 1992 (13.4 μg/L) (41), and two recent North Island studies (11 and 14 μg/L, respectively) (12, 42). This indicates there was little change over the years, in contrast to the increase of dietary intake shown in the current study. Mean selenium in breastmilk from women at three months postpartum in the MINI study was 13 ± 2.8 μg/L, which was consistent with the values reported from Liangshan women in China, a traditionally selenium-deficient area (28). For exclusively breastfed infants, breastmilk is the only source of selenium; in the current study, estimated selenium intake of most infants did not achieve the suggested AI. This suggests that most infants in our study were at risk of selenium deficiency.

Urinary selenium excretion can be used as a proxy measure for selenium status, especially, after adjusting for creatinine (43). The urinary selenium: creatinine (μg/g) at three months postpartum was associated with dietary selenium intake, breastmilk, and plasma selenium concentrations. Using creatinine to adjust urinary selenium is a better indicator of status than urinary selenium excretion alone due to variations in hydration status. When compared to a previous study of breastfeeding women carried out in the same region (12), the current results were double on average (22 μg/L), which was also reflected in an increased dietary intake from this current study cohort. In the present study, infant urinary selenium concentrations demonstrated strong correlations with maternal breastmilk selenium concentrations at three and six months, when breastmilk is the major food source. In contrast, it was difficult to estimate dietary selenium intakes from their urinary excretion since there is no research on selenium concentrations in infant urine, although infant kidney glomerular filtration rates are reported to be low at birth but gradually reach the adult level at two years of age (44).

### 8.5.4 Relationship to postnatal depression

Results from the MINI study were not atypical, with 18% having probable minor depression at three months postpartum. An earlier study found 16% of Pacific women living in Auckland, New Zealand, had EPDS scores 13 or higher indicative of probable major depression (45). About 8% to 16% of new mothers in New Zealand are reported to suffer from postnatal depression, the most common and serious disorder for mothers in the first year after childbirth (46, 47). Approximately one quarter of affected women are still depressed when their infant reaches their first birthday. In addition, mothers are often reluctant to seek available help (48). Results from the MINI study showed that at twelve months postpartum, three of the seventy-one participants (4%) continued to be classified with probable major depression, and another five withdrew, possibly indicating that other social factors prevented them from continuing the study. Our results also showed a deceased prevalence of both major and minor depression at six months postpartum which remained similar at twelve months postpartum. However,

there was a similar prevalence of anxiety found at three and twelve months, with a decrease at six months postpartum. It has been evident that minor depressive symptoms during postpartum periods may increase the risk of recurrence of depression throughout the childbearing years (49). In addition to socioeconomic status (50) and dietary patterns (51), micronutrients, including selenium have been suggested to inversely affect mental health (6, 7, 52).

In the MINI study, no significant association was observed between plasma selenium values and EPDS scores at three, six and twelve months postpartum. A possible reason may be that less than one third of participants showed low plasma selenium levels with the majority indicated to meet a saturation of GPx activity. A randomised clinical trial in Iran reported that a prenatal selenium supplementation at 100 μg daily may be effective in preventing early postnatal depression (eight weeks postpartum) (53). A previous study of young adults aged 17-25 years old found the lowest depressive symptoms prevalence occurred when serum selenium concentrations were between 82-85 μg/L and if both below or above this value presented higher rates of depressive symptoms (based on the Centre for Epidemiological Studies-Depression Scale) (52). The U shape relationship indicted in the young adult study was not observed in the current study, which may be due to all plasma selenium being above 85 μg/L. We only found a significant higher prevalence of minor depression among women with medium levels of plasma selenium levels (106 μg/L). The possible effects of selenium on postpartum women's mental health; and to a lesser degree, its mechanism, and possible effects on infant cognitive development, will necessitate further investigation.

### 8.5.5 Strengths and limitations

This study was an in-depth prospective study of selenium intake and status of women and their infants in the first year postpartum. The strength was to assess dietary intake, excretion and tissue selenium to provide an overall evaluation of selenium status in breastfeeding women and their infants. The chosen dietary method was the gold standard dietary assessment tool to examine selenium intake. A limitation was this

cohort consists of women who were mainly well educated and motivated in achieving optimum health, thus this study was not representative for New Zealand population. Further, the study did not measure functional selenium status, including serum or plasma selenoprotein P (23), plasma GPX3 and erythrocytes GPX1 (54), which are more reliable markers in identifying nutritional selenium deficiency and examining responses after supplementation trials (55, 56). However, plasma selenium at tissue level and selenium excretion in urine can indicate the degree of insufficient intake (56).

## 8.6 Conclusions

In the present study selenium intake was suboptimal for some of the mothers and infants, despite some observed increases in intake in recent studies; and some women had potentially insufficient selenium status. Further research is required to investigate whether these suboptimal intakes negatively affect maternal thyroid function, the development of postnatal depression and anxiety, and infant neurodevelopment.

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# Chapter 9 Discussion, Conclusions and Recommendations

*This chapter provides an overview of the major findings from both Study 1 – secondary data analysis of a previous Mother and Baby study, and Study 2 – Mother and Infant Nutrition Investigation (MINI). The overall aim of this PhD thesis and each research objective investigated in the two studies are considered closely, and summarised into five central aspects, including: 1) postpartum women's thyroid function and its relation to iodine, selenium and iron; 2) maternal and infants' iodine intake and status, and maternal use of iodine-containing supplements; 3) maternal and infants' selenium intake and status, and postnatal depression; 4) iron status of postpartum women; 5) acknowledgement of the strengths and limitations of the studies. These results have contributed to current published literature, are likely to have implications for public health practice, and have identified important research priorities for future investigation.*

## 9.1 Thyroid function, its relation to iodine, selenium, and iron

*Hypothesis 1: Suboptimal iodine, selenium or iron status will impede maternal thyroid function at six months postpartum.*

Abnormalities in thyroid function have been associated with anxiety, depression, cognitive deficit (1), and negative effects on reproductive health (2). Thyroid dysfunction during the first year after childbirth is defined as postpartum thyroiditis (PPT). The prevalence of PPT in a general population normally ranges from 1.1% to 16.7% (3). Thyroid dysfunction is a significant health issue in New Zealand, with women suffering five times the prevalence of men (4, 5). However, few studies have investigated the prevalence of thyroid dysfunction in postpartum women in New Zealand. This has become of increased importance due to changes in dietary patterns, and the establishment of two government interventions to improve iodine status.

In the MINI study, at six months after childbirth, 18% of women were experiencing thyroid dysfunction, including 3% with overt hypothyroidism and 15% with subclinical hyperthyroidism. The resulting overall prevalence was higher than that reported by a previous Australian study (n = 748) which identified 12% thyroid dysfunction among women at six months postpartum (6). This higher rate in our study could have occurred: 1) because women who participated had undiagnosed thyroid dysfunction during previous perinatal periods which may have increased their risk of thyroid dysfunction at this time; 2) because they could have a family history of thyroid dysfunction which may have encouraged their participation in this study; 3) because women who had autoimmune diseases that were previously unknown may have an increased risk of developing PPT (3). Additionally, the risk of developing PPT may increase in women who presented positive thyroid peroxidase antibodies (TPOAb) during early pregnancy (7) or at childbirth (8). Our study also found positive TPOAb at six months postpartum was associated with abnormal TSH concentrations. After examining a cohort of Iranian women with subclinical and overt PPT, who were undergoing T4 therapy, Azizi reported that a trend of increasing numbers of women

with subclinical PPT developed permanent thyroid dysfunction than those with overt PPT, but such differences were not found to be statistically significant (9). However, Azizi suggested that identifying both subclinical and overt cases of PPT early can allow timely interventions.

This MINI study has examined thyroid function in relation to iodine, selenium and iron status in postpartum women. The result has shown that selenium status measured as plasma selenium was the only significant predictor of the likelihood that women had abnormal thyroid stimulating hormone (TSH) concentrations. Women with a lower plasma selenium (< 95 μg/L) were more likely to experience thyroid dysfunction (41.2%), when compared to 10.5% of women with optimal plasma selenium to saturate glutathione peroxidases (GPx) activity. Numerous research studies have established the roles of selenium in thyroid function, including converting thyroxine ($T_4$) to triiodothyronine ($T_3$) via iodothyronine deiodinases; catalysing the reduction of hydrogen peroxide and protecting the thyroid gland from oxidative stress via GPx and selenoprotein P (10). Intervention studies have investigated selenium supplementation in the general population including pregnant women; selenium treatment may reduce TPOAb levels, improve thyroid echogenicity, and decrease the incidence of postpartum thyroid dysfunction, but researchers did not find significant effects of selenium on thyroid hormone synthesis (10, 11). However, this study finding raises questions as to who might benefit most from selenium supplementation, and what should be an effective dose and duration.

In addition, iodine status determined by the urinary iodine concentration (UIC) and breastmilk iodine concentration (BMIC) did not predict thyroid dysfunction. One possible reason may be the high intra-individual variations in these measures, which predict status of populations rather than individuals. Alternative explanations may include that thyroid hormones remain in the normal range even in a mild-to-moderate iodine deficient population (12). Further, the MINI study has found a high prevalence of subclinical hyperthyroidism (15%) in this cohort of iodine deficient postpartum women. This is consistent with the reports from Danish population

studies where the rate of hyperthyroidism was higher among those who were iodine deficient than those having adequate or excessive iodine intake (13, 14, 15). The MINI study found women using iodine-containing supplements (9/74) presented with normal TSH concentrations, while 20% (13/65) of women who did not use iodine-containing supplements recorded abnormal TSH concentrations. This indicated a beneficial effect of iodine supplementation on optimal thyroid function in an iodine deficient population. However, the number of iodine-containing supplement users was too small to generate sufficient statistical power.

Iron status in our study was not associated with thyroid hormone concentrations or thyroid volumes, which may be due to the predominantly adequate iron status of the women. However, a prospective study of iron deficient and anaemic (IDA) Turkish women of childbearing age (n = 66) found a significant reduction of thyroid volume after taking iron supplementation (16). This group of Turkish women were euthyroid and iodine deficient (MUIC, 83 μg/L). They were given equivalent to 100 mg elemental iron twice daily. Results showed that after a six-month treatment, their mean thyroid volume was significantly reduced from a baseline of 17 mL to 13 mL. Consequently, the authors suggested that iron status played a substantial role in the thyroid volume, possibly due to increased blood flow to thyroid gland, resulting in its subsequent enlargement occurring under iron deficiency anaemia (16). We may have found an association if more participants had ID or IDA.

Data analysis from the MINI study confirms that hypothesis 1 was partly accepted, as selenium status assessed by plasma selenium was the only significant predictor of thyroid dysfunction among this cohort of women who were iodine deficient and who mostly achieved adequate iron status.

## 9.2 Maternal and infants' intake and status of iodine over the first postpartum year

*Hypothesis 2: Breastfeeding women who used iodine-containing supplements will achieve better iodine status for themselves and their breastfed infants.*

*Hypothesis 3: Postpartum women and their infants will remain iodine deficient, despite two New Zealand government initiatives to improve iodine status.*

To combat re-emerged iodine deficiency, mandatory fortification of bread (all commercial bread other than organic and unleavened) with iodised salt (25-65 mg iodine/kg salt) was implemented in New Zealand in 2009. Post-fortification studies showed an increase in the iodine status of adults [median UIC (MUIC) from 53 μg/L to 103 μg/L], and women of childbearing age (MUIC from 48 μg/L to 104 μg/L) (17), as well as children (MUIC from 68 μg/L to 113 μg/L), which is indicative of iodine adequacy (18). However, such fortification may not sufficiently meet the increased needs for pregnant and breastfeeding women (19). Thus, in 2010, using a 150 μg/day iodine supplement was recommended for all pregnant and breastfeeding women in New Zealand. Few studies in New Zealand have investigated the iodine status of pregnant and lactating women.

The MINI study found iodine deficiency was present in the sample of New Zealand breastfeeding women at three months postpartum, despite the establishment of the two government initiatives. The maternal MUIC was 82 μg/L, which showed an improvement from 74 μg/L reported in a pilot study (n = 36) a year after the government initiatives (20). Furthermore, median BMIC was 69 μg/L in the MINI study, below the suggested index of 75 μg/L for adequacy (21). This remained similar to the mean BMIC of 63 μg/L reported in a New Zealand pilot study in 2011 (20).

Both Denmark and Australia have implemented mandatory fortification of bread with iodised salt since 2000 (22) and 2009 (5), respectively. In addition, mandatory iodine fortification in household salt takes place in Denmark (23), however, in Australia and New Zealand, iodised fortification in household salt remains voluntary. Both Australia (24) and New Zealand (25) recommend iodine supplementation for all pregnant and breastfeeding women. In Denmark, currently, there remains no official recommendation of iodine supplementation for pregnant and lactating women.

A comparison study between pre- and post-fortification in Demark reported that breastfeeding women were still iodine deficient, following the establishment of mandatory iodised salt fortification, although an increment was observed from 50 μg/L to 72 μg/L in MUIC (23). A small sample of pre- (n = 32) and post-fortification (n = 36) in New Zealand reported that iodine status of breastfeeding women was improved (34 to 74 μg/L), but still deficient (20). A recent cross-sectional study in South Australia compared the iodine status of breastfeeding women pre- and post-fortification, the results of which showed an increase in BMIC from 105 to 137 μg/L, confirming iodine adequacy (26).

In the MINI study, women who took iodine-containing supplements achieved better iodine status than non-users. Women who used iodine-containing supplements obtained higher MUIC (111 vs 68 μg/L, $P = 0.023$) and median BMIC (84 vs 62 μg/L, $P < 0.001$) compared with non-users. This trend is consistent with a small sample post-fortification study of 60 breastfeeding Australian women, recording a higher MUIC of 206 μg/L in iodine supplement users than non-users (97 μg/L) (27). Similarly, another large sample post-fortification study of breastfeeding women in South Australia (n = 653) reported a significantly higher BMIC of 195 μg/L in iodine supplement users compared to non-users (137 μg/L) (26).

A post-fortification study of breastfeeding Danish women (n = 209) reported a higher median BMIC (112 vs 72 μg/L) and MUIC (83 vs 65 μg/L) of iodine supplement users than non-users (28). Despite no official recommendation of iodine supplementation in Demark, half of the postpartum women studied took supplements, and Danish researchers have suggested iodine supplementation for breastfeeding women in Denmark is essential (28). In the MINI study, despite of a government recommendation of using iodine supplementation during lactation, only 46% used iodine-containing supplements. This rate is lower than the rate (90%) reported in the South Australian post-fortification study (26), and slightly lower than the 50% reported in the Danish study. High rates of iodine-containing supplement use may have contributed to the achievement of adequate iodine status of breastfeeding

women and their infants in South Australia (29). Our findings together with other research showing an improvement in iodine status of breastfeeding women who took iodine containing supplements supports the need for greater uptake of iodine supplements by breastfeeding women in New Zealand.

In addition, infants of iodine-containing supplement users achieved a higher MUIC compared to those from non-users (150 vs 86 μg/L, $P$ = 0.036). This result is comparable to the improved iodine status of Moroccan infants after their mothers were provided with a one-off supplementation of 400 mg iodine in early lactation (30). Therefore, urinary iodine results suggest that maternal iodine supplementation may benefit both breastfeeding mothers and infants.

In the MINI study, supplementation advice from health professionals was communicated to pregnant women (86% used iodine-containing supplements), however, such practices reduced to 46% during lactation. A recent online survey of New Zealand women during their pregnancy phase (n = 442) and during the 0-6 months postpartum period (n = 284) reported 95% and 63% (respectively) using iodine-containing supplements (31). These percentages are higher than was seen in our study, and higher than those reported previously (52% in 2017 for postpartum women) (32). However, the dosage, duration and frequency of iodine supplement use was not reported in the online survey, thus, it is difficult to know the adherence of iodine recommendations and whether iodine supplement use is sufficient (31). In the current study, the main predictor for using an iodine-containing supplement was iodine knowledge; however, 66% of women had nil or low iodine knowledge, including the importance of iodine in heath and good dietary sources of iodine; and 79% and 54% were unaware of the 1$^{st}$ and 2$^{nd}$ government initiatives. One of the main reasons given by women for not using iodine supplements during this time was that very little advice concerning iodine supplementation during lactation was given by health professionals. A 2014 Australian study investigating antenatal care reported that a majority of Australian General Practitioners questioned did not openly recommend iodine supplement to their patients (33). Furthermore, a more recent

Australian nation-wide survey of health professionals (including general practitioners, obstetricians, gynaecologists, midwives, and dietitians, n = 396) reported a lack of awareness of the appropriate dose and duration of iodine supplementation that is recommended for pregnant and breastfeeding women (34). Although such data in New Zealand are at present unavailable, this Australian nation-wide survey highlighted a need for iodine specific nutritional education initiatives to be brought to the attention of all primary health care providers (34).

To the best of our knowledge, only five studies have investigated iodine status at different time points from early lactation to the end of the first postpartum year, in Sudan (35), Switzerland (36), Norway (37), Sweden (38), and New Zealand (39) (Table 9.1). The New Zealand study was carried out over twenty years ago with a small sample of 35 breastfeeding women, the MUIC (24 hours urine samples) recorded as 37, 25, and 47 μg/L at three, six and twelve months postpartum, indicating iodine deficiency (39).

In the MINI study, maternal MUIC of 82, 85, and 95 μg/L at three, six and twelve months postpartum, respectively were below the WHO cut-off of 100 μg/L, suggesting iodine deficiency. A trend for an increase in MUIC across three time points in the MINI study was also found in a study of Sudanese breastfeeding women (n = 47) living in an area with 17.5% goitre rate, where MUIC increased from three (51 μg/L) to nine (63 μg/L) months postpartum (35). Similarly, a recent Norwegian longitudinal study of postpartum women (n = 915) reported the lowest MUIC at six weeks postpartum (57 μg/L), which increased through six, twelve and eighteen months postpartum (70 μg/L, 79 μg/L, 87 μg/L) (37). The Norwegian authors suggested the increase was possibly due to a decrease of iodine excretion in breastmilk during the postpartum period. Both decreased use of iodine-containing supplements and cessation of breastfeeding in the MINI study contributed to the increase of maternal UIC from 3MPP to 12 MPP. However, these studies on Norwegian and Sudanese women did not examine the breastmilk iodine concentrations.

**Table 9.1 Comparison of iodine status between four longitudinal studies and the MINI study.**

| Country | Iodine fortification at the time of study | Sample size | Postpartum time | Maternal MUIC (µg/L) | Median BMIC (µg/L) | % using iodine supplement | Tg (µg/L) | Infant MUIC (µg/L) | Year of the study | Authors |
|---|---|---|---|---|---|---|---|---|---|---|
| New Zealand | Voluntary iodised salt (25-65 mg iodine/kg salt) | 35 | 3 months | 37 | n/a | n/a | n/a | n/a | Prior to 1999 | Thomson et al. 2001 (39) |
| | | | 6 months | 25 | | | | | | |
| | | | 12 months | 47 | | | | | | |
| Sudan | Voluntary iodised salt (15 mg/kg), 14% household uses (40) | 47 | 3 months | 51 | n/a | n/a | 29 | n/a | 1993-1995 | Eltom et al. 2000 (35) |
| | | | 6 months | 30 | | | 27 | | | |
| | | | 9 months | 63 | | | 24 | | | |
| Switzerland | Voluntary Iodised salt (20mg/kg), 90% household uses (41) | 196 | 6 months | | 51 | | | 91 | 2005-2007 | Andersson et al. 2010 (36) |
| | | | 12 months | 75 | 42 | 3 | n/a | 103 | | |
| Norway | Voluntary iodised salt (5 mg/kg) (42) | 915 | 6 weeks | 57 | n/a | n/a | n/a | n/a | 2011-2014 | Aakre et al. 2020 (37) |
| | | | 6 months | 70 | | | | | | |
| | | | 12 months | 79 | | | | | | |
| | | | 18 months | 87 | | | | | | |
| Sweden | Voluntary iodised salt (50µg/g salt) (43) | 84 | 0.5 month | | 90 | 19 | 11.8 (non EBF) vs 22.3 (EBF) | n/a | 2008-2011 | Manousou et al. 2020 (38) |
| | | | 4 months | 78 | | 13 | | | | |
| | | | 12 months | 107 | | 11 | | | | |
| New Zealand | Mandatory iodised salt in bread (25-65 mg iodine/kg salt) and recommendation of consuming 150 µg/day iodine | 87 | 3 months | 82 | 69 | 40 | n/a | 115 | 2017-2018 | Jin et al. 2020 |
| | | | 6 months | 85 | 59 | 11 | 11.4 | 120 | | |
| | | | 12 months | 95 | 35 | 6 | n/a | 118 | | |

In the MINI study, a continuous decrease of BMIC was detected as 69, 59, and 35 µg/L at three, six and twelve months postpartum. This pattern suggests reduced transport of iodine to breastmilk. A similar reduction was reported in the observational Switzerland study of women from six (51 µg/L) to twelve (42 µg/L) months postpartum (36). Past research reported a sharp decrease of iodine concentration from colostrum to mature milk, which could be due to the differences in volume levels (44). A further reduction was observed during the six to twelve months postpartum period in the MINI study, which was possibly due to infants starting complementary food, and therefore becoming less reliant on iodine supply in breastmilk. However, for infants (exclusively or partially breastfed), breastmilk remains an important source of iodine.

A wide range of BMIC was observed in women in iodine-sufficient countries from 50 µg/L in Finland to 270 µg/L in the United States, however, there was no consensus to adopt an adequate BMIC cut-off (45). In a systematic review published in 2009, Azizi reviewed BMIC from iodine-sufficient and -deficient countries and suggested a value above 75 µg/L as an index for adequate infant iodine intake (21). Based on the suggested average breastmilk consumption of 0.78 L (during the first six months of age) (45), estimated iodine intakes for infants aged three and six months in the MINI study was 54 and 46 µg/day, respectively. These are below the suggested Adequate Intake (90 µg/day) by the Australian New Zealand Nutrient Reference Value (46), thus, breastfed infants aged three and six months recorded insufficient iodine intake.

In addition to indirectly estimating infant iodine intake from breastmilk, UIC in spot urine was used to evaluate infant's iodine status in the MINI study. The WHO suggests a median MUIC of at least 100 µg/L for population adequacy in infants (47), based on an estimated iodine intake is 55 µg/day (using approximately 0.5 L daily urine volume and 92% of dietary iodine excreted into urine) (48). This is much lower than the Institute of Medicine suggested adequate intake (AI) of 110 µg/day for infants up to age of six months (49). Furthermore, results from a 2016 dose-response crossover iodine balance study of euthyroid Swiss infants aged two to five months, suggested 125 µg/L as a MUIC cut-off for population sufficiency (based on the estimated average

requirement of 72 μg/day for infants aged two to five months) (48). Using this cut-off, the MUIC for infants aged three (115μg/L) and six months (120μg/L) in the current study would suggest iodine deficiency, which was consistent with the estimated suboptimal intake from BMIC. Compared to adults, there is less variation in infant hydration status. Despite this, to assess individual status requires multiple samples and the number needed is similar for infants and adults (50, 51). Further investigation of health outcomes related to such measures may provide additional useful evidence to confirm an optimal cut-off for infant MUIC. Future studies need to explore the iodine status of young infants in relation to their neurodevelopment, as iodine deficiency is the most preventable cause for brain damage in early childhood (52, 53).

Infant receiving complementary food may be at an increased risk of iodine deficiency owing to their high requirement of iodine per body weight and the recommendation of not given infants neither iodised nor non-iodised salt up to one-year (54). WHO recommends infants aged six months should receive complementary food in addition to breastmilk (55). In the present study, a significantly lower MUIC was found in infants aged six months who were exclusively breastfed, compared to infants who were mixed fed (80 vs 147 μg/L). Consumption of any complementary food or/and infant formula may contribute to such differences. Prior to the mandatory fortification of bread with iodised salt, the 2009 New Zealand Total Diet Survey (NZTDS) reported the mean iodine intake of infants aged 6-12 months was 66 μg/day which was mainly contributed by infant and follow on formula (56); after the fortification this was increased to 83 μg/day in the 2016 NZTDS , (57). This is possibly due to changes of iodine concentration in complementary foods. The 2016 NZTDS reported infants gained just under 70% of their iodine intake from infant formula and commercial infant foods, the balance was from other sources of dairy, grains, CEFM (chicken, eggs, fish, meat), and suggested that iodine intake of infants aged six to twelve months were less affected by the mandatory fortification of bread with iodine when compared to other populations, because infants have less consumption of grain-based foods (57). In addition, the NZTDS only includes infants on formula and not those who are

breastfeeding, there is limited data on iodine intake from complementary food sources in New Zealand. The current study results suggest the importance of iodine concentrations in breastmilk to enable adequate iodine intake for those who are not yet adding complementary foods. Recommended iodine supplementation of 150 μg/L for breastfeeding women in New Zealand may improve BMIC.

Data analysis from the MINI study broadly accepts hypotheses 2. However, hypothesis 3 is accepted in part since those women who used iodine-containing supplements and their breastfed infants both achieved adequate iodine status.

## 9.3 Maternal intake and status of selenium, and postnatal depression

*Hypothesis 4: Suboptimal selenium intake and insufficient selenium status exist among New Zealand postpartum women.*

*Hypothesis 5: Selenium intakes of breastfed infants aged three and six months are suboptimal.*

*Hypothesis 6: Lower plasma selenium will increase the risk of postnatal depression at three, six and twelve months postpartum.*

Numerous studies investigated selenium intake in New Zealand during the decade of 1980-1990. However, a few studies have monitored selenium intake in recent years in children (58), women of childbearing age (59), and older adults (60, 61). Only two recent studies in 2004 (62) and 2012/2013 (63) from the South Island of New Zealand have explored the selenium intake and status of postpartum women and their infants.

A secondary data analysis of the Mother and Baby study (2009/2011) found lactating women at three months postpartum had inadequate selenium intake [51 (36, 80) μg/day] when compared to the EAR (65 μg/day). In the 2017/2018 MINI study, a higher selenium intake of breastfeeding women was observed [62 (51, 85) μg/day], indicating a possible improvement in selenium intake in New Zealand women over time. The

2009/2011 dietary data were collected via repeated 24-hour dietary recalls, while data from the MINI study were collected from the weighed four-day diet diaries which are the gold standard method for estimating dietary intake.

Using a weighed three-day diet diary, the 2016 study of postpartum women (n = 53) living in the South Island of New Zealand reported mean selenium intake was 47 μg/day, which was much lower than that as reported in our studies (63). The differences may be due to the different methodologies. The importation of Australian wheat (containing ten times higher selenium content than New Zealand wheat) (64) has dramatically improved dietary selenium intake in the North Island of New Zealand, with less effect being observed in the South Island of New Zealand. From the results of the 2016 Total Diet Survey, bread from the North Island (0.1 mg selenium per kg bread) contains higher selenium than those from the South Island (< 0.005 mg selenium per kg bread) (57). Australian imported wheat is mainly used for making bread in the North Island, while locally produced wheat is mostly used for bread-making in the South Island (57). The most recent New Zealand Total Diet Survey (2016) reported an increased selenium intake among females aged over 25 years from 2009 (56 μg/day) to 2016 (68 μg/day) (57). This survey sampled 132 foods (the most consumed foods in New Zealand), and eight different samples of each food item were analysed. Food consumption data were based on 14 day simulated typical diets for different age-gender cohorts. This survey suggested increased consumption of selenium rich foods may contribute to the improvement of selenium intake since selenium concentrations of the key food contributors had remained unchanged (57).

At three months postpartum, median urinary selenium concentration (MUSC) of lactating women in the MINI study [22 (9, 34) μg/L] was almost double from that recorded in the 2009/2011 Mother and Baby study [12 (8, 20) μg/L], and this may be due to an increased dietary selenium intake from 51 to 62 μg/day, and the sample size of the Mother and Baby study was much smaller. Further, in the MINI study, selenium concentrations were measured in spot urine samples, while 24-hour urine specimens had been used in the previous study conducted across 2009 and 2011. However, as

shown in Table 9.2, breastmilk selenium concentration (BMSC) in the MINI study [13 (11, 14) μg/L] was slightly higher than 11 (10, 13) μg/L in the 2009/2011 study. It was also slightly higher than 11 μg/L in another 2012/2013 study conducted in the South Island of New Zealand (63). BMSC remained like those concentrations reported in the 1992 study from the South Island of New Zealand (13 μg/L) (65), and a more recent study from the North Island of New Zealand (14 μg/L) (66). In contrast to the increase of dietary intake shown in the MINI study, these comparative results indicate little change of BMSC over the period stated above. It is arguable that, relatively constant selenium secretions in breastmilk (11 to 14 μg/L) could be due to adjustments in the supply of adequate selenium to breastfed infants. Thus, extra selenium from increased dietary intakes would be excreted into urine. BMSC are assumed to reflect maternal dietary intake, however whether mammary tissue sequesters selenium to provide for the infant is unclear, the lowest of 2.6 μg/L being recorded in an area of endemic Keshan disease in China (with 7.6 μg/L reported in New Zealand in 1983) increasing to the highest at 283 μg/L in an area of endemic human selenosis in China (67). The BMSC at three months postpartum in the MINI study was similar to the concentration from Liangshan women in China in 2016 (12 μg/L), living in a traditionally selenium-deficient area (68). These BMSCs below the international reference range of 18.5 μg/L which provide adequate selenium intake for infants aged 0-6 months (13.9 μg/day) (69). To conclude, selenium intakes of breastfeeding women at three months postpartum were suboptimal in the 2009/2011 study and the 2017/2018 MINI study.

**Table 9.2 Selenium status between current and other New Zealand studies**

| | **PhD Study 1 (70)** | **PhD Study 2 (71)** | **Kendall (2015) (63)** | **Dolmore et al (1992) (65)** | **Butts et al (2018) (68)** |
|---|---|---|---|---|---|
| Location | North Island | North Island | South Island | South Island | North Island |
| Data collection (year) | 2009/2011 | 2017/2018 | 2012/2013 | n/a | n/a |
| Participants (n) | 68 | 87 | 53 | 70 | 78 |
| Timing (postpartum) | 4 months | 3 months | 8 weeks | up to 12 months | 6-8 weeks |
| MUSC, μg/L (median/mean) | 12 | 22 | n/a | n/a | n/a |
| BMSC, μg/L (median/mean) | 11 | 13 | 11 | 13 | 14 |

The MINI study explored both the MUSC and the BMSC of women at three, six and twelve months postpartum. Their maternal MUSC at three months postpartum (22 μg/L) remained the same at six months postpartum but increased to 26 μg/L at 12 months postpartum ($P$ = 0.016). This significant increase could be due to less selenium being excreted in breastmilk to meet infants' needs. Their BMSCs showed a slight variation from 13 μg/L at three months postpartum to 11 and 12 at six and twelve months postpartum, respectively. Reduction from three to six months postpartum was statistically significant ($P$ = 0.003). Based on two review articles published in 1989 and 2002 (67, 72), selenium is generally found to be higher in colostrum (26 ug/L), which then decreases to nadir levels in mature milk (1-3 months, 15 ug/L). Hence, this decrease of selenium concentrations may be both due to very low volume and high content of proteins in breastmilk during the early lactation period (72).

In the MINI study, maternal selenium status was measured in blood plasma, the most common biomarker for selenium status in the published literature (73). It allows an ease of comparison of selenium status with other countries. In a systematic review of 18 supplementation studies by Ashton et al. (2009) (73), plasma selenium significantly increased after selenium supplementation, suggesting it is useful in assessing selenium depletion. All women in the MINI study demonstrated adequate plasma selenium to achieve optimal activity of iodothyronine 5' deiodinases (> 65 ug/L) (74), but 23% had inadequate selenium status with plasma selenium below the 95 μg/L required for the saturation of GPx activity (75). Further, 59% of women did not achieve 110 μg/L which is needed to achieve full expression of selenoprotein P (76). Median plasma selenium of lactating women in the MINI study (106 μg/L) was higher than 72 μg/L reported by McLachlan et al. (2004) in the South Island of New Zealand (62), but remained lower than North American women at six months postpartum (138 μg/L) who lived in a selenium sufficient area (77).

The current study is not able to evaluate the use of selenium supplementation due to the small number of women who took selenium-containing supplements (5/87).

Selenium supplementation studies are inconclusive as to the degree of health benefits. Also, supplementation requires caution because of the "U" shaped relationship between selenium status and health outcomes, for example, selenium deficiency may increase the risks of having thyroid autoimmune disease, viral infection, declined cognitive functions, and prostate cancer, while excessive selenium may increase skin cancer, prostate cancer, and type 2 diabetes risks. (78, 79). Hence, further investigation is required to determine who will benefit from selenium supplementations, to assess what a safe dose of supplementation might be (possible randomised placebo-controlled trials), and to identify what long-term effects may exist after the cessation of using supplements (longitudinal or retrospective studies).

Hypothesis 5 states selenium intakes of breastfed infants aged three and six months are suboptimal. The current AI for infants aged 0-6 months (12 μg/day) was calculated from the average intake of 0.78 L breastmilk/day and BMIC of 15 μg/L [New Zealand study in 1992 (65) and Australian studies in 2000 (80)]. The estimated median infant selenium intake from the 2009/2011 cohort and the MINI study were identical at 9 μg/day, which is below the recommended AI of 12 μg/day (AI) (46). The results also showed that 70% (45/64) in the 2009/2011 study and 70% (49/72) in the MINI study did not achieve an intake of 10 μg/day based on the suggested adequate value extrapolated from adults (67); thus, breastfed infants aged around three months were at risk of selenium deficiency. The estimated intake of infants aged six months in the MINI study was 8 (7, 10) μg/day, and 90% did not achieve AI, which suggests suboptimal selenium intake in this cohort. The current estimated selenium intake from breastmilk presents some difficulties due to the complexity of accurately measuring daily breastmilk consumption for infants. When infants start receiving complementary foods, it may become even more difficult to estimate their dietary intakes due to the variety of complementary foods and diverse of feeding practices.

The MINI study has explored infant urinary selenium concentrations in spot urine samples. Infant MUSC was 8 (6, 13) μg/L at three months, 11 (6, 15) at six months, and 24 (10, 40) at twelve months ($P$ = 0.004). The significant increment from six to twelve

months may due to increased complementary food intake containing a more varied dietary selection of organic and inorganic selenium. Urinary selenium excretion directly reflects recent dietary intake, in adults, a 50-60% excretion rate is used to estimate selenium intake (81). There is limited research of young infants on their excretion rates of selenium in urine, therefore, it is difficult to estimate their selenium intake. Currently, there is no available cut-off for infant urinary selenium concentration to enable identification of selenium depletion in groups. It may be useful to investigate selenium excretion in urine from infants living in selenium-sufficient countries to establish an optimal value for future research purposes.

Infant selenium status can be measured using serum/plasma, or erythrocyte GPx activity in blood samples (80). However, it may be difficult to obtain parents' consents to collect blood samples from their healthy infants. One of less invasive methods is to analyse selenium concentrations in the nail clippings of infants. Measuring selenium in nail clippings has been used in evaluating the relation to lung and prostate cancer in adults (81), and in assessing the preeclampsia risk for pregnant women (82). The main advantage of the method is to measure selenium intake over a long exposure time. This measurement may provide opportunity to explore selenium exposure in utero since nails grow at a slow rate and can be collected from participants over time. However, it is worth noting that one mm of nail samples can reflect approximately one-month accumulation of nutrient status (81), and there is also a need to acknowledge the varied nail growth rates which are affected by age, gender, metabolic rate, and health conditions.

Prevalence of postnatal depression in the MINI study was typical, when compared to the results from a large longitudinal study - Growing Up in New Zealand - suggesting that 5% of women were experiencing depression symptoms at nine months postpartum (83). In the MINI study, the relation between selenium and postnatal depression was explored, but results remained inconclusive. No significant association was observed between plasma selenium and the Edinburgh Postnatal Depression Scale (EPDS) scores taken at three, six and twelve months postpartum. This may be

due to most women having plasma selenium higher than the 95 μg/L required for maximum GPx activity. In the event, our study found the highest prevalence of minor depression at three months postpartum and was only observed in women with a mean plasma selenium at 106 μg/L (the middle tertile), but not observed in other tertiles which may due to a small sample size. Another New Zealand study of young adults aged 17-25 years found a "U" relationship between serum selenium and the prevalence of depression, and the lowest depressive symptom prevalence occurred when serum selenium ranged between 82-85 μg/L (84). This phenomenon was not observed in the MINI study, since plasma selenium concentrations of all postpartum women were higher than 85 μg/L. The possible effects of suboptimal selenium on postpartum women's mental health and its mechanism and possible effects on infant cognitive development, requires ongoing investigation.

The data presented provides support for the hypotheses 4 and 5. Whether low plasma selenium increases the risk of postnatal depression (hypothesis 6), however, was inconclusive.

## 9.4 Maternal iron status

*Hypothesis 7: High prevalence of iron deficiency and iron deficiency anaemia exist among women at six months postpartum.*

Hypothesis 7 was proposed in anticipation that most women would have inadequate iron status at six months postpartum. However, our findings showed that only 4% were iron deficient without anaemia (ID), and none were classified as iron deficiency anaemia (IDA). The low rate of ID in this current study cohort could be due to the protective benefits from six-month lactational amenorrhoea. The possible consumption of iron supplements during pregnancy (69% reported taking iron supplements although exact compliance was not measured) may also contribute to this low rate. Additionally, most women who experienced postpartum haemorrhage were immediately treated with iron or blood transfusion (62%), which may contribute

to low rates of ID, as severe blood loss is strongly related to an increased risk of postpartum ID and IDA (85). In an iron status study of America women at six months postpartum, after controlling for confounding variables, the risk of ID in postpartum women, compared to non-pregnant women, was highest among those from low-income groups (86). If our study had included a more representative sample in household income status than 62% of women reported acceptable household income above the median New Zealand household income, the prevalence of ID may have been higher. While the data collected does not provide support for the hypothesis 7, it does not exclude it. More work is needed to investigate the iron status of those in New Zealand who are less wealthy.

## 9.5 Strengths and limitations

To the best of our knowledge, the MINI study was the first study to examine iodine, selenium, and iron concurrently in relation to the thyroid function of postpartum women. It is one of a few studies to investigate the prevalence of thyroid dysfunction in a New Zealand postpartum cohort. This study examined the iodine status of mother-infant dyads during the transitional period from exclusive, to partial, and in some cases, cessation of breastfeeding at three time points after partition. Results from this current study add to the existing limited knowledge of iodine status throughout the postpartum period. A triangular method was used to assess iodine status, including UIC, BMIC and serum Tg. Furthermore, to evaluate selenium status in breastfeeding women, the excretion of selenium in urine and breastmilk (short-term dietary selenium intake) and plasma selenium (the most common biomarker) were both assessed. Finally, both iron storage measuring SF and functional iron deficiency measuring serum sTfR which is less likely affected by inflammation status were assessed to provide a better understanding of iron status (87), and allow early detection of ID before the occurrence of IDA (88). Repeated measures on iodine and selenium concentrations in urine and breastmilk samples have rarely been carried out in other published literature. These measures applied in the MINI study provided a

continuous picture of iodine and selenium status in mothers and infants during their first postpartum year.

A limitation of this study is selection bias, where women with pre-existing iodine knowledge may have been attracted to participate. Women in this study were affluent and highly educated, and, typically, women who volunteer to participate in such studies are often more interested and likely to be motivated about health than the general population (89). If the iodine and selenium status of these women were inadequate, it is likely that women in the community who are less well-educated and under-represented may present further reduced status.

Since the study sample size was small, the results need to be interpreted with caution. However, the retention rates (90% and 82% at six and twelve months postpartum respectively) were sufficient to enable further analysis within follow-up visits. The MINI study did not measure functional selenium status, including serum or plasma selenoprotein P, plasma GPX3 and erythrocytes GPx1 in identifying selenium deficiency due to the cost and low stability. Biological samples cannot be measured immediately after sampling but need to be stored and measure in batches. However, plasma selenium is the most used biomarker in other published research which allowed comparison. Few participants reported the use of dietary supplements containing selenium (1% -1/78) or iron (8% - 6/78) during postpartum, leading to this study having insufficient statistical power to examine the effects of such supplementation on thyroid function.

## 9.6 Final Conclusions

A high prevalence of thyroid dysfunction (assessed by serum TSH, fT4 and fT3) was observed in this cohort of women at six months postpartum, with 15% presenting with subclinical hyperthyroidism. Selenium status was the only significant predictor of thyroid dysfunction, while iodine status (measured by UIC and BMIC) and iron status (serum sTfR) were not implicated. Most postpartum women achieved adequate iron status with only 4% having ID. This cohort of women throughout the first year after

partition were iodine deficient irrespective of the amount or duration of breastfeeding, and despite two New Zealand government iodine interventions initiated in 2009 and 2010. The iodine status of their breastfed infants aged three and six months may be less than adequate. Women who used iodine-containing supplements were more likely to achieve adequate iodine status for themselves and their breastfed infants. Maternal selenium intake remained suboptimal, and some women may have insufficient selenium status. Most infants were at risk of selenium deficiency. Prevalence of postnatal depression was typical for women domiciled in New Zealand. Although minor depression at three months postpartum was significantly different across tertiles of plasma selenium concentrations, any close relationship between selenium status and risk of postnatal depression and anxiety was inconclusive.

## 9.7 Implications for public health practice

A high prevalence of thyroid dysfunction was found in this cohort of iodine-deficient postpartum women with suboptimal selenium intake. Literature has suggested that women with PPT might develop permanent hypothyroidism or experience PPT again after the subsequent pregnancies, and such risk increases if women had positive TPOAb during early pregnancy (3). However, screening for thyroid dysfunction is not routinely recommended for women who are either planning a pregnancy or become pregnant in New Zealand (90). Early screening of TPOAb may be useful in identifying women who are at a high risk of experiencing PPT, which can allow timely interventions. In addition, it would be useful for health professionals who care for postpartum women to offer a thyroid function check at six months postpartum, especially for women who had existing autoimmune diseases such as type 1 diabetes. This may help detect postpartum thyroid dysfunction and provide effective intervention to enhance future maternal reproductive health. However, a cost and benefit analysis is needed prior to implement this practice.

In the MINI study, low plasma selenium increased the risk of thyroid dysfunction of postpartum women. Literature has shown some positive effects of selenium supplementation for thyroid function, especially for patients with Graves ophthalmopathy (79). Although using selenium supplementation to treat thyroid dysfunction is presently inconclusive, it is worth considering the narrow window of optimal selenium intake and selenium status when managing maternal thyroid dysfunction.

Despite recommendations made to date, the use of iodine-containing supplements was low in our lactating women. In the specific New Zealand context, mothers and their infants are cared for by midwives or obstetricians during the first six weeks after childbirth, then Plunket nurses, general practitioners (GPs), and lactation consultants all play an essential role in the postnatal care of mothers during the first postpartum year and beyond. Consequently, these health care workers are placed in an ideal position to provide timely advice to postpartum women. Here, we suggest promoting iodine specific nutrition education to all health professionals, especially midwives, obstetricians, Plunket nurses, and GPs. It is then recommended that these health professionals, as a trusted source of information, encourage the routine use of government subsidised iodine-only supplements during lactation. The MINI study found that using an iodine-containing supplement was strongly associated with maternal iodine knowledge. Such information should be considered for framing and targeting further educational interventions around iodine, as well as considering removing barriers for women to access these supplements. Providing practical advice, such as integrating iodine-related food sources in sample meal plans when developing nutrition specific educational resources, may be effective in improving maternal iodine status.

The MINI study also found most women at six months postpartum achieved adequate iron status, with only 4% having ID and none classified as IDA. However, as discussed earlier, the women in this study cohort were typically affluent and highly educated. As such, iron status of women in New Zealand who have low socioeconomic

backgrounds and are less well-educated may be of greater concern, since they may not be able to afford iron rich foods, such as meat. Also, postpartum haemorrhage is one of the significant risk factors in developing postpartum iron deficiency. In our study, most women who experienced haemorrhage at childbirth were treated with either iron or blood transfusion, however, their iron status was not followed up. The 2016 WHO guideline recommends oral iron supplementation may be provided to women at six to twelve weeks postpartum where a prevalence of gestational anaemia is higher than 20% (91). It is important to note that iron deficiency negatively impacts on maternal physical and mental health and assessing iron status in the early lactating period is pivotal. Overall, early detection of any stages of iron deficiency is important to ensure women have adequate iron status, especially before conceiving again. We recommend maternal iron status checks take place routinely at the six weeks postpartum medical check-up, as it is an opportune time when mothers and infants are individually assessed for their postnatal health by GPs. In practice, a full blood test examining both haemoglobin and serum ferritin is recommended. Alternatively, point of care equipment such as the HemoCue Hb 201+ system could be used to identify women at risk in the first instance, before referring them for a full blood test to further examine their iron status if necessary. However, women who have low iron stores without being anaemic may fail to benefit from this alternative approach.

## 9.8 Research Recommendations

1. Further investigation should be undertaken to examine the effects of iodine and/or selenium supplementation on maternal and infant thyroid function; for example, a randomised controlled trial with lactating women by dispensing only iodine or a combined iodine-selenium supplementation.
2. To achieve optimal iodine status is crucial during the first two years in supporting optimal neurodevelopment. Thus, further research should focus on investigating maternal postpartum iodine status in relation to infants' neurodevelopment for those early years after childbirth.

3. The current study confirmed that women who used iodine-containing supplements achieved adequate iodine status for themselves and their infants. Future cohort studies with iodine-depleted populations are indicated to confirm if iodine supplementation during conception and pregnancy can enhance future beneficial health outcomes for both mothers and their infants.
4. Future quality evidence from health research is needed to further clarify the optimal cut-off of MUIC to ensure iodine adequacy during infancy. For example, a large cohort study might investigate infant MUIC in iodine sufficient populations and use neonatal heel blood TSH to measure infant thyroid function as an indicator of optimal iodine status.
5. Future research studies should confirm how much iodine intake is contributed by infant formula or complementary foods in New Zealand; such results could help provide useful guidelines for improving infant iodine intake and status, particularly for infants having complementary feedings.
6. Research is required to better clarify the association between maternal selenium status and the development of postnatal depression and anxiety; for example, a case control study to examine selenium status (by measuring serum or plasma selenoprotein P, plasma GPX3 and erythrocytes GPx1) in women who have been confirmed with PND, conjunctively with women without PND.
7. Nail clippings have been used to determine selenium concentrations in large cohorts or epidemiological studies. Measuring selenium in nail clippings would be a useful biomarker representing long-term selenium exposure, from three to twelve months. Notably, in the MINI study, nail clippings have been collected, and further analysis presents a future opportunity to measure in utero selenium exposure for both mothers and their infants.
8. Larger cohort studies are required to provide a greater understanding of the role of selenium status, when combined with other micronutrients, needed for optimal thyroid function, such as zinc, copper, and vitamin A.

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# Appendices

## Appendix 1 – MINI Study Ethics Approval

Health and Disability Ethics Committees

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.

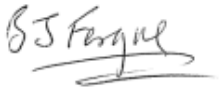
Your next progress report is due by 15 December 2016.

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

Health and Disability Ethics Committees

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

04 816 3985
hdecs@moh.govt.nz

04 May 2016

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/AM01** |
| | Study title: | Mother and Infant Nutrition Investigation |

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

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 2 – MINI Study MDHB Locality Approval

MidCentral District Health Board

# 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 | DHRST | 15/9/16 |

MidCentral District Health Board

# 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 : Womens 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.***

## 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: | Ying Jin |
| 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: | Actng C.D. |

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

MidCentral District Health Board
Te Pae Hauora o Ruahine o Tararua

**3. If the application is for a student project, the supervisor should sign the declaration.**

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

| | | | |
|---|---|---|---|
| Name (please print): | LOUISE BROUGH | | |
| Signature: | L. Brough | Institution: | ~~Palmerston North Hospital/MCH~~ MASSEY UNIVERSITY |
| Date: | 26/7/16 | Designation: | SENIOR LECTURER. |

**4. Declaration by relevant Operations Director**

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): | NICHOLAS GHOBB | | |
| Signature: | | Institution: | Palmerston North Hospital/MCH |
| Date: | 23.9.16 | Designation: | Ops Director |

# Appendix 3 – 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).

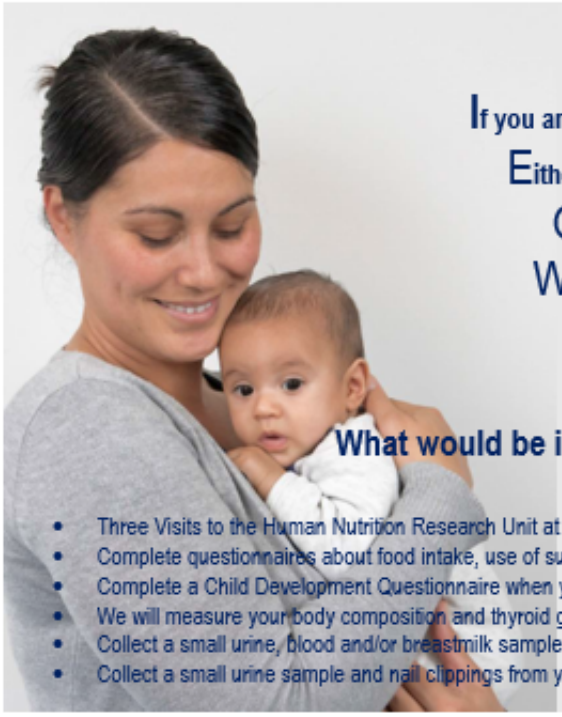
If you have any enquiries please send a message to info@actr.org.au or telephone +61 2 9562 5333.

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 4 – 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


TE KURA HAUORA TANGATA
MASSEY UNIVERSITY
COLLEGE OF HEALTH

## Appendix 5 – MINI Study Health Screening Questionnaire

## 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?**

# Appendix 6 – MINI Study 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**<br>**Register your interest – www.massey.ac.nz/ministudy**<br>**Phone: +64 (06) 9517556**<br>**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 breastmilk 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 2nd visit will be when your baby is 6 months old. The 3rd visit will be when your baby is 12 months old. We will ask you to complete questionnaires to assess your child's development at 4, 8 and 12 months. A Flow Chart is included in 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 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 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 monitoring of your wellbeing 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

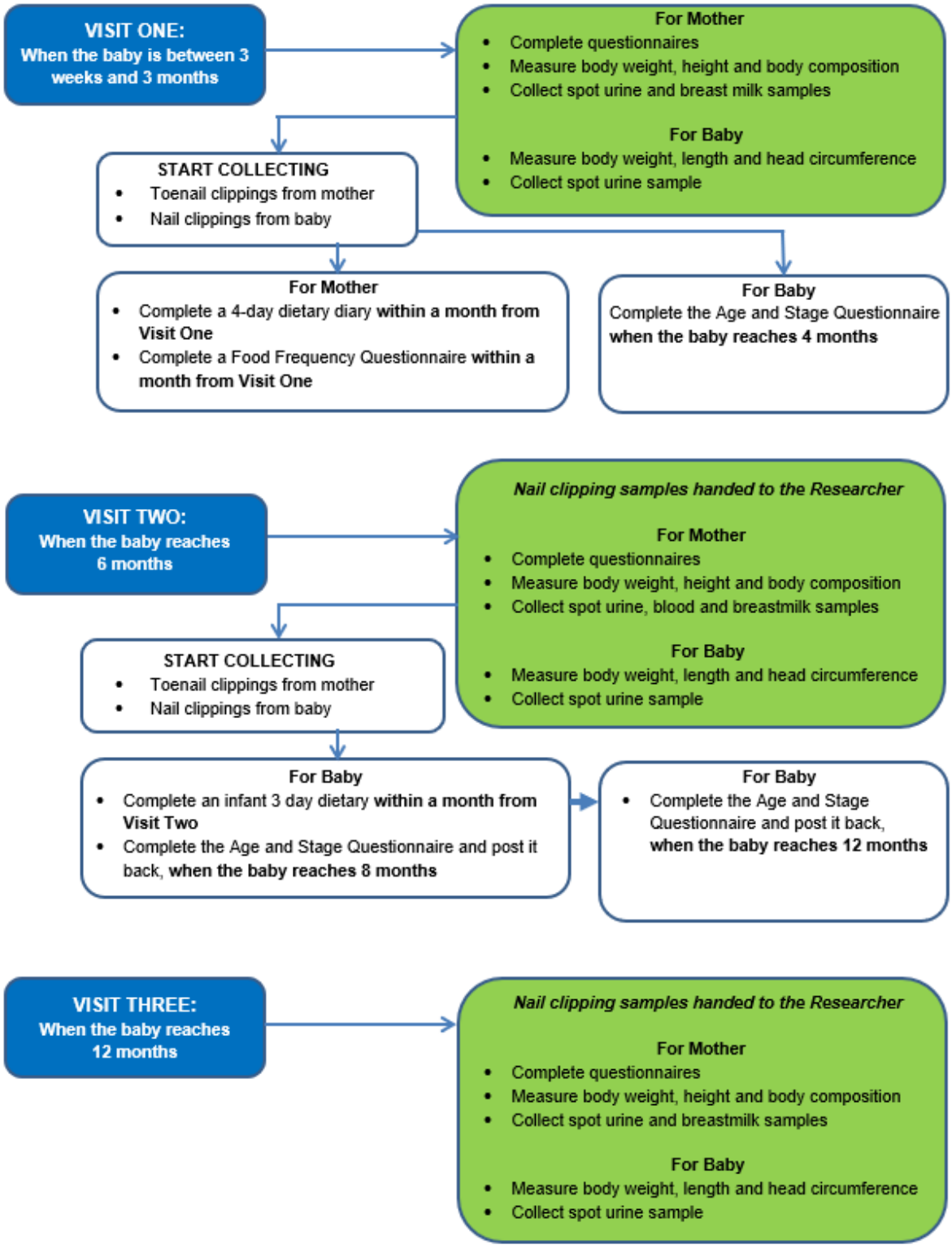
If you feel you would like to talk to a Māori health support person, please contact:

***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

## Appendix 7 - MINI Study Flow Chart



## Appendix 8 - 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 9 - MINI Study General Questionnaire - when your child is born

**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 REGNANCY.**

11. Did you take any supplements?

☐ Yes **(go to Q13)**

☐ No

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, 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 <u>the FIRST WEEK</u> 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 24hour period) do you currently breastfeed our 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

☐ Breastmilk 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 breastmilk was not convenient/possible

☐ New pregnancy

☐ Baby was old enough that the difference between breastmilk 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 | ☐ | ☐ | ☐ | ☐ |

**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 10 – MINI Study General Questionnaire – when your child is around 6 months old

**Date of visit: _____ Day_____ Month_____ Year**

### Questionnaire – when your child is around 6 months old

1. 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
2. How often do you eat red meat?
   - ☐ Never
   - ☐ Less than once a week
   - ☐ 1-2 times per week
   - ☐ 3-4 times per week
   - ☐ 5-6 times per week
   - ☐ 7+ times per day
3. How often do you use a cast-iron fry pan, wok or pot when preparing your meals?
   - ☐ Never or less than once a month
   - ☐ 1-3 times per month
   - ☐ Once a week
   - ☐ 2-3 times per week
   - ☐ 4-6 times per week
   - ☐ Once a day
   - ☐ 2-3 times per day
   - ☐ 4+ times per day

4. Some foods and drinks have iron added to them (eg. Some breakfast cereals) when you are choosing foods and drinks, how often do you choose the product with added iron instead of the product without?

   ☐ Whenever I can

   ☐ Usually

   ☐ Sometimes

   ☐ Never

   ☐ I do not know

   ☐ I do not consider whether the product has iron added

5. On average, how many slices of bread/toast or bread rolls do you eat per day?

   ☐ None, I do not eat bread or toast

   ☐ Less than one per day

   ☐ 1-2 per day

   ☐ 3-4 per day

   ☐ 5-6 per day

   ☐ 7+ per day

6. Have you had any of the following symptoms since the baby was born? If you know symptoms are due to allergy and not infection, do not check. Please check the correct answer: **(**Carr Infection Symptom Checklist)

0 = No symptoms 1 = Mild symptoms 2 = Moderate symptoms

3 = Strong symptoms 4 = Severe symptoms

| | **0** | **1** | **2** | **3** | **4** |
|---|---|---|---|---|---|
| Cold sores | | | | | |
| Canker sores | | | | | |
| Nasal stuffiness | | | | | |
| Sore throat | | | | | |
| Sinus drainage | | | | | |
| Sinus pain/pressure | | | | | |
| Swollen glands | | | | | |
| Diarrhea | | | | | |
| Abdominal cramps | | | | | |
| Burning on urination | | | | | |
| Dark, smelly urine | | | | | |
| Earache | | | | | |
| Hoarseness | | | | | |
| Styes | | | | | |
| Runny nose | | | | | |
| Skin infections | | | | | |
| Acne | | | | | |
| Red eyes | | | | | |
| Vaginal itching | | | | | |
| Vaginal yeast infection | | | | | |
| Vaginal herpes | | | | | |
| Fever | | | | | |
| Fingernail infection | | | | | |
| Wheezing | | | | | |
| Cough | | | | | |
| Shingles | | | | | |
| Generalized flu-like | | | | | |
| Breast infection | | | | | |
| Episiotomy infection | | | | | |
| Dental abscess | | | | | |

7. Apart from when you were in hospital immediately after having your baby, have you experienced any of the following?

| | Never | Rarely | Occasionally | Often |
|---|---|---|---|---|
| Extreme tiredness/exhaustion | | | | |
| More frequent coughs/colds/minor illness than usual | | | | |
| Severe headache or migraines | | | | |
| Lower back pain | | | | |
| Upper back pain | | | | |
| Painful perineum | | | | |
| Pain from caesarean section wound | | | | |
| constipation | | | | |
| hemorrhoids | | | | |
| Breast problems | | | | |
| Pelvic pain | | | | |

8. Overall, how would you describe your physical health at the moment?
   - ☐ Excellent
   - ☐ Very good
   - ☐ Good
   - ☐ Fair
   - ☐ Poor

9. Do you take or have taken cod liver oil, vitamins or other dietary supplements since the previous questionnaire?
   - ☐ No
   - ☐ Yes

   9a. If yes, which product, when did you take it and how often (one line for each product)

| 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** |
| | | | | | | | | | | | |

10. How often are you physically active at present?

| | Never | 1-3 times a month | Once a week | Twice a week | Three times or more a week |
|---|---|---|---|---|---|
| walking | | | | | |
| brisk walking | | | | | |
| running/jogging/orienteering | | | | | |
| cycling | | | | | |
| training studio/weight training | | | | | |
| special gymnastics/aerobics for women | | | | | |
| aerobics/gymnastics/dancing without running and jumping | | | | | |
| aerobics/gymnastics/dancing with running and jumping | | | | | |
| dancing (swing, rock, folk) | | | | | |
| skiing | | | | | |
| ball sport | | | | | |
| swimming | | | | | |
| riding | | | | | |
| other | | | | | |

11. Have you ever suffered from low iron stores, iron deficiency or iron deficiency anemia?

☐ No

☐ Yes

| Diagnosis date | Diagnosed by | Any further details |
|---|---|---|
| | | |

12. Have you ever been treated for iron deficiency or iron deficiency anemia?

| Type of treatment | Duration | Any further details |
|---|---|---|
| | | |

13. Have you had a severe blood loss during delivering your baby?
☐ No
☐ Yes

14. Do you have or have you had any medical condition which has resulted in blood loss?

If yes, please describe it and give approximate date_____

15. Have you had a blood donation during the last 6 months?
☐ No
☐ Yes

15a. If yes, how many times did you donate your blood? _____.

16. Have you had a blood transfusion during the last 12 months?

☐ No
☐ Yes

16a. If yes, do you know why you received the blood transfusion _____

17. Have you noticed any form of blood loss during the last 6 months?
☐ Not at all
☐ Yes, in stools
☐ Yes, in urine
☐ Yes, when brushing my teeth
☐ Yes, from a wound

18. Are you pregnant at the moment?

☐ No
☐ Yes

18a. If yes, how many weeks have you been pregnant? _____.

19. Has your period started again?

☐ No
☐ Yes

19a.If yes, please give an approximate date _____.

**About your child**

20. How old was your baby when you stopped breastfeeding?

☐ _____months_____ weeks_____ days

☐ I continue to breastfeed (**go to Q 22**)

21. Tick the reason (s) you chose not to breastfeed or stop breastfeeding your baby *(Tick all that apply)*

☐ Have breastfed long enough

☐ Baby had trouble latching on

☐ Did not have enough milk

☐ Breastmilk 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 breastmilk was not convenient/possible

☐ New pregnancy

☐ Baby was old enough that the difference between breastmilk and formula was minimal

☐ Others _____

22. Including times of weaning, what is the total time your baby was breastfed?

☐ Weeks

☐ Months

☐ Less than one week

23. At what age was your baby first given infant formula regularly?

_____Weeks _____ Months

24. At what age was your baby first given solid food regularly?

_____Weeks _____ Months

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

| | Never /seldom | 1-3 times/week | 4-6 times/week | At least once a day |
|---|---|---|---|---|
| Breastmilk | | | | |
| Pasteurised Cow's milk | | | | |
| Pasteurised Goat's milk | | | | |
| Evaporated milk | | | | |
| Organic milk products (milk, yoghurt) | | | | |
| Standard infant formula/formula milk | | | | |
| Standard formula milk with Omega-3 | | | | |
| Hypoallergenic formula | | | | |
| Water | | | | |
| Gripe water | | | | |
| Sugar water | | | | |
| Cold flavored milk drinks | | | | |
| Fizzy (carbonated) drinks | | | | |
| Squash, artificially sweetened | | | | |
| Baby cordial, artificially sweetened | | | | |
| Fruit juice | | | | |
| Herbal drinks | | | | |
| Tea/Coffee | | | | |
| Others _____ | | | | |

26. Do you give your child cod liver oil, vitamins, iron or any other dietary supplements?

☐ No

☐ Yes

26a. if yes, specify

| Name of product | How many teaspoons/time | How often | How old was your child at first time consumption (months) |
|---|---|---|---|
| | | | |
| | | | |

27. Has your child had the following health problems?

| | Has your child had health problems | | Number of times | Did you visit a doctor/clinic | | Has your child been admitted to hospital for this | |
|---|---|---|---|---|---|---|---|
| Common cold | ☐ Yes | ☐ No | | ☐ Yes | ☐ No | ☐ Yes | ☐ No |
| Throat infection | ☐ Yes | ☐ No | | ☐ Yes | ☐ No | ☐ Yes | ☐ No |
| Ear infection | ☐ Yes | ☐ No | | ☐ Yes | ☐ No | ☐ Yes | ☐ No |
| Bronchitis pneumonia | ☐ Yes | ☐ No | | ☐ Yes | ☐ No | ☐ Yes | ☐ No |
| Diarrhea | ☐ Yes | ☐ No | | ☐ Yes | ☐ No | ☐ Yes | ☐ No |
| wheezing | ☐ Yes | ☐ No | | ☐ Yes | ☐ No | ☐ Yes | ☐ No |
| vomiting | ☐ Yes | ☐ No | | ☐ Yes | ☐ No | ☐ Yes | ☐ No |
| High temperature | ☐ Yes | ☐ No | | ☐ Yes | ☐ No | ☐ Yes | ☐ No |
| Urinary tract infection | ☐ Yes | ☐ No | | ☐ Yes | ☐ No | ☐ Yes | ☐ No |
| Colic | ☐ Yes | ☐ No | | ☐ Yes | ☐ No | ☐ Yes | ☐ No |
| Nappy rash | ☐ Yes | ☐ No | | ☐ Yes | ☐ No | ☐ Yes | ☐ No |
| An accident | ☐ Yes | ☐ No | | ☐ Yes | ☐ No | ☐ Yes | ☐ No |

28. How would you describe the health of your baby now:

☐ Very healthy

☐ Healthy, but a few minor problems

☐ Sometimes quite ill

☐ Almost always unwell

29. Since your last visit, have you changed your smoking habits?

☐ No

☐ Yes, specify _____.

30. Since your last visit, have you changed your drinking habits?

☐ No

☐ Yes, specify _____.

## Thanks for completing this questionnaire

## Appendix 11 – MINI Study General Questionnaire – when your child is around 12 months old

**Date of visit: _____ Day_____ Month_____ Year**

### Questionnaire – when your child is around 12 months old

**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 indicates 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

5. Do you take or have taken cod liver oil, vitamins, or other dietary supplements since the previous questionnaire?

   ☐ No

   ☐ Yes

   5a. If yes, which product, when did you take it and how often (one line for each product)

| 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** |
| | | | | | | | | | | | |
| | | | | | | | | | | | |

6. Have you had any of the following symptoms since the baby was born? If you know symptoms are due to allergy and not infection, do not check. Please check the correct answer:
   0 = No symptoms  1 = Mild symptoms  2 = Moderate symptoms
   3 = Strong symptoms  4 = Severe symptoms

| | **0** | **1** | **2** | **3** | **4** |
|---|---|---|---|---|---|
| Cold sores | | | | | |
| Canker sores | | | | | |
| Nasal stuffiness | | | | | |
| Sore throat | | | | | |
| Sinus drainage | | | | | |
| Sinus pain/pressure | | | | | |
| Swollen glands | | | | | |
| Diarrhea | | | | | |
| Abdominal cramps | | | | | |
| Burning on urination | | | | | |
| Dark, smelly urine | | | | | |
| Earache | | | | | |
| Hoarseness | | | | | |
| Styes | | | | | |
| Runny nose | | | | | |
| Skin infections | | | | | |
| Acne | | | | | |
| Red eyes | | | | | |
| Vaginal itching | | | | | |
| Vaginal yeast infection | | | | | |
| Vaginal herpes | | | | | |
| Fever | | | | | |
| Fingernail infection | | | | | |
| Wheezing | | | | | |
| Cough | | | | | |
| Shingles | | | | | |
| Generalized flu-like | | | | | |
| Breast infection | | | | | |
| Episiotomy infection | | | | | |
| Dental abscess | | | | | |

7. How often are you physically active at present?

| + | Never | 1-3 times /month | Once/ week | Twice/ week | 3 times or more/week |
|---|---|---|---|---|---|
| Walking | | | | | |
| Brisk walking | | | | | |
| Running/jogging/orienteering | | | | | |
| cycling | | | | | |
| Training studio/weight training | | | | | |
| Special gymnastics/aerobics for women | | | | | |
| Aerobics/gymnastics/dancing without running and jumping | | | | | |
| Aerobics/gymnastics/dancing with running and jumping | | | | | |
| Dancing (swing, rock, folk) | | | | | |
| Skiing | | | | | |
| Ball sport | | | | | |
| Swimming | | | | | |
| Riding | | | | | |
| Other | | | | | |

8. Overall, how would you describe your physical health at the moment?
   - ☐ Excellent
   - ☐ Very good
   - ☐ Good
   - ☐ Fair
   - ☐ Poor, specify _____

**About your child**

9. How old was your baby when you stopped breastfeeding?

   ☐ _____months_____ weeks_____ days

   ☐ I continue to breastfeed (**go to Q 11**)

10. Tick the reason (s) you chose not to breastfeed or stop breastfeeding your baby *(Tick all that apply)*

    ☐ Have breastfed long enough

    ☐ Baby had trouble latching on

☐ Did not have enough milk

☐ Breastmilk 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 breastmilk was not convenient/possible

☐ New pregnancy

☐ Baby was old enough that the difference between breastmilk and formula was minimal

☐ Others _____

11. At what age was your baby first given infant formula regularly?

    _____Weeks _____ Months

12. At what age was your baby first given solid food regularly?

    _____ Weeks _____ Months

13. Do you give your child cod liver oil, vitamins, iron or any other dietary supplements since your last visit?

    ☐ No

    ☐ Yes, specify

| Name of product | How much each time | How often | How old was your child at first time consumption (months) |
|---|---|---|---|
| | | | |
| | | | |
| | | | |

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

| | Never /seldom | 1-3 times/week | 4-6 times/week | At least once a day |
|---|---|---|---|---|
| Breastmilk | | | | |
| Pasteurised Cow's milk | | | | |
| Pasteurised Goat's milk | | | | |
| Evaporated milk | | | | |
| Organic milk products (milk, yoghurt) | | | | |
| Standard infant formula/formula milk | | | | |
| Standard formula milk with Omega-3 | | | | |
| Hypoallergenic formula | | | | |
| Water | | | | |
| Gripe water | | | | |
| Sugar water | | | | |
| Cold flavored milk drinks | | | | |
| Fizzy (carbonated) drinks | | | | |
| Squash, artificially sweetened | | | | |
| Baby cordial, artificially sweetened | | | | |
| Fruit juice | | | | |
| Herbal drinks | | | | |
| Tea/Coffee | | | | |
| Others _____ | | | | |

15. Has your child had the following health problems since your last visit?

| | Has your child had health problems | | Number of times | Did you visit a doctor/clinic | | Has your child been admitted to hospital for this | |
|---|---|---|---|---|---|---|---|
| Common cold | ☐ Yes | ☐ No | | ☐ Yes | ☐ No | ☐ Yes | ☐ No |
| Throat infection with confirmed streptococcal infection | ☐ Yes | ☐ No | | ☐ Yes | ☐ No | ☐ Yes | ☐ No |
| Other type of throat infection | | | | | | | |
| Ear infection | ☐ Yes | ☐ No | | ☐ Yes | ☐ No | ☐ Yes | ☐ No |
| Pseudcroup | | | | | | | |
| Bronchitis pneumonia | ☐ Yes | ☐ No | | ☐ Yes | ☐ No | ☐ Yes | ☐ No |
| Gastric flu/Diarrhea | ☐ Yes | ☐ No | | ☐ Yes | ☐ No | ☐ Yes | ☐ No |
| Urinary tract infection | | | | | | | |
| Wheezing/ whistling in the chest | ☐ Yes | ☐ No | | ☐ Yes | ☐ No | ☐ Yes | ☐ No |
| Vomiting | ☐ Yes | ☐ No | | ☐ Yes | ☐ No | ☐ Yes | ☐ No |
| High temperature | ☐ Yes | ☐ No | | ☐ Yes | ☐ No | ☐ Yes | ☐ No |
| Urinary tract infection | ☐ Yes | ☐ No | | ☐ Yes | ☐ No | ☐ Yes | ☐ No |
| Colic | ☐ Yes | ☐ No | | ☐ Yes | ☐ No | ☐ Yes | ☐ No |
| Nappy rash | ☐ Yes | ☐ No | | ☐ Yes | ☐ No | ☐ Yes | ☐ No |
| An accident | ☐ Yes | ☐ No | | ☐ Yes | ☐ No | ☐ Yes | ☐ No |

16. How would you describe the health of your baby now:

☐ Very healthy

☐ Healthy, but a few minor problems

☐ Sometimes quite ill

☐ Almost always unwell

17. Are there any changes in your living situation since your last time completing the questionnaire?

    ☐ No

    ☐ Yes, please specify_____

18. Since your last visit, have you changed your smoking habits?

    ☐ No

    ☐ Yes, specify _____.

19. Since your last visit, have you changed your drinking habits?

    ☐ No

    ☐ Yes, specify _____.

**Thanks for completing this questionnaire**

## Appendix 12 - MINI Study Maternal Health Questionnaire – Edinburgh Postnatal Depression Scale

**Date of visit: _____ Day_____ Month_____ Year**

## Maternal Health Questionnaire

As you have recently had a baby, we would like to know how you are feeling. Please check the answer that comes closest to how you have felt **IN THE PAST 7 DAYS**, not just how you feel today.

Before you start, I will show you an example question that has already been completed.

I have felt happy:
☐ Yes, all the time
☒ Yes, most of the time
☐ No, not very often
☐ No, not at all

This would mean: "I have felt happy most of the time" during the past 7 days. Please complete the other questions in the same way.

In the past 7 days:

1. I have been able to laugh and see the funny side of things

   ☐ As much as I always could
   ☐ Not quite so much now
   ☐ Definitely not so much now
   ☐ Not at all

2. I have looked forward with enjoyment to things

   ☐ As much as I ever did
   ☐ Rather less than I used to
   ☐ Definitely less than I used to
   ☐ Hardly at all

3. I have blamed myself unnecessarily when things went wrong

   ☐ Yes, most of the time
   ☐ Yes, some of the time
   ☐ Not very often
   ☐ No, never

4. I have been anxious or worried for no good reason

   ☐ No, not at all
   ☐ Hardly ever
   ☐ Yes, sometimes
   ☐ Yes, very often

5. I have felt scared or panicky for no very good reason

   ☐ Yes, quite a lot
   ☐ Yes, sometimes
   ☐ No, not much
   ☐ No, not at all

6. Things have been getting on top of me

   ☐ Yes, most of the time I have not been able to cope at all
   ☐ Yes, sometimes I have not been coping as well as usual
   ☐ No, most of the time I have coped quite well
   ☐ No, I have been coping as well as ever

7. I have been so unhappy that I have had difficulty sleeping

   ☐ Yes, most of the time
   ☐ Yes, sometimes
   ☐ Not very often
   ☐ No, not at all

8. I have felt sad or miserable

   ☐ Yes, most of the time
   ☐ Yes, quite often
   ☐ Not very often
   ☐ No, not at all

9. I have been so unhappy that I have been crying

   ☐ Yes, most of the time
   ☐ Yes, quite often
   ☐ Only occasionally
   ☐ No, never

10. The thought of harming myself has occurred to me

    ☐ Yes, quite often
    ☐ Sometimes
    ☐ Hardly ever
    ☐ Never

## Appendix 13 - MINI Study Maternal and Infant data collection sheets

**Date of visit: _____ Day_____ Month_____ Year**

## Maternal Data

| DOB: | Age: | Weeks after giving birth: |
|---|---|---|
| | | |

**Prior to Pregnancy:** Estimated usual body weight _____ kg

**Pregnancy:** Blood hemoglobin _____

Any complications:

| **Maternal delivery Information** | |
|---|---|
| Date of delivery: | _____ Day _____ Month |
| Time of delivery: | _____ |
| Method of delivery: | _____ |
| Usage of iodine containing sanitizer: | _____ |
| Any severe blood loss | _____ |
| Pain relief used | _____ |
| **Baby's summary** | |
| Gestation: | _____ weeks |
| Gender: | _____ |
| Apgar score at 1 minute | _____ |
| Apgar score at 5 minutes | _____ |
| Birth weight: _____ kg | Head circumference: _____ cm |
| Body length:_____ cm | |
| Guthrie test : | _____(positive or negative) |

**Have you given birth to any other children?**

Yes = 1/ No = 0

| **Number** | **Gender** | **Age** | **Method delivery** | **Method of feeding** *(probe duration of bf)* |
|---|---|---|---|---|
| | | | | |
| | | | | |
| | | | | |

**Anthropometric measurement - Visit One**

Body weight _____kg Average _____ kg

_____ kg

_____ kg

Body height _____ cm Average _____ cm

_____ cm

_____ cm

BMI _____

BIA results

BodPod Results

**Anthropometric measurement - Visit Two**

| | | |
|---|---|---|
| Body weight | _____ kg | Average _____ kg |
| | _____ kg | |
| | _____ kg | |
| Body height | _____ cm | Average _____ cm |
| | _____ cm | |
| | _____ cm | |
| BMI | _____ | |

BIA results

BodPod Results

**Anthropometric measurement - Visit Three**

| | | |
|---|---|---|
| Body weight | _____ kg | Average _____ kg |
| | _____ kg | |
| | _____ kg | |
| Body height | _____ cm | Average _____ cm |
| | _____ cm | |
| | _____ cm | |
| BMI | _____ | |

BIA results

BodPod Results

# Infant Data

**Infant Anthropometric measurement – Visit One**

Body weight: _____ kg

_____ kg

_____ kg Average _____ kg

Body length: _____ cm

_____ cm

_____ cm Average _____ cm

Head circumference: _____ cm

_____ cm

_____ cm Average _____ cm

**Infant Anthropometric measurement – Visit Two**

Body weight: _____ kg

_____ kg

_____ kg Average _____ kg

Body length: _____ cm

_____ cm

_____ cm Average _____ cm

Head circumference: _____ cm

_____ cm

_____ cm Average _____ cm

**Infant Anthropometric measurement – Visit Three**

Body weight: _____ kg

_____ kg

_____ kg Average _____ kg

Body length: _____ cm

_____ cm

_____ cm Average _____ cm

Head circumference: _____cm

_____cm

_____cm Average _____ cm

| | |
|---|---|
| **First week** | |
| Body weight: _____ kg | |
| **2-4 weeks** | |
| Body weight: _____ kg | Head circumference: _____cm |
| **4-6 weeks** | |
| Body weight: _____ kg | Head circumference_____ cm |
| Body length: _____ cm | |
| **8-10 weeks** | |
| Body weight: _____ kg | Head circumference_____ cm |
| Body length: _____ cm | |
| **3-4 months** | |
| Body weight: _____ kg | Development |
| **5-7 months** | |
| Body weight: _____ kg | Development |
| **9-12 months** | |
| Body weight: _____ kg | Body length: _____ cm |
| Development | |

**(Notes taken from the Well Child Book)**

## Appendix 14 - MINI Study Maternal Thyroid Gland Measurement sheet

**Date of visit: _____ Day_____ Month_____ Year**

**Left Lobe**

**Width:** _____

**AP Diameter:** _____

**Length:** _____

**Tvol (L):** _____

**Right Lobe**

**Width:** _____

**AP Diameter:** _____

**Length:** _____

**Tvol (R):** _____

**Calculation of Thyroid Volume**

**Tvol Lobe:** **Tvol Lobe (L) + Tvol Lobe (R)**

_____

**Isthmus:** _____

**Note: Tvol Lobe = Width x AP Diameter x Length x 0.479**

## Appendix 15 – MINI Study - 4-Day Dietary Diary (Maternal)

**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<br>Milk (fresh, blue top)<br>Sugar white<br><br>Toast, multigrain bread<br>Marmalade | Robert Harris<br>Anchor<br>Pams<br><br>Pams<br>Pams | Mug<br>A dash<br>1 level teaspoon<br><br>1 slice<br>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<br>tearoom | Ham salad sandwich from home:<br>Bread wholemeal thick sliced<br>Margarine light<br>Smoked ham thin sliced<br>Lettuce, iceberg<br>Cucumber with skin | <br>Pams<br>Sunlight<br>Supermarket | <br>2 slices<br>1 tablespoon<br>2 slices<br>1 leaf<br>4 thin slices |
| **2pm to 5pm** | | | | |
| 3pm | Meeting<br>room | Herbal tea<br>Louise slice | Healthiers<br>bakery | 1 cup<br>1 regular slice |

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

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

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

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

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

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

Breakfast cereal



Spaghetti/ noodles




Rice




Chips



Broccoli or cauliflower




Stew or curry

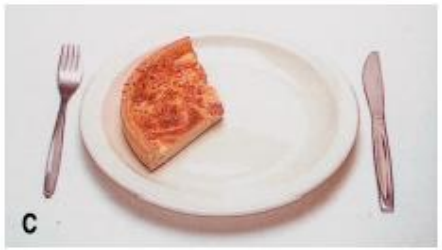



Battered fish



Quiche or pie





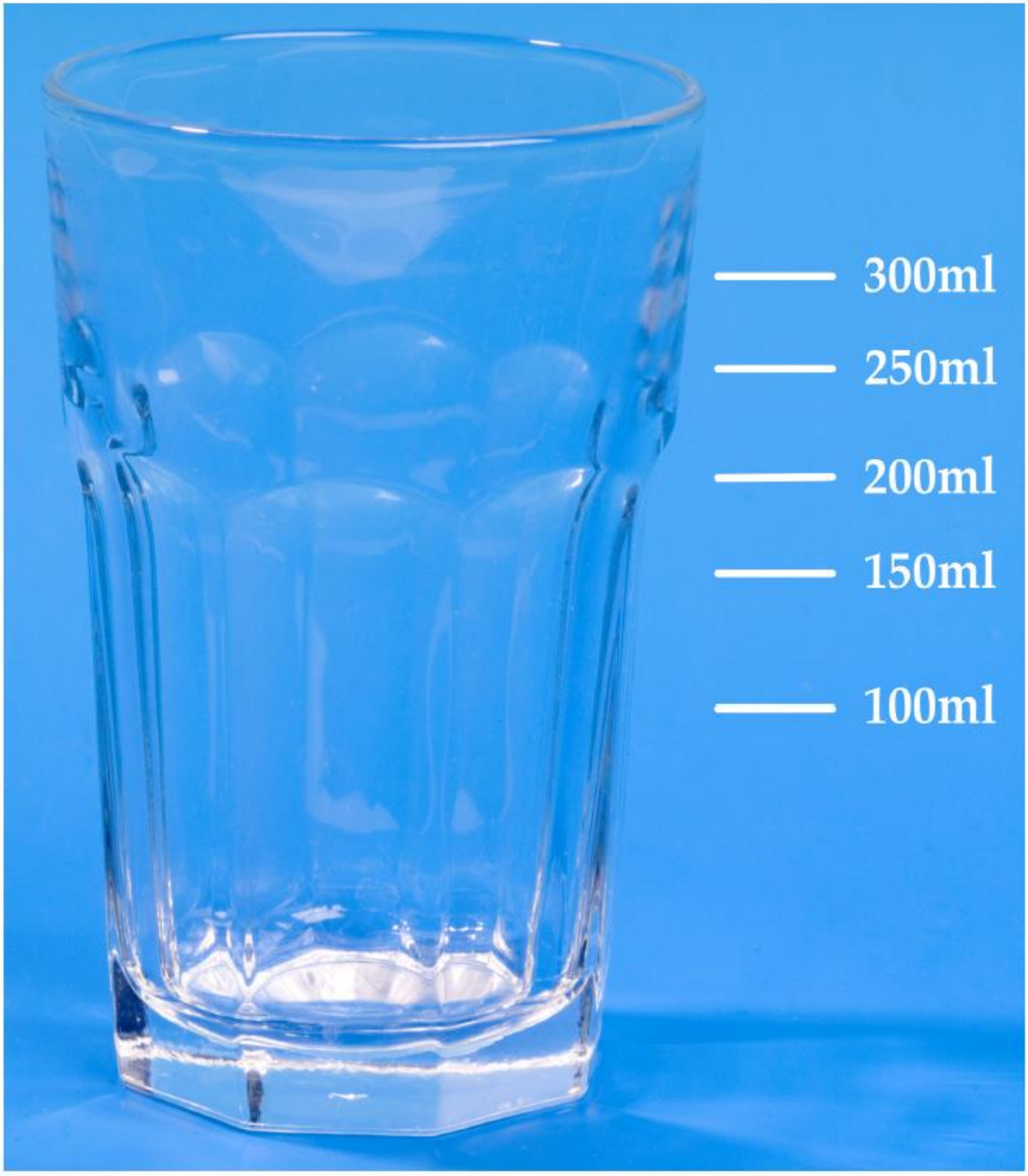

Cheese




Spongy cake



**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<br>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 16.1 – Selenium in 24-hour urine samples in breastfeeding women using Spearman's rho

| n=68 | | Urine Volume L | Urine Selenium µg/L | Urine Selenium µg/day | Urine Creatinine g/L | Urine Creatinine g/day | Selenium: Creatinine ratio µg/g | Milk Selenium µg/L | Dietary Selenium µg/day |
|---|---|---|---|---|---|---|---|---|---|
| **Urine Selenium µg/L** | r | -0.631 | | | | | | | |
| | p | .000 | | | | | | | |
| **Urine Selenium µg/day** | r | | 0.642 | | | | | | |
| | p | ns | .000 | | | | | | |
| **Urine Creatinine g/L** | r | -0.871 | 0.657 | | | | | | |
| | p | .000 | .000 | ns | | | | | |
| **Urine Creatinine g/day** | r | | | | | | | | |
| | p | ns | ns | ns | ns | | | | |
| **Selenium Creatinine ratio µg/g** | r | | 0.580 | 0.879 | | | | | |
| | p | ns | .000 | .000 | ns | ns | | | |
| **Milk Selenium µg/day** | r | | | 0.269 | | | 0.280 | | |
| | p | ns | ns | .000 | ns | ns | .025 | ns | |
| **Dietary Selenium µg/day** | r | | | | -0.357 | | | | |
| | p | ns | ns | ns | .003 | ns | ns | ns | |
| **Total Energy Intake** | r | .247 | | | | | | | .405 |
| | p | .043 | ns | ns | ns | ns | ns | ns | .001 |

## Appendix 16.2 - Selenium in 24-hour urine samples in pregnant women using Spearman's rho

| n=59 | | Urine Volume L | Urine Selenium µg/L | Estimated Selenium intake (urine) µg/day | Urine Creatinine g/L | Urine Creatinine g/day | Selenium: Creatinine ratio µg/g | Estimated Selenium intake (dietary data) µg/day |
|---|---|---|---|---|---|---|---|---|
| **Urine Selenium µg/L** | r | -0.719 | | | | | | |
| | p | .000 | | | | | | |
| **Estimated Selenium intake (urine) µg/day** | r | | 0.485 | | | | | |
| | p | ns | .000 | | | | | |
| **Urine Creatinine g/L** | r | -0.863 | 0.760 | | | | | |
| | p | .000 | .000 | ns | | | | |
| **Urine Creatinine g/day** | r | | | 0.380 | 0.259 | | | |
| | p | ns | ns | .003 | 0.047 | | | |
| **Selenium Creatinine ratio µg/g** | r | | 0.466 | 0.804 | | | | |
| | p | ns | .000 | .000 | ns | ns | | |
| **Estimated Selenium intake (dietary data) µg/day** | r | | | .230 | | | | |
| | p | ns | ns | .079 (ns) | ns | ns | ns | |
| **Total Energy intake kJ** | r | | | | | | | 0.263 |
| | p | ns | ns | ns | ns | ns | ns | 0.044 |

## Appendix 17 – Maternal UIC, urinary iodine creatinine ratio, and BMIC using Spearman's rho

| | | Maternal UIC_V1_ug/L | Urinary I:Cr_V1_ugg | BMIC_V2 _ug/L | Maternal UIC_V2_ug/L | Urinary I:Cr_V2_ugg | BMIC_V3 _ug/L | Maternal UIC_V3_ug/L | Urinary I:Cr_V3_ugg |
|---|---|---|---|---|---|---|---|---|---|
| BMIC_V1 | Correlation Coefficient | .275** | .441** | .563** | .125 | .279* | -.101 | .007 | .110 |
| _ug/L | Sig. (2-tailed) | .010 | .000 | .000 | .277 | .016 | .576 | .952 | .393 |
| | N | 87 | 79 | 72 | 78 | 74 | 33 | 71 | 62 |
| Maternal | Correlation Coefficient | | .276* | .075 | .225* | -.051 | -.043 | .171 | -.197 |
| UIC_V1 | Sig. (2-tailed) | | .014 | .533 | .048 | .664 | .813 | .155 | .125 |
| _ug/L | N | | 79 | 72 | 78 | 74 | 33 | 71 | 62 |
| Urinary | Correlation Coefficient | | | .168 | .005 | .454** | -.039 | -.047 | -.050 |
| I:Cr_V1_ugg | Sig. (2-tailed) | | | .182 | .968 | .000 | .835 | .713 | .708 |
| | N | | | 65 | 70 | 67 | 31 | 65 | 58 |
| BMIC_V2 | Correlation Coefficient | | | | .253* | .552** | .015 | .062 | .156 |
| _ug/L | Sig. (2-tailed) | | | | .032 | .000 | .936 | .621 | .246 |
| | N | | | | 72 | 69 | 32 | 66 | 57 |
| Maternal | Correlation Coefficient | | | | | .185 | .068 | .381** | .045 |
| UIC_V2 | Sig. (2-tailed) | | | | | .115 | .706 | .001 | .728 |
| _ug/L | N | | | | | 74 | 33 | 71 | 62 |
| Urinary I:Cr | Correlation Coefficient | | | | | | -.074 | .077 | .193 |
| _V2_ugg | Sig. (2-tailed) | | | | | | .692 | .537 | .146 |
| | N | | | | | | 31 | 67 | 58 |
| BMIC_V3 | Correlation Coefficient | | | | | | | .301 | .577** |
| _ug/L | Sig. (2-tailed) | | | | | | | .089 | .001 |
| | N | | | | | | | 33 | 29 |
| Maternal | Correlation Coefficient | | | | | | | | .340** |
| UIC_V3 | Sig. (2-tailed) | | | | | | | | .007 |
| _ug/L | N | | | | | | | | 62 |

# Appendix 18 – Statement of Contribution Doctorate with Publications/Manuscripts

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|---|---|
| Name of candidate: | Ying Jin |
| Name/title of Primary Supervisor: | Dr. Louise Brough |
| In which chapter is the manuscript /published work: | Chapter 3 |

Please select one of the following three options:

(●) The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

Jin Y, Coad J, Weber J, Thomson J, Brough L. Selenium Intake in Iodine-Deficient Pregnant and Breastfeeding Women in New Zealand. Nutrients. 2019;11(1):69. doi:10.3390/nu11010069

( ) The manuscript is currently under review for publication – please indicate:

- The name of the journal:

- The percentage of the manuscript/published work that was contributed by the candidate:

- Describe the contribution that the candidate has made to the manuscript/published work:

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Please select one of the following three options:

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- Please provide the full reference of the Research Output:

Jin Y, Coad J, Zhou SJ, Skeaff S, Benn C, Kim N, Pond RL, Brough L. Mother and Infant Nutrition Investigation in New Zealand (MINI Project): Protocol for an Observational Longitudinal Cohort Study. JMIR Res Protocol.2020;9(8): e18560. DOI: 10.2196/18560. PMID: 32852279

( ) The manuscript is currently under review for publication – please indicate:

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- Please provide the full reference of the Research Output:

◉ The manuscript is currently under review for publication – please indicate:

- The name of the journal:

The Clinical Endocrinology

- The percentage of the manuscript/published work that was contributed by the candidate: 70.00
- Describe the contribution that the candidate has made to the manuscript/published work:

Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing-Original draft preparation, Writing-reviewing and Editing

○ It is intended that the manuscript will be published, but it has not yet been submitted to a journal

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| Date: | 4-Dec-2020 |

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| In which chapter is the manuscript /published work: | Chapter 6 |

Please select one of the following three options:

(●) The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

Jin, Y, Coad, J, Zhou, JS, Skeaff, S, Benn C, Brough L. Use of iodine supplements by breastfeeding mothers is associated with better maternal and infant iodine status. Biological Trace Element Research. 2020. DOI: 10.1007/s12011-020-02438-8 PMID: 33094447

( ) The manuscript is currently under review for publication – please indicate:

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- The percentage of the manuscript/published work that was contributed by the candidate:
- Describe the contribution that the candidate has made to the manuscript/published work:

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| In which chapter is the manuscript /published work: | Chapter 7 |

Please select one of the following three options:

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- Please provide the full reference of the Research Output:

◉ The manuscript is currently under review for publication – please indicate:

- The name of the journal:
  British Journal of Nutrition
- The percentage of the manuscript/published work that was contributed by the candidate: 70.00
- Describe the contribution that the candidate has made to the manuscript/published work:
  Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing-Original draft preparation, Writing-reviewing and Editing

○ It is intended that the manuscript will be published, but it has not yet been submitted to a journal

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(●) The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

Jin Y, Coad J, Pond R, Kim N, Brough L. Selenium intake and status of postpartum women and postnatal depression during the first year after childbirth in New Zealand – Mother and Infant Nutrition Investigation (MINI) Study. Journal of Trace Elements in Medicine and Biology 2020; 61:126503. DOI: 10.1016/j.itemb.2020.126503. PMID: 32442890

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