School of Public Health

Longitudinal Investigation of the Biomarkers of Iodine Status in Pregnant and Post-partum Women in Perth

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Human Ethics: The research presented and reported in this thesis was conducted in accordance with the National Health and Medical Research Council National Statement on Ethical Conduct in Human Research (2007) – updated March 2014. The proposed research study received human research ethics approval from the Curtin University Human Research Ethics Committee (EC00262), Approval Number #HR 47/2013 and from the Western Australian Health Human Research Ethics Committee, Approval Number #2014075EW.

Signature: ……

Date: 31/07/2018

Abstract

The importance of adequate maternal iodine intake during pregnancy and the early neonatal period for optimal fetal and infant development is well-established. Less clear is the effect of advancing gestation on maternal iodine status, with studies showing conflicting results based on trends for urinary iodine excretion. Contributing to the situation is the lack of truly longitudinal data investigating this issue. To date, no studies have examined the gestational changes in iodine status in WA women, nor used biochemical indicators to assess iodine status of pregnant and post-partum women in Perth.

This longitudinal study explored the biomarkers of iodine status and the effect of advancing gestation on urinary iodine excretion in pregnant (n=61) and post-partum women (n=48) in WA in 2013-15. In addition, the use of iodised salt and iodine-containing supplements and consumption of six key iodine-containing foods were evaluated. Data collection occurred at 10-14 or 18-22 (Stage 1), 26-28 (Stage 2) and 36-38 (Stage 3) wk gestation and 4-6 wk (Stage 4) post-partum.

Participants provided spot urine samples to determine iodine and creatinine concentrations, and dietary intake information at Stages 1-4. Blood samples to evaluate thyroid function [thyroid stimulating hormone (TSH) and free thyroxine (FT4)] were provided at Stage 1 or 2 and Stage 4. Breast milk samples were provided by 45 women during Stage 4. There was no significant change in urinary iodine-to-creatinine ratio, as measured by actual gestational week of sample (range 10-39 wk), with advancing gestation (p=0.073 unadjusted; p=0.11 adjusted). In addition, the median urinary iodine concentration (MUIC) from urine samples collected at all time points during pregnancy (n=183) was 182 µg/L, indicating adequate iodine intake. There was no significant difference (p=0.476) in MUICs between daily iodine-containing supplement users [176 (IQR 103, 295) µg/L; n=142] and non-daily users [194 (IQR 114, 321) µg/L; n=40] during pregnancy, with results indicating adequate intake for both groups.

For breastfeeding women, the MUIC and median breast milk iodine concentration (BMIC) were 105 and 180 µg/L, respectively, indicating adequate iodine intake. For both women who consumed iodine-containing supplements daily during lactation and those who did not, adequate iodine intake was indicated by the median BMIC in both

groups but it was significantly higher in women taking an iodine-containing supplement (193 vs 134 µg/L, respectively; p=0.033). MUIC also indicated adequate iodine intake for both groups but the difference was not significant (110 vs 106 µg/L, respectively; p=0.798). Mean TSH and FT4 concentrations were within the respective reference ranges throughout the study.

Study findings indicate that there was no significant change in iodine status with advancing gestation in this cohort of Perth women. In addition, these women in Perth had adequate iodine intake despite the non-universal use of iodine supplementation. These results challenge the NHMRC recommendation for all Perth pregnant and breastfeeding women to use daily iodine-containing supplements. However, future research with larger and more representative samples should be conducted to validate these outcomes.

Dedication

This thesis is dedicated to my Aunty Kath who, as a school teacher and deputy principal for 43 years, understood the value of education better than anyone.

If it is to be, it is up to me.

RIP KJH

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List of Abbreviations

ACT	Australian Capital territory
AI	adequate intake
AJ	Anita Jorgensen
ATA	American Thyroid Association
BMIC	breast milk iodine concentration
EAR	estimated average requirement
FFQ	food frequency questionnaire
FSANZ	Food Standards Australia New Zealand
FT3	free triiodothyronine
FT4	free thyroxine
hCG	human chorionic gonadotropin
ICPMS	inductively coupled plasma mass spectrometry
IDD	iodine deficiency disorders
IIH	iodine-induced hyperthyroidism
IQR	interquartile range
KEMH	King Edward Memorial Hospital
MITCH	maternal iodine supplementation and effects on thyroid function
MUIC	median urinary iodine concentration
NHMRC	National Health and Medical Research Council
NHMS	national health measures survey
NINS	national iodine nutrition survey
NIS	sodium/iodide symporter
NSW	New South Wales
NT	Northern Territory
NZ	New Zealand
RCT	randomised controlled trials
RDI	recommended dietary intake
SA	South Australia
SD	standard deviation
SPSS	Statistical package for the social sciences

T3	3,5,3’-triiodothyronine
T4	3,5,3’,5’-tetraiodothyronine (thyroxine)
TBG	thyroxine binding globulin
Tg	thyroglobulin
TPO-Ab	thyroperoxidase antibody
TSH	thyroid stimulating hormone
Tvol	thyroid volume
UCC	urinary creatinine concentration
UIC	urinary iodine concentration
UIC/Cr	urinary iodine concentration-to-creatinine ratio
UL	upper limit
USI	universal salt iodization
WA	Western Australia
WHO	World Health Organisation

Chapter 1 Introduction

1.1 Statement of the problem

Adequate iodine intake is important for optimal health in all stages of life. A deficiency in this essential trace element, resulting in inadequate production of thyroid hormones, can have profound effects on normal growth, development and functioning. Collectively, these deleterious consequences are termed iodine deficiency disorders (IDD)(Hetzel, 1983; WHO/UNICEF/ICCIDD, 2007). The most serious outcomes occur with chronic, severe insufficiency during pregnancy and early infancy and include increased incidence of abortion, stillbirth, congenital abnormalities, perinatal mortality, and severe neurological damage resulting in irreversible mental and physical impairment (known as cretinism). However, the population effects of more subtle degrees of iodine deficiency are of even greater public health significance. Worldwide, iodine deficiency is one of the most common causes of preventable intellectual impairment (Rohner et al., 2014; WHO/UNICEF/ICCIDD, 2007; Zimmermann, 2012). Recent WHO estimates suggest that approximately one-third of both the global population and all school-aged children have inadequate iodine nutrition (Zimmermann, 2012).

Population iodine status is most commonly assessed by measuring casual/spot urinary iodine concentration (UIC) in a representative sample from the population, typically of school-aged children. The median UIC (MUIC) is compared against established WHO cut-offs to determine iodine status. Iodine status in the general population (including school-aged children) is optimal if the MUIC is between 100 and 200 µg/L; iodine deficiency is defined as a MUIC <100 µg/L. Iodine deficiency during pregnancy is defined as a MUIC <150 µg/L and <100 µg/L for lactation (WHO/UNICEF/ICCIDD, 2007). While UIC reflects recent iodine intake (days), other biomarkers often used in combination with UIC assess long-term iodine nutrition (months to years) including thyroid size, thyroid stimulating hormone (TSH) and thyroid hormones (triiodothyronine, T3 and thyroxine, T4) (Zimmermann & Andersson, 2012). Furthermore, there is increasing evidence to suggest that breast milk iodine concentration (BMIC) is a more accurate biomarker of iodine status than UIC in lactating women and that both biomarkers should be measured simultaneously in this subpopulation (Dold et al., 2017).

In recent decades there has been a re-emergence of iodine deficiency in some areas throughout the world (de Benoist, McLean, Andersson, & Rogers, 2008). Australia is one of a handful of countries globally now considered mildly iodine deficient after previously being considered sufficient. Factors contributing to this change included 1) a reduction in the use of iodine-containing sanitisers by the dairy industry in the 1990s which significantly decreased the iodine content of milk, 2) discontinuation of prior food fortification and supplementation initiatives, and 3) decreased consumption of iodised salt due to a trend towards increased consumption of foods prepared outside the home, which largely contained non-iodised salt (Australian Population Health Development Principal Committee, 2007; M. Li et al., 2006). Studies conducted in New South Wales (NSW), Victoria and Tasmania in the late 1990's confirmed the re-emergence of mild iodine deficiency in some parts of Australia and led to the introduction of a voluntary iodised salt in bread fortification program in Tasmania in 2001. A national investigation into population iodine status, the National Iodine Nutrition Survey (NINS), was conducted in 2003-04 and involved determining urinary iodine concentration (UIC) in spot urine samples from 1709 children aged 8-10 years from 88 schools across five Australian states [namely, NSW, South Australia (SA), Victoria, Queensland (Qld) and Western Australia (WA)]. Results showed that overall, children in mainland Australia were borderline iodine deficient. State-by-state results showed a range of iodine status from mild deficient in NSW and Victoria to borderline deficient in SA to sufficient in WA and Qld (M. Li et al., 2006).

The NINS provided evidence of the re-emergence of mild iodine deficiency in the Australian population and following a successful voluntary bread fortification program in Tasmania, a national mandatory bread fortification program was implemented by Food Standards Australia New Zealand (FSANZ) in October 2009. Overall results of the more recent 2011-12 National Health Measures Survey (NHMS), part of the 2011-2013 Australian Health Survey, which began 18 months after the introduction of mandatory fortification indicated adequate and improving iodine levels in the general Australian population (Australian Bureau of Statistics, 2013). Furthermore, the latest post-mandatory survey conducted in Tasmania in 2016 showed that iodine adequacy has been sustained in this state 7 years after mandatory bread fortification, based on results of studies conducted in school children (Hynes, Seal, Otahal, Reardon, & Burgess, 2018).

Pregnant and breastfeeding women are population subgroups particularly vulnerable to iodine deficiency due to their increased requirements for iodine to support normal fetal and infant growth and development (Zimmermann, 2012) . Despite this, to date there has been no national survey to determine the iodine intake or iodine status of Australian pregnant and breastfeeding women specifically. However, the 10 studies investigating the iodine status of pregnant women in individual states and territories [namely NSW (Blumenthal, Byth, & Eastman, 2012; Charlton, Gemmings, Yeatman, & Ma, 2010; Gunton, Hams, Fiegert, & McElduff, 1999; M. Li, Ma, Boyages, & Eastman, 2001; Travers et al., 2006) , Victoria (Hamrosi, Wallace, & Riley, 2005), Tasmania (Burgess et al., 2007; Stilwell et al., 2008), Northern Territory (NT) (Mackerras, Singh, & Eastman, 2011) and Australian Capital Territory (ACT) (Nguyen et al., 2010)] over recent decades prior to bread fortification all indicated inadequate iodine intake, based on MUIC <150 µg/L.

In addition, there were concerns that bread fortification alone would not increase iodine levels enough to meet the higher iodine requirements of pregnant women in Australia (Food Standards Australia New Zealand, 2009). Consequently, in January 2010 the National Health and Medical Research Council (NHMRC) introduced recommendations that all pregnant and breastfeeding women, as well as those planning a pregnancy, take a daily supplement containing 150 µg of iodine (National Health and Medical Research Council, 2010). Studies conducted post-mandatory fortification and iodine supplement recommendation showed improved iodine status for pregnant women in NSW and SA. However, both studies highlighted the importance of iodine supplementation during pregnancy to achieve adequate iodine status, by means of a MUIC >150 µg/L (Charlton et al., 2013; Condo et al., 2016). An important point to note is that no studies examining urinary iodine excretion have been conducted with pregnant women in WA or Qld, neither pre- nor post-fortification and iodine supplement recommendation.

Furthermore, while the importance of adequate iodine intake during pregnancy is well-established, the effect of advancing gestation on iodine status is poorly understood (Bath, Furmidge-Owen, Redman, & Rayman, 2015). This topic has been explored in longitudinal studies of populations of varying iodine status worldwide with the evidence supporting both an increase and decrease in urinary iodine excretion across

gestation (Fuse, Shishiba, & Irie, 2013). Such longitudinal studies conducted in Australia to date are limited in number, have been conducted in the same one state (SA) and have produced conflicting results (Clifton et al., 2013; Condo et al., 2016).

1.2 Benefits of the study

Population iodine status is considered adequate in WA, with results from the recent NHMS showing WA adults and school children have the highest MUICs of any state or territory (Australian Bureau of Statistics, 2013). However, there is a gap in knowledge regarding the iodine status of pregnant and post-partum/breastfeeding women in WA. This is the first study to investigate the iodine status of these vulnerable subgroups in WA using biochemical measures (namely UIC, TSH, FT4 and BMIC). It is also one of few Australian studies, and the first in WA, to conduct a longitudinal investigation of the gestational changes in iodine status in the same cohort of women. In addition, it is among the first in WA to examine the use of iodine-containing supplements in these subgroups and to determine the appropriateness of the national iodine supplement recommendation in this state.

1.3 Study aims and objectives

Aim

To investigate gestational changes in iodine status and to determine the overall iodine health of a cohort of pregnant and post-partum women in Perth.

Objectives

1. To investigate the changes in maternal urinary iodine excretion and blood indices of iodine status (based on thyroid function tests of TSH and free T4) throughout pregnancy and the post-partum period in Perth women.
2. To determine the iodine concentration of breast milk and relate this to maternal iodine status.
3. To investigate the differences in iodine status in women taking iodine supplements and those only receiving iodine in the diet.
4. To determine if study results support the NHMRC recommendation for iodine supplement during pregnancy and post-partum in Perth women.

Chapter 2 Literature Review

2.1 Scope of review

There are two main sections to this literature review. The first section contains background information on iodine including its importance, requirements, metabolism, status assessment and metabolism, with a particular emphasis on pregnancy. This also includes discussion on trends in iodine status in the Australian population over recent decades. The second part of this chapter is a critical review of longitudinal studies published between January 1997 and July 2017 on the changes in iodine status during pregnancy. The electronic databases used in the literature search for relevant articles were Medline, Cinahl, Informit and Scopus, using the key terms "iodine" or "iodine status" and "pregnancy" or "pregnant women" and "gestational changes" or "longitudinal". Relevant references cited in key articles have also been considered for inclusion in the review.

2.2 Introduction

Iodine is an essential trace element required for normal thyroid gland function, in particular for the production of the thyroid hormones triiodothyronine (3,5,3'-triiodothyronine; T3) and thyroxine (3,5,3',5'-tetraiodothyronine; T4). Thyroid hormones are vital for normal brain development, growth and maturation, reproductive function and regulation of energy metabolism (Rohner et al., 2014; Zimmermann, 2009a). Iodine accounts for 65% and 59% of the weights of T4 and T3, respectively, reflecting its importance in their composition. Structurally, the only difference between the two hormone forms is an extra iodine atom in T4 (see Figure 2.1). A healthy adult body contains 15-20 mg of iodine, with the vast majority (70-80%) in the thyroid (located at the base of the neck) and most of the remainder in blood (Zimmermann, Jooste, & Pandav, 2008). In contrast, thyroid iodine content may fall to <1 mg in chronic iodine deficiency (Zimmermann, 2012). In addition to iodine's established role in thyroid hormone synthesis, other suggested possible functions/associations for iodine include a link to fibrocystic breast disease, a role in the immune response and the ability to alter gastric cancer risk (Rohner et al., 2014).

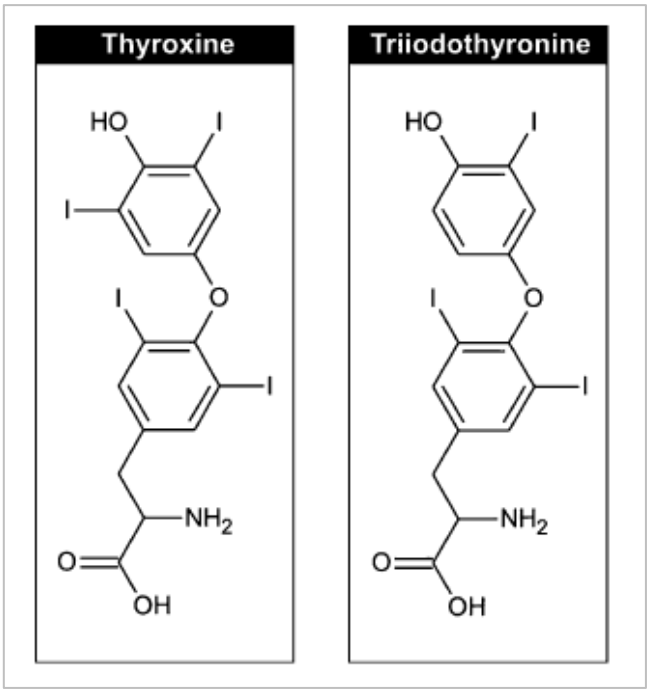

Figure 2.1 Chemical structure of thyroid hormones
(Zimmermann, 2012, p. 559)

2.3 Ecology and Dietary Sources of Iodine

Iodine (as iodide) is present in soils in widely varying concentrations. This uneven distribution of soil iodide may exist within regions and across wider geographic locations. Most iodide in the Earth's environment is found in the oceans (Zimmermann, 2012). Here, iodide is converted into elemental iodine which is released into the atmosphere and returned to the land (soil, groundwater and surface water) via rain and snow, thereby completing the ecological cycle. However, in many regions this process is slow and unreliable in terms of redistribution and does not overcome the iodine deficit in depleted soils and freshwater. Iodine-deficient surface soils are common in areas subjected to glacial leaching, frequent flooding and soil erosion, the later typically found in mountainous areas and in regions of excessive loss of vegetation due to agricultural production or overgrazing by livestock (Rohner et al., 2014).

As an essential nutrient, iodine cannot be made in the body and must be obtained via exogenous/dietary sources. Food sources of marine origin concentrate iodine from seawater, resulting in higher iodine content (Zimmermann, 2012). In contrast, the natural iodine content of most common foods is low at 3-80 µg/serving (Haldimann, Alt, Blanc, & Blondeau, 2005) and depends on the soil iodine content from where

foods originated. Therefore, plants and animals from iodine-deplete areas will in turn be low in iodine. The iodine content of foods is also influenced by the use of iodine-containing compounds, for example, in irrigation, fertilizers, livestock feed, iodophors for cleaning (Zimmermann, 2012) and processing aids such as calcium iodate and potassium iodide (National Health and Medical Research Council and New Zealand Ministry of Health, 2006). Furthermore, iodine is deliberately added to some foods such as dietary supplements and iodised salt to provide additional dietary iodine. Iodine losses may occur with cooking and canning, however, these losses are minimal (≤10%) in foods containing iodised salt (Zimmermann, 2012).

2.4 Iodine absorption and metabolism

2.4.1 Absorption

Iodine may be consumed and absorbed in three chemical forms, each with unique absorptive and metabolic characteristics (Rohner et al., 2014; Zimmermann, 2012).

1. Iodide (I^-) - is the main form of iodine in foods and supplemental sources and is rapidly and almost completely (>90% in healthy adults) absorbed in the stomach and duodenum (Rohner et al., 2014). This process is mediated by the sodium/iodide symporter (NIS), a transmembrane protein located on the apical surface (lumen facing) of the small intestine epithelial cells. This results in the inward movement of iodide against its concentration gradient, that is, active absorption. Similarly, NIS activity also results in the secretion of iodide into the gastrointestinal tract via gastric juice and saliva. While the functional significance of this is unknown, iodide released in this way is likely to be reabsorbed further down the gastrointestinal tract and recycled, thereby aiding in iodide conservation. This mechanism is most likely of greatest significant in iodine scarcity or deficiency (Nicola et al., 2009).
2. Iodate (IO_3^-) – is the main source of iodine in iodised salt. Ingested iodate is reduced to iodide in the gut prior to absorption in the duodenum (Rohner et al., 2014; Zimmermann, 2012).
3. Organically bound iodine – is typically digested and the released iodide absorbed, however, some forms are absorbed intact. For example, ~75% of an oral dose of thyroxine used to treat an underactive thyroid (hypothyroidism) is absorbed intact (Zimmermann, 2012).

Once absorbed, most iodine (as iodide) that enters the circulation is cleared by the thyroid and kidneys. While renal iodine clearance is relatively constant, thyroid iodine clearance varies considerably depending on iodine intake. In the situation of adequate iodine supply, ≤10% of absorbed iodine is removed by the thyroid. In contrast, a greater proportion of absorbed iodine (≥80%) is taken up by the thyroid in chronic iodine deficiency, leading to a corresponding reduction in renal iodine excretion (Rohner et al., 2014). Under normal conditions, circulating iodine is rapidly turned over with a half-life of ~10 hours. However, this turnover is shortened in the case of iodine deficiency or hyperthyroidism (excess production of thyroid hormones) when the thyroid is overactive (Rohner et al., 2014; Zimmermann, 2012).

2.4.2 Metabolism and excretion

To balance losses and maintain adequate thyroid hormone synthesis, the adult thyroid needs to take up 60-80 µg of iodine per day from circulation. This uptake is again facilitated by an NIS, on this occasion located in the basolateral membrane of the thyroid cell (Zimmermann, 2012). Via an active transport process, the NIS concentrates and transfers iodide at a gradient 20 to 50 times that of plasma (Eskandari et al., 1997). Once inside the thyrocyte, the iodide migrates to the apical membrane to begin the multi-step process leading to the production of thyroid hormones T4 and T3 (See Fig. 2.2):

- Iodide is oxidised by the enzymes thyroperoxidase (TPO) and hydrogen peroxide and attached to tyrosyl residues on thyroglobulin (Tg), a large glycoprotein and the carrier of iodine in the thyroid.
- This produces the precursors of thyroid hormone, namely monoiodotyrosine (MIT) and diiodotyrosine (DIT). Catalysed by TPO, the residues then couple within the Tg molecule in the follicular lumen to form thyroxine (T4; via linkage of two DIT molecules) and triiodothyrosine (T3; via linkage of one MIT molecule and one DIT molecule). A third product, biologically inactive reverse T3, is produced within the thyroid when DIT and MIT are coupled in the reverse order than for T3.
- The mature Tg is stored extracellularly, with iodine accounting for just 0.1 to 1.0% of the glycoprotein's weight. In iodine sufficiency, there is 3-4 times more T4 than T3 per Tg molecule (Dunn & Dunn, 2000).

- When required, Tg enters the thyrocyte by endocytosis and is degraded, releasing T4 and T3 into the circulation. The precursors MIT and DIT are not normally released into circulation. The iodide they contain is conserved and recycled for use within the thyroid (Zimmermann, 2012).

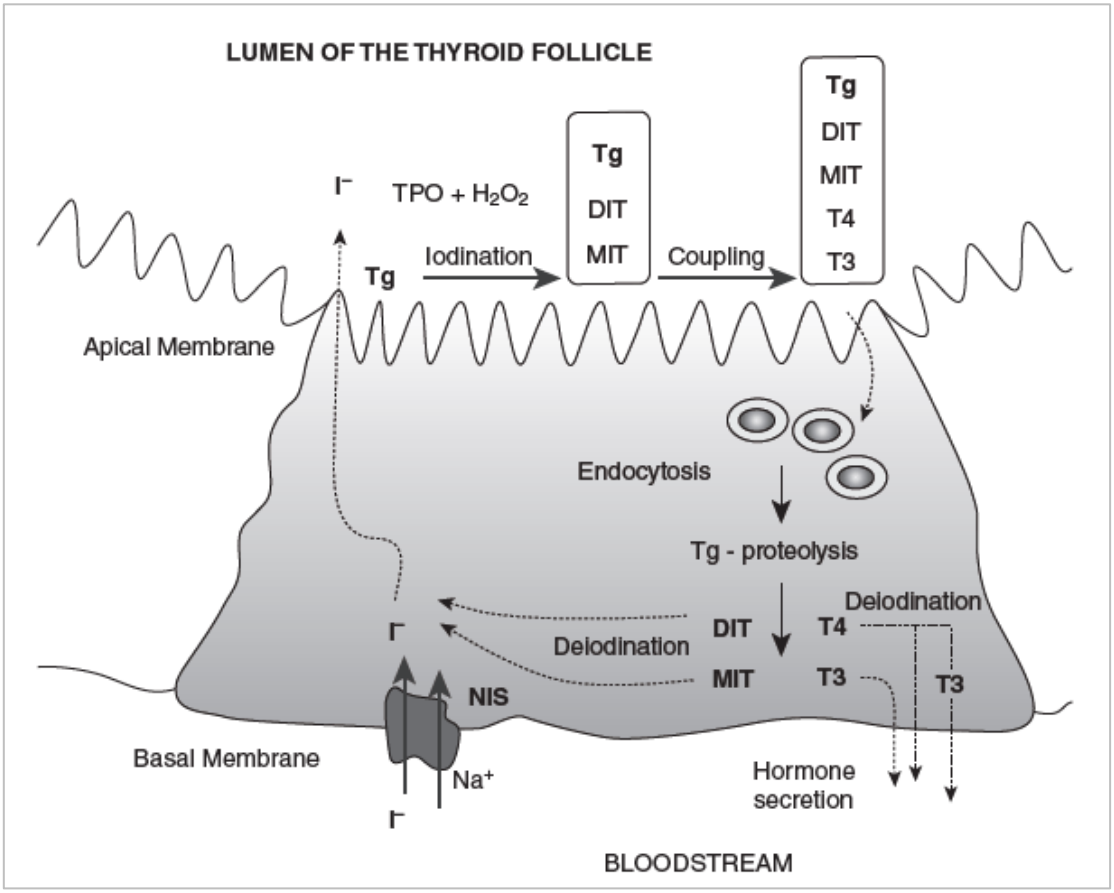

Figure 2.2 Pathway in thyroid cell
(Zimmermann, 2012, p. 558)

In the circulation, more than 99% of thyroid hormones are bound to plasma carrier proteins, primarily thyroxine-binding globulin, but also to transthyretin and albumin. However, it is only the small amount of unbound or free hormones that are bioactive and available for uptake by target tissues, namely liver, kidney, heart, muscle, pituitary and the developing brain. At receptors on the surfaces of target cells, various iodothyronine 5'-deiodinase enzymes remove an outer ring iodine from T4 converting it to T3, the main biologically active thyroid hormone. Within target tissue cells, T3 binds to nuclear receptors which in turn stimulates several pathways responsible for energy production involving ATP and the regulation of protein synthesis (Zimmermann, 2012). Both T3 and T4 are broken down in the periphery via a complex series of pathways. Their turnover is relatively slow, with the half-lives of T4 and T3

being ~5 and 1.5-3 days, respectively. The liberated iodide enters the plasma iodide pool for reuptake by the thyroid or is excreted by the kidney. Interestingly, >90% of ingested iodine is ultimately excreted in the urine of individuals in positive iodine balance (iodine sufficiency), with only a small amount lost in the faeces. Conversely, in chronic iodine deficiency, urinary excretion may be <20% of the ingested amount (Rohner et al., 2014; Zimmermann, 2012).

Thyroid hormone metabolism is primarily regulated by thyroid stimulating hormone (TSH) secreted by the pituitary. TSH secretion is stimulated by thyrotropin releasing hormone which is produced in the hypothalamus and both are under the negative feedback control of circulating thyroid hormones. TSH is released into the blood in response to a low level of circulating thyroid hormones and binds to TSH receptors on the follicular cells of the thyroid gland to initiate thyroid hormone production and secretion. In the first step, TSH binding to TSH receptors on the thyroid gland increases thyroid iodide uptake by stimulating NIS expression through promotion of NIS gene transcription. In addition, TSH also promotes breakdown of Tg and subsequent release of thyroid hormones into the blood. It is crucial that thyroid hormone secretion is under fine control as either excess or deficiency will have negative impact on normal function (Zimmermann, 2012).

More than 100 substances have been reported to negatively affect the thyroid's utilisation of iodine. Collectively, these substances are termed "goitrogens" and exert their deleterious effects on thyroid metabolism via several mechanisms. Some of the better understood goitrogens are naturally-occurring components of food and industrial pollutants (see Table 2.1) (Rohner et al., 2014) . The undesirable effects of most dietary goitrogens can be negated by simple food preparation. For example, cassava (a staple food in many developing countries) can be soaked or cooked to remove the linamarin, which is otherwise metabolised in the gut to thiocyanate. Thiocyanate, also found in cigarette smoke, is a potent competitor inhibitor of the thyroid NIS, thereby significantly reducing thyroidal uptake of iodide (Zimmermann, 2012).

In addition, some micronutrient deficiencies can also result in impaired iodine metabolism and thyroid function and worsen the effects of iodine deficiency:

- Selenium – the thyroid enzymes glutathione peroxidase and the deiodinases are selenium-dependent enzymes. Selenium deficiency results in an accumulation of peroxides which may damage the thyroid. Reduced deiodinase activity impairs thyroid hormone synthesis.
- Iron – deficiency results in impaired thyroid production due to reduced heme-dependent thyroperoxidase activity.
- Vitamin A – in iodine deficient children, vitamin A deficiency increases TSH stimulation and goitre risk, most likely through reduced vitamin A-mediated suppression of the pituitary TSHβ gene transcription (Rohner et al., 2014; Zimmermann, 2012).

Table 2.1 Goitrogens affecting iodine metabolism and thyroid function
(Rohner et al., 2014)

Goitrogen source	Goitrogenic substance	Mechanism
Food		
• Cassava, lima beans, linseed, sorghum, sweet potato	Cyanogenic glucosides are metabolised to thiocyanates	Compete with iodine for uptake by thyroid gland
• Cruciferous vegetables: cabbage, kale, cauliflower, broccoli, turnips, rapeseed	Glucosinolates	Compete with iodine for uptake by thyroid gland
• Soy, millet	Flavonoids	Impair thyroid peroxidase activity during production of thyroid hormones
Industrial pollutants (that enter food and water)	Percholate, nitrate	Competitive inhibitors of sodium/iodide symporter so decrease transfer of iodine into thyroid
	Disulfides (from coal processes)	Reduce thyroid iodine uptake

2.4.3 Physiologic changes in pregnancy and lactation

Pregnancy and lactation bring about three major changes in maternal thyroid function and iodine metabolism.

1. Increased demand on the maternal thyroid gland for T3 and T4 production – pregnancy has a substantial impact on the thyroid gland and its function. From early in the first trimester of gestation, T3 and T4 production is required to increase by approximately 50% to maintain adequate maternal levels (euthryoidism) and for placental transfer to the developing fetus. In early pregnancy, the fetus is entirely dependent on maternal thyroid hormones as the fetal thyroid is not yet functioning adequately (significant T4 production occurs from approximately the 20th week of gestation and T3 production rises by 30 weeks gestation). This increased production is influenced by high levels of circulating estrogen in addition to TSH levels. Furthermore, the alpha subunit of human chorionic gonadotrophin (hCG), a hormone produced by the placenta during pregnancy, also binds to and stimulates the thyroid TSH receptor (Yarrington & Pearce, 2011)(see Fig. 2.3).
2. Increased renal clearance of iodine – also starting in early pregnancy and persisting until parturition is an increase in renal blood flow and glomerular filtration rate, resulting in an increased iodide clearance from the plasma and kidneys of 30-50% (Glinoer, 2007). As renal iodide excretion is a passive process, this represents an obligatory loss of iodine. However, this loss is somewhat compensated for as the renal 'leaking' tends to lower the circulating plasma iodide concentration which in turn stimulates increased thyroidal clearance of iodide (Glinoer, 2007) (see Fig 2.3).
3. Iodine concentrating ability of mammary gland tissue – iodide is strongly concentrated by the lactating mammary gland to meet the iodine demands of the developing infant, via breast milk. This avid uptake of iodine, due to the presence of NIS (Semba & Delange, 2001; Yarrington & Pearce, 2011), results in human milk with an iodine concentration 20-50 times higher than that of plasma (Azizi & Smyth, 2009). However, a number of chemicals competitively inhibit NIS-mediated iodide transport into the lactating mammary gland, the most common being thiocyanate from cigarette smoke. Consequently, smokers tend to have lower breast milk iodine concentrations (Laurberg, Nøhr, Pedersen, & Fuglsang, 2004).

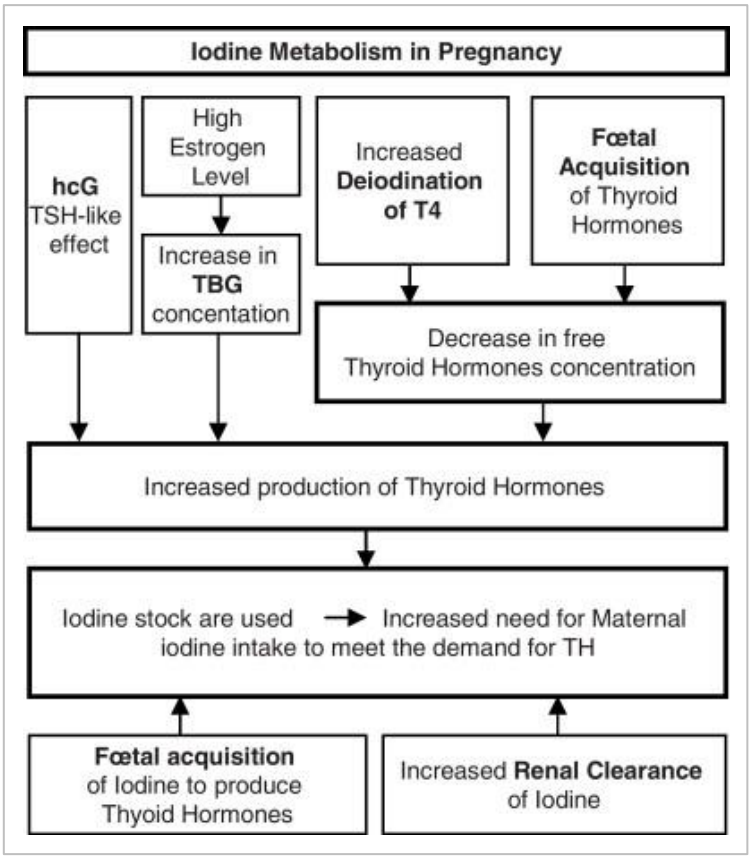

Figure 2.3 Iodine metabolism in pregnancy
(Trumpff et al., 2013, p. 177)

2.5 Iodine requirements

2.5.1 Dietary iodine requirements

Dietary iodine requirements vary by life stage, with infants and young children needing the lowest daily intake and lactating women the highest. Recommended iodine intakes for the Australian (and New Zealand) population are reflected in the Nutrient Reference Values released in 2006. Table 2.2 presents the iodine requirements for the life stages of particular interest in this study.

Table 2.2 Iodine NRV for Infants and Adults
(National Health and Medical Research Council and New Zealand Ministry of Health, 2006)

	EAR[a] (ug/day)	AI[b]/RDI[c] (ug/day)	UL[d] (ug/day)
Infants (AI) **0-6 months** **7-12 months**		90 110	Not possible to establish. Source of intake should be milk, formula or food only.
Men (RDI) **19->70 y**	100	150	1100
Women (RDI) **19->70 y**	100	150	1100
Pregnancy (RDI) **19-50 y**	160	220	1100
Lactation (RDI) **19-50 y**	190	270	1100

aEAR (Estimated Average Requirement) – A daily nutrient level estimated to meet the requirements of half the healthy individuals in a particular life stage and gender group.
bAI (Adequate Intake) – Used when an RDI cannot be determined. The average daily nutrient intake level based on observed or experimentally-determined approximations or estimates of nutrient intake by a group/s of apparently healthy people that are assumed to be adequate.
cRDI (Recommended Dietary Intake) – The average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all (97-98%) healthy individuals in a particular life stage and gender group.
dUL (Upper Limit) – The highest average daily nutrient intake level likely to pose no adverse health effects to almost all individuals in the general population. As intake increases above the UL, the potential risk of adverse effects increases.

2.5.1.1 Infants

The recommended iodine intake (AI) of infants 0-6 months of 100 µg/day was determined by multiplying the average intake (0.78 L/day) and average iodine concentration of breast milk 115 (µg/L), with rounding. A metabolic weight ratio was used to determine the AI for 7-12 months, based on that of younger infants (National Health and Medical Research Council and New Zealand Ministry of Health, 2006).

2.5.1.2 Adults

The EAR for men and women was determined from iodine balance studies that indicated a physiological requirement of approximately 100 µg/day. The RDI of 150 µg/day was derived by adding a coefficient of variation (CV) of 20% to the EAR, with rounding up to account for the potential influence of natural goitrogens. The UL was

based on the outcomes of two studies which showed elevated TSH concentrations, the first indicator of challenged thyroid function due to iodine excess, after supplemental iodine at amounts of 1800 µg/day and 1700 µg/day, respectively (Gardner, Centor, & Utiger, 1988; Paul et al., 1988). These study results, together with an uncertainty factor of 1.5, were used to derive the UL of 1100 µg/day (National Health and Medical Research Council and New Zealand Ministry of Health, 2006)

2.5.1.3 Pregnancy

The higher EARs and RDIs for pregnancy, compared with those for the non-pregnant state, reflect the greater need for iodine during this life stage. As discussed in Section 2.4.3, this increased requirement is necessary to meet maternal and fetal demands for thyroid hormone production and to account for increased maternal renal losses due to physiologic changes that occur in iodine metabolism during pregnancy. Later in pregnancy, this increase in iodine requirement provides for iodide for fetal synthesis of thyroid hormone, as well as a decreasing amount of preformed thyroid hormone (Glinoer, 2007; Morreale de Escobar, Obregon, & Escobar del Rey, 2004). The EAR of 160 µg/day was determined from data on the newborn thyroid content, iodine balance studies and studies of iodine supplementation in pregnancy. A CV of 20% for the EAR was used to set the RDI as 220 µg/day. As evidence suggests there is no increased sensitivity to excess intake during pregnancy, the same adult UL of 1100 µg/day applies (National Health and Medical Research Council and New Zealand Ministry of Health, 2006).

2.5.1.4 Lactation

The EARs and RDIs for lactation are higher again than those for pregnancy at 190 µg/day and 270 µg/day, respectively. The EAR was set to account for adult female needs (100 µg/day) and as discussed in 2.4.3, to replace iodine secreted in breast milk (90 µg/day). The RDI was based on the EAR with CV of 20%. For the same reason as pregnancy, the lactation UL was set at the adult UL of 1100 µg/day (National Health and Medical Research Council and New Zealand Ministry of Health, 2006).

2.5.2 Assessment of dietary iodine intake

The main aim of dietary iodine assessment is to measure the average, usual long-term intake of iodine-containing foods, taking into account amounts consumed and frequency of consumption. The three primary tools used for this are food frequency questionnaires (FFQ), food diaries or 24-hr recalls, and weighed food records, each with their own strengths and limitations for use (Rohner et al., 2014; Subar et al., 2015; Zimmermann & Andersson, 2012). One major limitation common to all dietary assessment methods is the inability to accurately determine the contribution of iodine from iodised salt used at the table or in cooking, due to difficulties in quantifying amounts used (Skeaff, 2012). Additionally, all three methods require information on the iodine content of foods to determine iodine intake. This is provided by food-composition tables/nutrient-composition databases, the use of which introduces further factors that impact on the accuracy of dietary iodine intake assessment (Subar et al., 2015). The large day-to-day variation in individual intake is another issue challenging the assessment of 'usual' iodine intake. Nonetheless, dietary data are useful in determining the main food sources of iodine which can in turn be used to develop or modify intervention strategies, for example, for iodine fortification (Rohner et al., 2014; Zimmermann & Andersson, 2012).

2.6 Iodine balance

Iodine balance or status is directly influenced by habitual dietary iodine intake (see Fig. 2.4). The daily iodine intake must be adequate to enable thyroid turnover (uptake and release) of approximately 95 µg iodine per day, thus achieving iodine balance and maintaining normal thyroid function (euthyroidism). Short-term deficits in iodine intake can be overcome by utilising intrathyroid stores (up to 20 mg in iodine-sufficient areas) and increased thyroidal uptake of circulating iodine and stimulation of NIS transcription under the influence of TSH. However, in the case of chronically low iodine intakes, thyroid iodine stores will be gradually exhausted, resulting in thyroid dysfunction and reduced thyroid hormone production (see Section 2.7). The actual levels of habitual daily iodine intake at which these adverse consequences occur is unknown (Zimmermann & Andersson, 2012) but is likely to be ~ <60 µg/day and to vary significantly among individuals (Rohner et al., 2014).

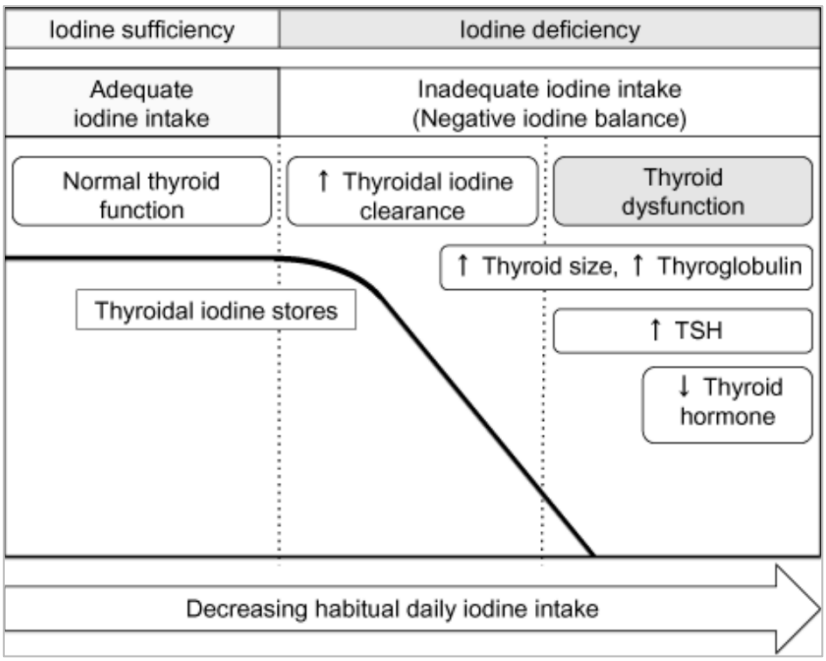

Figure 2.4 Physiological stages of iodine status
(Zimmermann & Andersson, 2012, p. 563)

2.7 Iodine Deficiency

Iodine deficiency results from iodine intakes below requirements. Globally, iodine deficiency is a significant public health issue. Recent WHO estimates suggest that approximately 2 billion individuals worldwide (approximately one-third of the global population) have inadequate iodine nutrition, with many of these in developing countries. This includes 266 million school-aged children, which is 31.5% of all school-aged children globally (Zimmermann, 2012). In many parts of the world, iodine deficiency occurs where the presence of certain geographical or ecological characteristics reduce the iodine content of the soil, as discussed in Section 2.3. More recently however, iodine deficiency has been shown to exist in many areas without these characteristics, including large cities, highly developed countries, coastal areas and where iodine deficiency was previously thought to have been eliminated. This includes countries like Australia, the United Kingdom and the United States (WHO/UNICEF/ICCIDD, 2007).

2.7.1 Consequences of iodine deficiency

Iodine deficiency, leading to inadequate production of thyroid hormones, results in a spectrum of deleterious effects on growth and development. Collectively, these are termed the iodine deficiency disorders (IDD), with all age groups potentially at risk of being affected (see Table 2.3) (Hetzel, 1983; WHO/UNICEF/ICCIDD, 2007) . The degree of adversity is dependent on both the timing and severity of deficiency (Rohner et al., 2014).

Table 2.3 Iodine deficiency disorders by age group
(Zimmermann, 2012)

Age group	Health consequences of iodine deficiency
All ages	Goitre Increased susceptibility of the thyroid gland to nuclear radiation
Fetus	Abortion Stillbirth Congenital anomalies Perinatal mortality
Neonate	Infant mortality Endemic cretinism
Child and adolescent	Impaired mental function Delayed physical development
Adults	Impaired mental function Reduced work productivity Toxic nodular goitre; iodine-induced hyperthyroidism Hypothyroidism in moderate to severe iodine deficiency

The classic and most obvious sign of (chronic) iodine deficiency is thyroid enlargement or goitre. This is the body's adaptive response when iodine intakes are habitually ~ <50 µg/day. At such low intakes, increased TSH secretion stimulates several changes within the thyroid gland, including an increase in the number of thyroid cells (hyperplasia) and their size (hypertrophy), resulting in an increase in the overall size of the gland. These changes attempt to maximise uptake of available circulating iodine and improve the efficiency of its use for thyroid hormone

production. Goitres can affect all ages, with large ones not only being aesthetically unattractive, they may also obstruct the trachea and esophagus and damage the laryngeal nerves (Zimmermann, 2012).

However, the most serious outcomes of iodine deficiency occur due to chronic, severe insufficiency during pregnancy and include a greater incidence of stillbirth, abortion, congenital abnormalities and perinatal mortality. This is because the developing fetal brain is particularly susceptible to iodine deficiency. Adequate amounts of thyroid hormones are vital for neuronal migration and myelination of the central nervous system during fetal and early postnatal life. Cretinism is the term used to describe the most severe form of neurological damage due to a lack of fetal thyroid hormones (hypothyroidism). The condition is characterised by irreversible mental impairment, together with varying degrees of short stature, deaf mutism, goitre and spasticity. In areas of severe, long term iodine deficiency, as many as 10% of the population may be cretins (Zimmermann, 2012). Furthermore, a high prevalence of goitre in the population is highly suggestive of inadequate maternal iodine intakes (Skeaff, 2012).

Nevertheless, of even greater public health importance are the population effects of more subtle degrees of cognitive impairment due to inadequate iodine intake. Iodine deficiency is one of the most common causes of preventable mental impairment globally (Rohner et al., 2014; WHO/UNICEF/ICCIDD, 2007; Zimmermann, 2012) . Moderate to severe deficiency may reduce mean IQ scores by 13.5 points, as concluded by a meta-analysis involving 18 studies (Bleichrodt et al., 1996). This reduced cognitive capacity diminishes the performance and productivity of otherwise normal children and adults, thereby reducing the quality of life and potential of entire communities. Without appropriate measures to overcome the situation, the self-perpetuating cycle continues (WHO/UNICEF/ICCIDD, 2007).

Given that maternal reserves of iodine decrease by 40% during pregnancy, it is not surprising that mild iodine deficiency is common in pregnancy, even in areas which are generally considered iodine replete (Marchioni et al., 2008). Three recent observational studies have shown that mild maternal iodine deficiency during pregnancy is associated with decreased child cognition and development, as evidenced by lower intelligence quotient and reading scores at age 8 y (Bath, Steer, Golding, Emmett, & Rayman, 2012)(United Kingdom), reduced educational assessment scores

at age 9 y (Hynes, Otahal, Hay, & Burgess, 2013)(Tasmania) and most recently, symptoms of child language delay, behaviour problems and reduced fine motor skills at 3 y of age (Abel et al., 2017)(Norway). Despite these findings, the extent to which mild to moderate iodine deficiency during pregnancy influences child neurobehavioural development still remains in question (Pearce, Lazarus, Moreno-Reyes, & Zimmermann, 2016).

2.7.2 Correction of iodine deficiency

Efforts to overcome iodine deficiency have been on the global health agenda for decades. As early as 1952 a WHO technical group recommended iodization of all 'food salt' in iodine-deficient areas. However, it wasn't until some forty years later in 1990, that at the United Nations World Summit for Children and the World Health Assembly, and again in 1991 at the Conference on Ending Hidden Hunger, world leaders established the ambitious goal of global elimination of iodine deficiency (Zimmermann & Andersson, 2012). Prior to this time, only several countries globally were considered completely iodine sufficient, namely Australia, the United States and Canada (Zimmermann, 2012). In 1993, the WHO and UNICEF Joint Committee on Health Policy reaffirmed universal salt iodization (USI) as a safe, cost-effectiveness and sustainable strategy to ensure adequate iodine intake by all individuals. USI involves the iodisation of all salt for human and livestock consumption (WHO/UNICEF/ICCIDD, 2007). Salt iodization is generally considered adequate at amounts of 15-20 mg iodine per kg salt, assuming that daily per capital salt consumption is approximately 10 g (Rohner et al., 2014).

Programs of USI, conducted in accordance with recommendations of the WHO and the Iodine Global Network (formerly the International Council for the Control of Iodine Deficiency Disorders), have been adopted as the first-line strategy against iodine deficiency by almost all countries where it exists. In 2012, 71% of the global population had access to iodized salt, up from just 20% in 1990 (Zimmermann & Andersson, 2012). This has resulted in substantial improvement in iodine nutrition worldwide over the past two decades, to the extent that severely iodine-deficient regions and new cases of cretinism are now uncommon (Pearce et al., 2016). Furthermore, USI has been clearly shown to improve maternal health and infant developmental outcomes in iodine-deficient regions (Pearce et al., 2016).

Consequently, USI is well recognised as the most effective way to achieve the virtual elimination of IDD. However, sustained efforts in USI program monitoring and delivery are vital to ensure that IDD do not return after their elimination (WHO/UNICEF/ICCIDD, 2007).

In addition, in severely iodine-deficient countries and areas where USI programs are inadequate or not feasible, the WHO recommends that vulnerable groups receive oral supplementation, either in the form of a daily low dose or a high dose of iodised oil taken every 6-12 mo. Such groups are pregnant and lactating women, women of reproductive age, and children less than 2 y of age (Pearce et al., 2016; WHO/UNICEF/ICCIDD, 2007). The effectiveness of iodine supplementation for severely iodine-deficient pregnant women in improving obstetric and child outcomes is well established, with evidence dating back to the 1960s in Papua New Guinea (Pearce, 2013). This practise has since been successfully replicated in many other regions around the world (Zimmermann, 2009b).

With the success of USI and supplementation in reducing the prevalence of severe iodine-deficiency worldwide, public health attention and concern has shifted toward areas of mild to moderate iodine deficiency. For example, iodine supplementation for women who are pregnant, breastfeeding, or planning a pregnancy in areas of mild to moderate iodine deficiency has also been recommended by some medical and public health advisory groups worldwide (Pearce et al., 2016). However, it is unclear from the observational studies conducted to date whether maternal iodine supplementation improves psychomotor or mental development in children in such areas, as results from these studies are inconsistent (Abel et al., 2017; Pearce et al., 2016; Taylor, Okosieme, Dayan, & Lazarus, 2014; Zhou, Anderson, Gibson, & Makrides, 2013). Furthermore, some studies have even indicated adverse effects on child neurodevelopment with maternal supplemental iodine (Murcia et al., 2011; Rebagliato et al., 2013).

Consequently, data from well-designed, prospective, randomised controlled trials (RCT) are urgently required to examine this possible cause-and-effect relationship in mild to moderate deficiency regions (Pearce et al., 2016; Zhou et al., 2013). Unfortunately, an Australian/New Zealand RCT commencing in 2010 investigating this issue was aborted following the withdrawal of support from the funding body (NHMRC), who viewed a placebo-controlled trial inconsistent with its iodine

supplement recommendation during pregnancy (Zhou et al., 2015). The results of an RCT (the MITCH studies) recently conducted at two study sites (Bangkok, Thailand and Bangalore, India) are due imminently and will contribute vital evidence on this issue (Melse-Boonstra et al., 2012; Pearce et al., 2016). The current lack of definitive evidence on efficacy and safety is believed to be the reason why broad recommendations for iodine supplementation have not been more widely adopted by government health authorities in most regions of mild to moderate deficiency (Pearce et al., 2016). Conversely, there is evidence that providing iodine supplementation to school-aged children can at least partially reverse cognitive impairment, even in areas of mild to moderate iodine deficiency (Gordon et al., 2009; Zimmermann et al., 2006).

2.8 Clinical and biochemical assessment of iodine status

In view of the limitations of dietary assessment, iodine status is typically assessed by a number of clinical and biochemical biomarkers. Clinical assessment includes examining thyroid size for the presence of goitre. Biochemical indices used to assess iodine status include measures of TSH, thyroid hormones (T4 and T3) and Tg in blood, and iodine concentration in urine. The biomarkers provide insight into iodine status of differing timeframes. For example, exposure biomarkers, such as urinary iodine concentration (UIC), assess short-term iodine status (days) by exploring recent iodine intake, while functional biomarkers assess long-term iodine nutrition (months to years) by exploring thyroid status, including thyroid size (goitre) and thyroid hormones (Zimmermann & Andersson, 2012). The following section briefly describes each biomarker, while Table 2.6 presents some key advantages and limitations of each assessment method.

2.8.1 Urinary iodine concentration

Urinary iodine concentration (UIC) is the most practical and routinely used biomarker worldwide to assess iodine status in populations (Pearce & Caldwell, 2016; Skeaff, 2012). As previously mentioned, UIC directly reflects recent dietary iodine intake and can be expressed in a number of different and noninterchangeable ways. The most common format is as a concentration (in µg/L), expressed as the median, from non-fasting spot or random samples in the target population (Rohner et al., 2014). This

format overcomes the inherent difficulties and errors associated with 24-h urine collections which determine 24-h iodine excretion, in µg/d. However, UIC in spot samples is affected by state of hydration. If the urine is concentrated (as in dehydration), the UIC will be artefactually increased whereas overhydration will dilute UIC. To overcome this limitation, UIC can also be expressed in relation to creatinine excretion (in µg iodine/g creatinine), a product of muscle metabolism. Given that daily urinary creatinine concentration (UCC) is relatively constant in healthy, well-nourished adults (at ~1 g/day), expressing the UIC from spot samples in this ratio format (UIC/Cr) provides a reasonable representation of a 24-h collection (Rohner et al., 2014). In pregnancy, urinary creatinine excretion represents the amount of creatinine released from both maternal and fetal muscle turnover. In addition to correcting for differences in state of hydration/urine volume and muscle mass (as a marker for body size) between individuals, UCC also adjusts for variations in GFR (Pearce & Caldwell, 2016).

UIC is used to provide an accurate indication of general population iodine status. Typically this is determined by measuring casual/spot UIC in a representative sample of at least 200 school-aged children (defined as children 6-12 y) and comparing results (medians) with established WHO epidemiological criteria (see Table 2.4). The same cut-off criteria also apply to adults, excluding pregnant and lactating women. School-aged children are used for population iodine assessment due to their high physiological vulnerability to iodine deficiency and ease of access for sampling via schools (WHO/UNICEF/ICCIDD, 2007). Despite the suggestion that median UIC in school aged children is not a reasonable indicator of iodine status in the general population (Zimmermann & Andersson, 2012), the following WHO criteria remain in use:

- iodine status is optimal if the median UIC is between 100 µg/L and 200 µg/L
- a population is iodine deficient if the median UIC is <100 µg/L, and
- no more than 20% of the population should have iodine concentrations <50 µg/L (WHO/UNICEF/ICCIDD, 2007).

Caution needs to be taken in interpreting spot UIC measurements for populations as it cannot be assumed that all individuals with a spot UIC <100 µg/L are iodine deficient, due to high day-to-day intra-individual variability for spot UIC values

(Zimmermann, 2012). Consequently, it is estimated that 10 UIC measurements from spot samples or 24-h collections are needed to determine an individual's iodine status with 20% precision (König, Andersson, Hotz, Aeberli, & Zimmermann, 2011; Pearce & Caldwell, 2016).

Table 2.4 Epidemiological criteria for assessment of population iodine nutrition based on urinary iodine concentrations
(WHO/UNICEF/ICCIDD, 2007)

Population group and median UIC (µg/L)	Iodine intake	Iodine status
School-aged children and adults		
<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	More than adequate	Risk of iodine-induced hyperthyroidism in susceptible groups
>300	Excessive[a]	Risk of adverse health consequences (iodine-induced hyperthyroidism, autoimmune thyroid disease)
Pregnant women		
<150	Insufficient	
150-249	Adequate	
250-499	More than adequate	
≥500	Excessive[a]	
Lactating women		
100	Insufficient	
≥100	Adequate	

[a]The term "excessive" means in excess of the amount required to prevent and control iodine deficiency. There may be an increased risk of adverse effects at this level.

2.8.1.1 UIC in pregnancy and lactation

By the third trimester, a woman's UIC is determined by her dietary iodine intake, her GFR and the amount of iodine that is not retained by the feta-placental unit which then transfers across the placenta into the maternal circulation. Prior to 2007, the recommendations for median UIC cut-offs for groups of pregnant women were the same as those for other groups in the population at 100 µg/L. In recognition of the importance of and greater requirement for iodine during this life stage, the median UIC value consistent with 'adequate' iodine intake during pregnancy was raised to 150 µg/L in 2007. Despite lactating women having higher iodine requirements than pregnant women, the median UIC cut-off is lower than that for pregnancy to account for the iodine excreted in breast milk. Furthermore, there is recent evidence to suggest that UIC measured in schoolchildren may not be an accurate indicator of iodine status or iodine deficiency risk among pregnant women in all populations (Pearce & Caldwell, 2016; Rohner et al., 2014; Wong, Sullivan, Perrine, Rogers, & Pena-Rosas, 2011). This is concerning, given pregnant women are the main target group for iodine interventions (Rohner et al., 2014). In addition, results are conflicting regarding the trend changes in UIC with advancing gestation, hence there are currently no trimester-specific reference ranges for UIC during pregnancy (Bath et al., 2015). This issue will be discussed in detail in the second part of this review (see Section 2.10).

2.8.2 Thyroid stimulating hormone (TSH)

Measurement of serum TSH by highly sensitive, third generation, immunometric assays is considered to be the single best test of thyroid function, in both non-pregnant and pregnant subjects (C. Eastman, 2012; Glinoer & Spencer, 2010). As previously described in Section 2.4.2, TSH regulates thyroid hormone synthesis and secretion. TSH secretion is mainly determined by and inversely related to the level of circulating thyroid hormone. Consequently, serum TSH concentrations are increased when thyroid hormone concentrations are low (hypothyroidism) and are decreased when thyroid hormone concentrations are high (hyperthyroidism or thyrotoxicosis).

The advantages and limitations of using TSH as an iodine biomarker are presented in Table 2.6. A key consideration is that TSH is not a sensitive indicator of iodine status in children and adults, as due to tight homeostatic regulation, values can remain within

normal reference ranges even in individuals with inadequate iodine intake (Bath, Pop, Furmidge-Owen, Broeren, & Rayman, 2017; Rohner et al., 2014). Conversely, WHO has recommended measurements of neonatal TSH as a surrogate marker of iodine status in a population. According to the current WHO criteria, sensitive TSH measurements in neonatal blood collected 3-4 days after birth indicate iodine sufficiency in a population when <3% of the samples have TSH concentrations >5 mIU/L (WHO/UNICEF/ICCIDD, 2007). Importantly, measurement at this time reflects iodine status during the critical period of brain development (Zimmermann, 2012). Iodine deficiency during pregnancy may result in fetal hypothyroidism whereby fetal TSH is increased. Congenital hypothyroidism is among the most common treatable causes of intellectual impairment Newborn screening for the detection of congenital hypothyroidism is performed routinely (typically 2-5 days after birth via a heel prick) in Australia, as well as in the United States, Canada, Europe, Israel, Japan and New Zealand (Alexander et al., 2017).

Neonatal TSH concentrations are influenced by a number of factors including age at the time of blood sampling. Due to the introduction of expanded newborn screening, many countries have adopted an earlier sampling timeframe (as early as 48 h after birth), which has been shown to produce contrasting results than if a later blood collection timeframe was used, such as that of the current WHO recommendation. This recent evidence suggests that in a population considered iodine replete or borderline iodine deficient, the current WHO criteria involving the use of newborn TSH concentrations are not adequately robust to define 'iodine sufficiency'. These outcomes suggest a review of current WHO recommendations is required regarding the use of neonatal TSH for monitoring population iodine status, which considers recent changes in technology and clinical practises (Clapin, Lewis, Greed, Dawkins, & O'Leary, 2014). Furthermore, neonatal TSH concentration can also be used to indirectly assess iodine status in pregnant women (Skeaff, 2012) .

2.8.2.1 TSH in pregnancy

Due to the significantly increased demand on the thyroid during pregnancy, careful monitoring of thyroid function assessment via TSH frequently occurs during pregnancy. Typically, TSH levels are lower in pregnant women compared to nonpregnant women/before pregnancy (O'Leary, Boyne, Atkinson, Mileham, &

James, 1992) . The largest reduction is serum TSH is observed in early pregnancy (weeks 7-12) due to increased levels of placental human chorionic gonadotropin (hCG), which in turn stimulates thyroid hormone secretion. Studies have shown that up to 15% of healthy pregnant women have first trimester TSH levels below the nonpregnant lower limit of 0.4 mIU/L, with no clinical significance likely. Serum TSH levels gradually rise in the second and third trimesters but still remain lower than in non-pregnant women (Alexander et al., 2017).

The downward shift in pregnancy TSH values is observed in virtually all populations studied and supports the use of reduced TSH cut-offs for the normal range during pregnancy, relative to the non-pregnant TSH cut-offs. However, the degree of reduction in TSH concentrations during pregnancy varies considerably due to geographic location and ethnic diversity. This in turn makes it difficult to establish trimester-specific cut-offs for TSH applicable to all population groups (Alexander et al., 2017). Initial recommendations (in 2011) from the American Thyroid Association (ATA) for pregnancy TSH cut-offs were based on studies of pregnant women from the United States and Europe, with the first trimester upper limit of 2.5 mIU/L and the second and third trimester upper limit of 3.0 mIU/L used for the diagnosis of subclinical hypothyroidism (Alexander et al., 2017; Skeaff, 2012). However, evidence from more recent studies in pregnant women in areas including Asia, India and the Netherlands suggests only a modest reduction in the non-pregnant upper limit cut-off is required during pregnancy (Alexander et al., 2017). Furthermore, increased serum thyroperoxidase antibody (TPO-Ab) levels, as occurs with autoimmune thyroid disease, are associated with higher TSH values. This highlights the importance of measuring TPO-Ab levels in pregnant women when developing pregnancy reference ranges and when using TSH in the assessment of iodine status (Pearce et al., 2008).

In 2017, the American Thyroid Association recommends defining TSH cut-offs during pregnancy based on representative local population data (non-pregnant adult) where possible, with the first trimester (during weeks 7-12) lower reference limit reduced by ~0.4 mIU/L and the upper reference limit reduced by ~0.5 mIU/L. This should result in a TSH upper limit of 4.0 mIU/L for the typical pregnant woman in early gestation. Thereafter in the second and third trimesters, TSH levels should show a gradual increase towards the non-pregnant range (Alexander et al., 2017).

The current recommendation (set in 2012 and reviewed in 2015) of the Royal Australian and New Zealand College of Obstetricians and Gynaecologists (the College), a peak body concerned with maternal health in these countries, is to use pregnancy specific reference intervals from the individual laboratory if available. In addition, the College has adopted the initial ATA recommendations for use as guidance for appropriate trimester-specific reference intervals for TSH during pregnancy. An example of applying the College's recommendation is presented in Table 2.5, whereby the PathWest Laboratory Medicine WA reference ranges for TSH in pregnancy have been derived in part from local data (collected in 2006 from 1817 Western Australian pregnant women 9-13 weeks' gestation)(Gilbert et al., 2008) and ATA recommendations. The College's recommendations are due for review in 2018 (Royal Australian and New Zealand College of Obstetricians and Gynaecologists (Women's Health Committee), 2012).

Table 2.5 PathWest reference ranges for TSH in pregnancy and non-pregnancy

Trimester	Reference range (mIU/L)
First	0.02-2.5*
Second	0.3-3.0
Third	0.3-3.0**
Non-pregnant	0.4-4.0

*(Gilbert et al., 2008) **(Stagnaro-Green et al., 2011)

2.8.3 Thyroglobulin

As discussed in Section 2.4.2, thyroglobulin (Tg) is a carrier protein in the thyroid within which T3 and T4 are synthesised. Tg is thyroid-specific and small amounts of it may be released into circulation with T3 and T4. However, TSH stimulation in iodine deficiency results in an increased amount of Tg released into the blood due to greater thyroidal cell mass and increased Tg synthesis and breakdown (Rohner et al., 2014). Tg can be measured in serum or dried blood spots and shows increasing potential for use as a functional biomarker of iodine status, reflecting long-term iodine intake (weeks or months) (Zheng Feei Ma & Skeaff, 2014; Zimmermann & Andersson, 2012). Furthermore, serum Tg levels are positively correlated with thyroid volume in areas of iodine deficiency (Zheng Feei Ma & Skeaff, 2014). While there are no WHO-defined cut-offs for Tg in relation to iodine status, the widely accepted international

reference range is 4-40 µg/L. In addition, a median Tg concentration of <13 µg/L and/or <3% of Tg values >40 µg/L have been suggested to indicate adequate iodine status in children, and with caution, in adults (Zimmermann, Aeberli, Andersson, Assey, Yorg, Jooste, Jukic, et al., 2013). Based on these proposed cut-offs, recent studies have shown Tg to be a good biomarker of iodine status in groups of adults and children (Zheng Feei Ma & Skeaff, 2014; Zheng Feei Ma, Venn, Manning, Cameron, & Skeaff, 2016; Zimmermann, Aeberli, Andersson, Assey, Yorg, Jooste, Jukić, et al., 2013). However, further studies are required into the usefulness of Tg as a biomarker of individual iodine status (Zheng F. Ma, Venn, Manning, Cameron, & Skeaff, 2017). (see Table 2.6 for additional advantages and limitations of Tg use.)

2.8.3.1 Tg in pregnancy

In pregnancy, serum Tg concentrations are influenced by hCG and so may be elevated when hCG concentrations are high, typically during the first half of gestation (Glinoer et al., 1990; Pearce & Caldwell, 2016). Therefore, the effect of pregnancy on Tg serum levels should be considered when using Tg as a biomarker for iodine status (Zhang et al., 2017). However, there are currently no normative Tg reference ranges for pregnant women (Pearce & Caldwell, 2016). Despite this, and the fact that investigation into the relationship between iodine status and serum Tg in this population group is limited (Zheng Feei Ma & Skeaff, 2014), some results to date indicate that Tg shows considerable promise as a long-term biomarker of iodine status in pregnant women (Bath et al., 2017; Zheng Feei Ma & Skeaff, 2014; Orgiazzi, 2016). However, other results suggest limited use of Tg for this purpose (Zhang et al., 2017). Therefore, more larger observational studies involving pregnant women with adequate and inadequate iodine status, as well as interventional trials including both Tg and UIC, are required to draw stronger conclusions about the usefulness of Tg as an iodine status biomarker in this population group. A further consideration is the possible need for trimester-specific cut-offs for Tg, as is suggested for some other biomarkers (Zheng Feei Ma & Skeaff, 2014).

2.8.4 Thyroid hormones

Almost all (>99%) of the thyroid hormones, both the inert T4 and the active T3, present in the circulation are tightly bound to proteins, predominantly thyroxine binding

globulin (TBG). However, it is only the small amount of unbound or free hormone that is bioactive and available for tissue uptake. In subclinical (mild) hypothyroidism, serum total and free thyroxine (FT4) remain within the reference range while in the more advanced overt hypothyroidism, serum total and FT4 are low (note that TSH is increased in both cases). Typically, serum total and free T3 (FT3) concentrations do not decline until hypothyroidism is well advanced. This is because the associated high TSH levels stimulate T3 release from the thyroid (Pearce & Caldwell, 2016; Rohner et al., 2014).

2.8.4.1 Thyroid hormones in pregnancy

During pregnancy, thyroid hormone levels are also influenced by both hCG and estrogen. Serum FT4 levels increase during the first trimester when hCG levels are at their highest and typically decrease thereafter as hCG concentrations fall, yet FT4 levels remain elevated compared with the non-pregnant state (O'Leary et al., 1992). Furthermore, high estrogen levels during pregnancy increases serum total T3 and T4 concentrations via an increase in the circulating concentrations of TBG (Pearce & Caldwell, 2016). In turn, this rise in total T4 makes measuring the small fraction of FT4 even more challenging in pregnancy. The reduction in plasma albumin also impacts on the actual level of FT4 and FT3 in the woman's circulation (Alexander et al., 2017). As observed in the non-pregnant state, during pregnancy FT4 is typically within the reference range in subclinical hypothyroidism and decreased in overt hypothyroidism (Royal Australian and New Zealand College of Obstetricians and Gynaecologists (Women's Health Committee), 2012). A further consideration is that elevated TPO-Ab levels in pregnant women are associated with lower T4 values. Therefore, it is important to know the TPO-Ab status of women when developing and applying pregnancy-specific reference ranges for T4 (Pearce et al., 2008), especially if the TSH is increased.

In Australia currently, both FT4 and FT3 are measured in routine thyroid function tests during pregnancy, while total T4 and T3 measurements are no longer routinely used (Royal Australian and New Zealand College of Obstetricians and Gynaecologists (Women's Health Committee), 2012). However, a number of unfavourable factors raise questions over the use of FT4 measurements during pregnancy.

These include:

1. that there is no single international method for standardisation of free thyroid hormone tests (Royal Australian and New Zealand College of Obstetricians and Gynaecologists (Women's Health Committee), 2012),
2. the uncertainty regarding the validity of some of the many different assay methods used (Alexander et al., 2017; C. Eastman, 2012) and,
3. the use of pregnancy reference ranges (or even the use of non-pregnant reference ranges) as provided by assay manufacturers, rather than laboratories establishing their own FT4 reference ranges for pregnancy (Alexander et al., 2017; C. Eastman, 2012).

Therefore, measuring total T4 (and applying a pregnancy-specific reference range) is a more reliable means of estimating hormone concentration during the latter stage of pregnancy. If measuring FT4 during pregnancy, both assay method-specific and trimester-specific pregnancy references ranges should be used (Alexander et al., 2017). As this is often not the case, measurement of FT4 in pregnancy should only occur in addition to serum TSH determination (C. Eastman, 2012).

2.8.5 Goitre

There are two methods used in the clinical assessment of thyroid gland size (volume) to determine the presence of goitre (see Table 2.6 for advantages and limitations of each method).

1. Neck inspection and palpation – this is the traditional method used to evaluate iodine nutrition, dating back to the early 1500s (Zimmermann & Andersson, 2012). This method involves the examiner sitting or standing directly in front of the individual (child or adult) and using both thumbs simultaneously to feel at the base of the neck. With the neck in the normal position, the normal thyroid should not be visible. The goitre is then graded accordingly (WHO & UNICEF., 2007):

   Grade 0 thyroid not palpable nor visible
   Grade 1 thyroid palpable but not visible
   Grade 2 thyroid clearly visible and palpable.

   Furthermore, WHO recommends that a total goitre rate (TGR; number with goitres of grades 1 and 2 divided by total number examined), based on goitre prevalence

in school-aged children, is used to establish the degree of iodine deficiency in populations according to (WHO & UNICEF., 2007):

<5%	Iodine sufficiency
5.0-19.9%	Mild deficiency
20.0-29.9%	Moderate deficiency
>30%	Severe deficiency

2. Thyroid ultrasonography – this is a more precise and objective method of assessing thyroid volume than palpation. It is the preferred method to use in areas of mild to moderate iodine deficiency where the prevalence of visible goitres is low and for monitoring iodine control programmes, where the anticipated trend is for thyroid volumes to reduce over time. Ultrasonography results from a study population should be compared with reference data. This reference data includes values for thyroid volume measured by ultrasonography (using a documented standardized method) in schoolchildren of iodine-sufficient populations as a function of age, sex and body surface area (WHO & UNICEF., 2007). Despite the apparent advantages of this method, it has not been widely adopted as a field tool (Zimmermann & Andersson, 2012).

2.8.5.1 Goitre in pregnancy

Thyroid volume has been shown to increase during pregnancy in both iodine-sufficient and iodine-deficient areas, by 10-15% and ~25%, respectively (Pearce, 2013). However, there are currently no guidelines for monitoring this biomarker during pregnancy, making the routine use of this parameter impractical in this population subgroup (Skeaff, 2012).

2.8.6 Breast milk iodine concentration

There is increasing evidence to suggest that BMIC is a more accurate biomarker of iodine status than UIC in lactating women and that both biomarkers should be measured simultaneously in this subpopulation. As discussed in 2.4.3, this is because of the ability of the mammary glands to concentrate iodine in breast milk, even when iodine intake is low in lactating women from iodine-sufficient populations. Therefore, maternal UIC alone may not reliably reflect iodine status (Dold et al., 2017). Typically, BMIC (as a mean or median) may be as low as <50 µg/L in iodine-deficient areas (Mulrine, Skeaff, Ferguson, Gray, & Valeix, 2010) and as high as 150-180 µg/L in

areas of good iodine supply (Dorea, 2002; Semba & Delange, 2001). There is currently no scientific consensus on the BMIC indicating sufficient maternal iodine intake (Dold et al., 2017) and consequently no reference ranges for this biomarker have been specified. Despite this, values over 75 µg/L (Azizi & Smyth, 2009) or 100 µg/L (Semba & Delange, 2001) are considered indicative of adequate maternal iodine status. More recently, a metabolic balance study involving healthy infants (n=11; mean ± SD age 13 wk ± 3 wk) suggested a BMIC of ≥92 µg/L to meet exclusively breastfed infants' daily iodine requirements (including an allowance for accumulation of thyroidal iodine stores) at age 2-5 months. This was based on an EAR of 72 µg/d and ingestion of 0.78 L of breast milk/d (Dold et al., 2016).

2.8.7 Summary

In summary, there are advantages and limitations associated with each of the clinical and biochemical biomarkers used to assess iodine status. While UIC is the most commonly used method, it is not a functional biomarker of iodine status and only reflects recent iodine intake, which may not represent usual intake in an individual (Bath et al., 2017). Therefore, although UIC is a validated population marker for iodine status, its use in assessing individual status is limited (Pearce & Caldwell, 2016). In addition, while the thyroid hormones (T3 and T4) and TSH are functional measures, in most settings and population groups (including newborns according to Clapin et al., 2014) they are not sensitive indicators of iodine status. This is because values for these measures can remain within the normal reference range, even in the case of severe deficiency, due to the ability of the thyroid to adapt to suboptimal intakes and the tight regulation of homeostasis (Bath et al., 2017). Additionally, the functional biomarker thyroglobulin has been shown to be an accurate indicator of iodine status in some population groups while its usefulness in others is yet to be proven, including pregnant women (Pearce & Caldwell, 2016). For these reasons, a combination of biomarkers should ideally be used to assess population iodine status (Ristić-Medić et al., 2014). Furthermore, the effects of pregnancy on biomarkers should be considered when assessing iodine status in this population group, together with the use and development of pregnancy-specific reference ranges (Pearce & Caldwell, 2016).

Table 2.6 Advantages and limitations of iodine biomarkers
(Rohner et al., 2014)

Biomarker	Advantages	Disadvantages/Considerations
Urinary iodine concentration (UIC)	Relatively easy to collect in most population groups (except neonates and infants). Can be measured in spot urine specimens from a representative sample of the target population and expressed as the median, in µg/L. Widely accepted cut-offs exist for school-aged children, pregnant and lactating women, and infants Variations in hydration status between individuals generally even out in a large number of samples, therefore the median UI in spot samples correlates well with that from 24-h samples.	Needs sufficiently large sample size to even out inter- and intra-individual variations Limited usefulness for individuals (due to very high day-to-day variability in the dietary iodine intakes of individuals which results in same high daily variation in UIC). Thresholds not established for all population groups (eg. neonates). Results of assessment in one subgroup may not necessarily be representative of other groups in the same population. Median UIC does not provide direct information on thyroid function (however a lower value suggests a population is at higher risk of developing thyroid disorders). Important to avoid contamination.
Thyroid stimulating hormone (TSH)	A sensitive indicator of iodine status in the newborn period to detect congenital hypothyroidism (although questioned by Clapin et al., 2014). Neonatal TSH screening is an existing universal practice in most industrialized countries. The results have been used to assess the risk of iodine deficiency in the mothers during the pregnancy.	A relatively insensitive indicator of iodine nutrition in school aged children and adults. Serum TSH may be slightly increased in iodine deficiency, however, the values often remain within the normal range. Mean TSH values for these population groups do not reliably discriminate between iodine-deficient and iodine-sufficient populations.
Thyroglobulin (Tg)	Is well correlated with the severity of iodine deficiency as measured by UIC. Levels fall rapidly with iodine repletion and is a more sensitive indicator or iodine repletion than TSH or T4. A new assay for Tg has been developed for dried blood spots taken by a finger prick, simplifying collection and transport. An international reference range and a reference standard for DBS-thyroglobulin in iodine-sufficient school children (4-40 µg/L) is available.	Uncertainty about the need for concurrent measurement of antithyroglobulin antibodies to avoid potential underestimation of thyroglobulin. Update – Cannot be reliably measured in people with detectable anti-thyroglobulin antibodies, who comprise ~10% of the adult population (more frequent in women than men and more prevalent with increasing age) (Pearce & Caldwell, 2016) Large inter-assay variability and poor reproducibility, even with the use of standardisation.

Biomarker	Advantages	Disadvantages/Considerations
T3/T4	Concentrations are a direct reflection of thyroid function.	Except in areas of severe iodine deficiency, thyroid hormone concentrations are poor indicators of iodine status. Changes in T3/T4 are often within the normal range, and the overlap with iodine-sufficient populations is large enough to make thyroid hormone concentrations an insensitive measure of iodine nutrition.
Goitre	Thyroid ultrasound is non-invasive, quickly done (2-3 min per individual), and feasible even in remote and resource-limited areas using portable equipment. Reference ranges for thyroid volume (Tvol) are available for school-aged children.	Palpation of goitre in areas of mild deficiency has poor sensitivity and specificity; in such areas, measurement of Tvol by ultrasound is preferable. Thyroid ultrasound is expensive, somewhat subjective and requires judgement and experience. Differences in technique can produce large interobserver errors in Tvol.

2.9 Iodine status in Australia

The iodine status of the Australian population first became a public health concern in the early 1900s when endemic goitre was recorded in the Atherton Tablelands in Queensland, scattered regions of New South Wales (NSW) and Victoria and in Tasmania (C. J. Eastman, 1999). Also, goitre rates were on the increase in the Adelaide Hills in South Australia (SA) but reportedly this was not the case in Western Australia (WA) nor the Northern Territory (NT) (Australian Population Health Development Principal Committee, 2007). A number of interventions were introduced from the 1920s – 1960s to address iodine deficiency in Australia, with varying degrees of success:

- 1920 – introduction of iodised table salt to households
- 1947 – Australian government provided funding for iodine tablets for school children and pregnant and lactating women in Australian Capital Territory [ACT] and Tasmania as part of a goitre prevention program
- 1953 – introduction of potassium iodate into bread improvers (food fortification) in the ACT only.
- 1966 – as above, in Tasmania (Australian Population Health Development Principal Committee, 2007).

However, the use of iodine-containing bread improvers was relatively brief, ceasing in Tasmania in 1976 due to unsatisfactorily high rates of iodine-induced hyperthyroidism (IIH), most noticeably in those with chronic iodine deficiency. The ACT followed suit soon after and discontinued fortification of bread with iodine in the 1980s. Interestingly, the unforeseen high rates of IIH were in fact caused by iodine-containing sanitising agents (iodophores) used by the dairy industry. This resulted in residues of these agents unintentionally increasing the iodine content of milk, which was a major source of iodine in the Australian food supply from the 1960s. This advantageous contamination of milk is thought to have provided protection against iodine deficiency during the 1970s and 1980s. However, changes within the dairy industry in the 1990s saw iodine-containing sanitisers largely replaced by more effective chlorine-containing sanitisers, together with improved best practice standards which reduced sanitiser contamination in milk overall. These changes resulted in a significant lowering of the iodine content in milk (Australian Population Health Development Principal Committee, 2007) . Despite this, periodic surveys of iodine status conducted in the early 1990s indicated iodine sufficiency within parts of the Australian population, with MUIC levels above 200 µg/L recorded (C. J. Eastman, 1999; M. Li et al., 2006).

Nevertheless, population iodine levels in Australia declined considerably during the next decade. Numerous studies from Victoria, NSW and Tasmania consistently showed MUIC levels <100 µg/L, indicating iodine deficiency. Some of these studies included pregnant women, with MUIC levels considerably below the 150 µg/L cut-off (M. Li et al., 2006). In addition to the reduction in the use of iodine-containing dairy sanitisers, other factors suggested to contribute to the reduction in iodine levels in Australia were discontinuation of prior food fortification and supplementation initiatives, and decreased consumption of iodised salt (Australian Population Health Development Principal Committee, 2007; M. Li et al., 2006). The latter factor occurred due to a trend towards increased consumption of foods prepared outside the home, which largely contained non-iodised salt (Australian Population Health Development Principal Committee, 2007).

Unfavourable results of studies conducted at the time prompted a national survey to investigate population iodine status in 2003/04. The National Iodine Nutrition Survey

(NINS) measured spot UIC from 1709 children aged 8-10 years from 88 schools across five Australian states, namely NSW, SA, Victoria, Queensland and WA. Results showed that overall, children in mainland Australia were borderline iodine deficient, with a MUIC of 104 µg/L. However, state-by-state results showed a range of iodine status from mild deficient in NSW and Victoria to borderline deficient in SA to sufficient in WA and Qld (see Table 2.7)(M. Li et al., 2006). In addition, there were several limitations with the survey. Firstly, the survey was not nationally representative as it did not include children from Tasmania, ACT and NT (Australian Population Health Development Principal Committee, 2007). However, in Tasmania, an area with a well-established history of low iodine soils and iodine deficiency, a 3-year study of iodine status in school aged children was already underway following the introduction of a state-based voluntary iodine fortification program in 2001 (Seal, Doyle, Burgess, Taylor, & Cameron, 2007). Furthermore, the NINS data were not weighted according to population. Had this been the case, the sample MUIC would have been even lower at 98.0 µg/L, albeit still indicating borderline mild deficiency (Australian Population Health Development Principal Committee, 2007).

Table 2.7 Iodine status from the National Iodine Nutrition Survey
(M. Li et al., 2006)

State	Sample size	Median urinary iodine concentration (µg/L) (interquartile range)	Population iodine status
NSW	427	89.0 (65.0-123.5)	Mildly deficient
Vic	348	73.5 (53.0-104.3)	Mildly deficient
SA	317	101.0 (74.0-130.0)	Borderline deficient
WA	323	142.5 (103.5-214.0)	Sufficient
Qld	294	136.5 (104.3-183.8)	Sufficient
Total	**1709**	**104.0 (71.0-147.0)**	**Borderline deficient**

Results from NINS provided evidence of the re-emergence of mild iodine deficiency in the Australian population and were influential in the decision to implement a national mandatory fortification program by Food Standards Australia New Zealand (FSANZ) in October 2009. This occurred in response to advice from Australian Health Ministers in 2008, who agreed that mandatory fortification is the most efficient and cost-effective strategy to address population iodine deficiency (Australian Population Health

Development Principal Committee, 2007). This also followed the successful implementation of the voluntary iodine fortification program in Tasmania in 2001, which resulted in a modest but significant improvement in population iodine status, as measured by UIC in schoolchildren. However, a noted limitation of the voluntary fortification initiative was the inability to meet the increased iodine requirements of pregnant and breastfeeding women (Burgess et al., 2007; Seal et al., 2007). The primary purpose of the national regulatory initiative was to reduce the prevalence of iodine deficiency in Australia to the greatest extent possible, thereby reducing the risk of physical and mental impairment in children and thyroid disease across all age groups. The specified target groups for the initiative were young children aged 2-3 years, breast-feeding women and women of child-bearing age (16-44 years) (Food Standards Australia New Zealand, 2009).

The national mandatory iodine fortification program (FSANZ Standard 2.1.1) remains current and requires the use of iodised salt in general bread and bread products where salt is usually used, except in organic bread and bread mixes for making bread at home (Food Standards Australia New Zealand, 2009). According to FSANZ (2009, p.3), bread is defined as

> "the product made by baking a yeast-leavened dough prepared from one or more cereal flours or meals and water…including yeast-leavened bread made from all cereals flours, not solely wheat flour eg. rye flour." (Food Standards Australia New Zealand, 2009).

The level of iodisation of salt used for this purpose must be in the range of 25-65 mg iodine/kg salt, with the target level being the mid-point of 45 mg iodine/kg salt. Typically, potassium iodate is used for salt iodisation in Australia. Bread was chosen as the food for fortification because it is consumed regularly by a large proportion of the Australian population, including the target groups, and across all socio-economic subgroups (Food Standards Australia New Zealand, 2009). In terms of outcomes, this strategy is estimated to increase mean iodine intake by 46 µg/d in the general population of women aged 16-44 years, thereby reducing the proportion of women with inadequate iodine intakes from 59% to 9% (Food Standards Australia New Zealand, 2008).

However, there were concerns that bread fortification alone would not increase iodine levels enough to meet the higher iodine requirements of pregnant women in Australia (Food Standards Australia New Zealand, 2009). Consequently, in January 2010 the

National Health and Medical Research Council (NHMRC) introduced recommendations that all pregnant and breastfeeding women, as well as those planning a pregnancy, take a daily supplement containing 150 µg of iodine (National Health and Medical Research Council, 2010). This has led to the development or reformulation of many commercial dietary supplements with sufficient amounts of iodine to meet the recommendation.

The most recent information regarding the iodine status of the Australian population is provided by the 2011-12 National Health Measures Survey (NHMS), which was part of the 2011-2013 Australian Health Survey. The survey began 18 months after the introduction of mandatory fortification and by including the measure of urinary iodine excretion, it provides key evidence in determining the efficacy of the intervention in improving population iodine status. Important outcomes of the study include:

- Australian adults are iodine sufficient according to WHO criteria, with a population MUIC of 124.0 µg/L and 12.8% with a UIC <50 µg/L (Australian Bureau of Statistics, 2013), well below the WHO recommendation of no more than 20% (WHO/UNICEF/ICCIDD, 2007).
- MUIC for adults varied across states and territories, ranging from 108 µg/L in Tasmania to 157.4 µg/L in WA (see Figure 2.5). Tasmania also had the highest percentage of adults with a UIC <50 µg/L, with 14.9%.
- for children aged 8-10 years, MUIC levels had increased significantly in all five states since the 2003-04 NINS and post-mandatory fortification. Iodine levels in WA school children remained the highest of all states at 261.3 µg/L.
- MUIC of 121.0 µg/L for women aged 16-44 years (childbearing age) indicated adequate iodine levels overall. However, a greater proportion of women (18.3%) had iodine levels <50 µg/L compared to the adult population national average (12.8%). In addition, almost two thirds (62.2%) (Australian Bureau of Statistics, 2013) had a UIC less than the WHO recommended level for pregnant and breastfeeding women of 150 µg/L (WHO/UNICEF/ICCIDD, 2007).

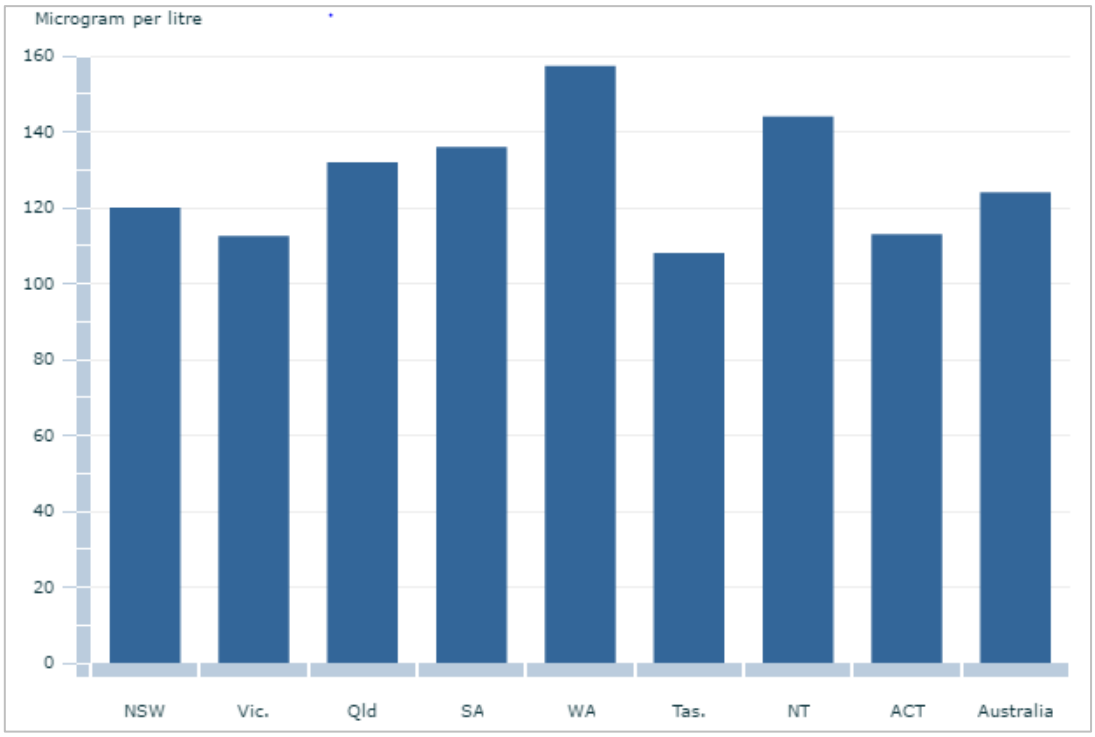

Figure 2.5 Adult Median Urinary Iodine Concentrations by state/territory, NHMS 2011-12 (Australian Bureau of Statistics, 2013)

Overall, results of the NHMS indicate adequate and improving iodine levels in the general Australian population following the mandatory fortification of bread with iodised salt. However, as suspected, the results also indicate that mandatory fortification alone is unlikely to provide the additional iodine required to meet the increased needs of pregnant and breastfeeding women (Australian Bureau of Statistics, 2013). This conclusion is consistent with the findings of other Australian research which used two available food consumption surveys, namely the 1995 National Nutrition Survey and 2003 Australian Longitudinal Study on Women's Health, to estimate the dietary iodine intake of pregnant women post-fortification (Mackerras, Powers, Boorman, Loxton, & Giles, 2011). In addition, a recent FSANZ post-fortification monitoring report detailed an estimated 52% (51 µg/d) improvement in the mean daily iodine intake for females of child-bearing age compared with pre-fortification intake values (Food Standards Australia New Zealand (FSANZ), 2016). While this rise matched the increase projected for this subgroup during the fortification development stage (Food Standards Australia New Zealand, 2008), it still results in inadequate iodine intakes for pregnant and breastfeeding women.

2.9.1 Iodine status of pregnant women in Australia

To date there has been no national survey to determine the iodine intake or iodine status of Australian pregnant and breastfeeding women specifically. Despite this, numerous studies have investigated the iodine status of pregnant women in individual states and territories (namely NSW, Victoria, Tasmania, NT and ACT) over recent decades (see Table 2.8). The outcomes of all 10 studies conducted with this population subgroup prior to mandatory bread fortification and the iodine supplementation recommendation indicated inadequate iodine status, based on median UICs <150 µg/L (Blumenthal et al., 2012; Burgess et al., 2007; Charlton et al., 2010; Gunton et al., 1999; Hamrosi et al., 2005; M. Li et al., 2001; Mackerras, Singh, et al., 2011; Nguyen et al., 2010; Stilwell et al., 2008; Travers et al., 2006).

In addition, a further four studies have been conducted with pregnant women post-fortification of bread and iodine supplement recommendation, with mixed outcomes. Two of these studies, undertaken in Victoria and SA, indicated mild iodine insufficiency with MUICs of 96 µg/L (n=86) and 82 µg/L (n=196), respectively (Clifton et al., 2013; Rahman, Savige, Deacon, Chesters, & Panther, 2011) (See Table 2.8). However, the timing of these studies incorporated the introduction of mandatory fortification and/or iodine supplement recommendation. Consequently, and perhaps not surprisingly, Rahman et al. (2011) reported no significant difference between pre- and post-mandatory fortification MUIC values (n=24, 96.0 µg/L vs n=62, 95.5 µg/L, respectively; p=0.51). Furthermore, the inclusion of samples collected both pre- and post-mandatory fortification and iodine supplement recommendation (and without distinction in timeframe) in the study by Clifton et al. (2013) makes drawing clear conclusions about the impact of these initiatives difficult. While the two remaining and most recent studies showed improved iodine status for pregnant women in NSW and SA, both studies highlighted the importance of iodine supplementation during pregnancy to achieve adequate iodine status, by means of a median UIC >150 µg/L (Charlton et al., 2013; Condo et al., 2016). An important point to note is that no studies have been conducted with pregnant women in WA or Qld, neither pre- nor post-fortification and iodine supplement recommendation. This is a gap in knowledge that the present study aims to address for WA.

Table 2.8 Studies of iodine status of pregnant women in Australia (1980 to present)

Author, Publication date (Date of study)	State or Territory	Sample size (n)	Gestation stage (wk)	MUIC (µg/L)
Gunton et al., 1999 (1998-1999)	NSW	81	Approx 30	104
Li et al., 2001 (1998-1989)	NSW	88	Full-term	88
Hamrosi et al., 2005 (1998-2001)	Vic	802 (from 3 ethnic groups)	14-20	52-61
Stilwell et al., 2008 (1999-2001)	Tas	431 (686 urine samples)	Mean = 19.4 (8.7-40.7)	75
Travers et al., 2006 (2004)	NSW	815	≥28	85
Burgess et al., 2007 (2000-2006)	Tas	802 (pre-and post-voluntary fortification)	≤13	76-86
Mackerras et al., 2011 (2005-2008)	NT	24	Not reported	49
Blumenthal et al., 2012 (2007-2009)	NSW	367	7-11	81
Charlton et al., 2010 (2008)	NSW	110	All; Mean = 33	87.5
Nguyen et al., 2010 (2009)	ACT	100	Not reported	62
*Rahman et al., 2011 (2009-2010)	Vic	24 (pre-fortification) 62 (post-fortification)	≥28	96 95.5
*Clifton et al., 2013 (2009-2010)	SA	196 (longitudinal)	Various (12, 18, 30, 36)	82
**Charlton et al., 2013 (2011-2012)	NSW	106 (2011) 95 (2012)	All All	145.5 166
**Condo et al., 2016 (2011-2012)	SA	781 730 (longitudinal)	<20 28	189 172

MUIC: Median urinary iodine concentration

*conducted during the introduction of mandatory fortification/iodine supplementation recommendation

**conducted post-mandatory fortification/iodine supplementation recommendation

2.10 Longitudinal assessment of iodine status during pregnancy

As discussed in Section 2.4.3, pregnancy brings about a number of physiological changes in maternal thyroid function and iodine metabolism. However, the effect of advancing pregnancy on maternal iodine status remains poorly understood (Bath et al., 2015). Most studies examining the changes in maternal iodine status during pregnancy to date are cross-sectional in design, whereby measures of iodine status occur in different individuals at each assessment stage. In contrast, a considerable strength of longitudinal studies is that measures occur in the same individuals at each assessment stage (Bath et al., 2015). In this way, individuals serve as their own controls, thereby reducing the likelihood of confounding variables influencing outcomes.

Table 2.9 below presents details of 20 international longitudinal studies conducted during the past two decades investigating changes in maternal iodine status throughout pregnancy. Specifically, only studies which provide results of serial measurements (minimum of two) of UIC from the same women during pregnancy have been considered. As discussed in Section 2.8.1, UIC is the most reliable and universally used indicator of population iodine status (Pearce & Caldwell, 2016; Skeaff, 2012; WHO/UNICEF/ICCIDD, 2007) and the measure of most interest in the present study. The timing and number of iodine status assessments varied between studies, with most studies assessing status 2-3 times across the different trimesters. Furthermore, the studies have occurred in areas with different population iodine statuses, using a range of clinical and biochemical biomarkers for assessment. Outcomes of studies based on these different measures are discussed below.

2.10.1 Assessment of iodine status

2.10.1.1 Urinary iodine concentration

As Table 2.9 indicates, all but one study has assessed UIC via spot samples, as opposed to 24-hour collections only (a further two studies used a combination of spot samples and 24-hour collections). Presumably this was for practical reasons as previously discussed in Section 2.8.1. Similarly, most authors (from 16 out of 20 studies) have reported outcomes based solely on measures of UIC, rather than UIC and urinary

iodine-to-creatinine ratio (UIC/Cr). However, results of these studies based on UIC are conflicting, showing both a significant increase (Aguayo et al., 2013; Bath et al., 2017; Clifton et al., 2013; Kung, Lao, Chau, Tam, & Low, 2000) and decrease (Ainy, Ordookhani, Hedayati, & Azizi, 2007; Amouzegar, Khazan, Hedayati, & Azizi, 2014; Brander et al., 2003; Condo et al., 2016; De Zoysa, Hettiarachchi, & Liyanage, 2016; Fuse et al., 2013; C. Li et al., 2016; Mehdi, Hoque, Zinnat, Shirin, & Khan, 2009; Menon et al., 2011; Smyth et al., 2005; Zhang et al., 2017) with advancing gestation. In addition, another six studies showed no significant change in UIC across gestation (Alvarez-Pedrerol et al., 2009; Elnagar, Eltom, Wide, Gebre-Medhin, & Karlsson, 1998; Fuse et al., 2011; Lean et al., 2014; Luton et al., 2011; Vila et al., 2008).

Inconsistencies in UIC results continue to exist when the iodine status of the general population is considered. For example, those studies which found an increasing trend over gestation were from both iodine-deficient and iodine-sufficient areas, namely the UK (Bath et al., 2015), and Australia (South Australia)(Clifton et al., 2013) and China (Hong Kong)(Kung et al., 2000), respectively. Furthermore, while the majority of studies that showed a significant decrease in UIC with advancing gestation were conducted in areas with adequate population iodine status (Ainy et al., 2007; Amouzegar et al., 2014; Brander et al., 2003; Condo et al., 2016; De Zoysa et al., 2016; Fuse et al., 2013; C. Li et al., 2016; Smyth et al., 2005; Zhang et al., 2017), one involved areas where population iodine status was deficient (Menon et al., 2011). Likewise, studies that showed no significant change in UIC across gestation have occurred in both iodine-sufficient (Elnagar et al., 1998; Fuse et al., 2013) and iodine-deficient regions (Elnagar et al., 1998; Lean et al., 2014; Luton et al., 2011; Vila et al., 2008). Unfortunately, population iodine status was not reported for three studies and no comparison groups (non-pregnant women or school-aged children) were included. Two of these studies were conducted in Spain, with one showing an increase in UIC with advancing gestation (Aguayo et al., 2013) and the other no change (Alvarez-Pedrerol et al., 2009). The remaining study of unknown population iodine status was conducted in Bangladesh and showed a decreasing trend with advancing gestation (Mehdi et al., 2009).

Just five studies have assessed changes in iodine status during pregnancy via UIC/Cr in addition to UIC (see Table 2.9). Interestingly, two of these showed the same

directional change for both measures. This was an increasing trend in UIC and UIC/Cr in UK (Bath et al., 2015), considered a mild-moderate deficiency area, and a decreasing trend in UIC and UIC/Cr in a borderline sufficient area, namely Switzerland (Brander et al., 2003). As discussed in Section 2.8, UIC values cannot be used to estimate individual iodine status due to large day-to-day variability (König et al., 2011; Rasmussen, Ovesen, & Christiansen, 1999). However, UIC/Cr is considered a better measure to use, particularly when age and sex of the individual is taken into consideration and cohorts of selected groups are investigated (Knudsen, Christiansen, Brandt-Christensen, Nygaard, & Perrild, 2000). Furthermore, UCC is used to correct UIC for variations in urine volume and therefore dilution of spot urine samples from well-nourished subjects (Bourdoux, 1988). In addition, as the renal clearance of iodine and creatinine are altered to the same degree in pregnancy (Brander et al., 2003), UIC/Cr ratio represents a valid indicator of UIC in longitudinal studies during gestation (Brander et al., 2003; C. Li et al., 2016).

However, when taking population iodine status into account, studies measuring urinary iodine excretion via UIC/Cr have also shown inconsistencies in the changes in iodine status during gestation. Specifically, three studies that have shown an increasing trend in UIC/Cr were from areas of both mild-moderate iodine deficiency (Bath et al., 2015) and iodine sufficiency (Fuse et al., 2011; C. Li et al., 2016). Additionally, the two remaining studies were conducted in iodine-sufficient regions and showed both a decrease (Brander et al., 2003) and no change (Fuse et al., 2013) in UIC/Cr with advancing gestation. Furthermore, variable trends in gestational changes of iodine status are seen when taking into account the overall iodine status of women in the study cohorts, based on median UIC/Cr. For example, increasing trends in UIC/Cr were shown in studies involving women with mild-moderate iodine deficiency (Bath et al., 2015), sufficient iodine status (Fuse et al., 2011) and where women were initially iodine sufficient and then developed mild deficiency as pregnancy progressed (C. Li et al., 2016). Similar inconsistencies are shown in studies with a decreasing trend (Brander et al., 2003). These results tend to suggest that the iodine statuses of both the general population and study cohort are not major factors influencing changes in iodine status during gestation. However, further research needs to be conducted to determine if this is in fact the case.

The cited reasons for the observed trends in iodine status with advancing gestation are both physiologic and behavioural. For example, possible explanations for a decreasing trend include:

1. increased maternal thyroid hormone production (Zhang et al., 2017),
2. the transfer of part of the available iodine pool from maternal circulation to the fetus (Brander et al., 2003; Fuse et al., 2011; Zhang et al., 2017),
3. increased glomerular filtration rate and increased renal clearance of iodine (Brander et al., 2003; Zhang et al., 2017) resulting in increased thyroidal iodide uptake to compensate for the lower levels of circulating iodide (Brander et al., 2003),
4. increased GFR possibly leading to increased urine volume which results in urine dilution during pregnancy (C. Li et al., 2016), and
5. inadequate initial thyroidal stores become further depleted over time with insufficient intake (Ainy et al., 2007).

In contrast, an increasing trend in iodine status across gestation may reportedly be explained by:

1. the increased use of iodine-containing supplements as gestation progresses (Aguayo et al., 2013; Clifton et al., 2013), and
2. the increased dietary iodine intake from food, especially from dairy products (Bath et al., 2015).

While the above suggestions appear reasonable explanations for the trend changes in maternal iodine status observed throughout gestation, many of the physiologic changes that occur are not well understood. Specifically, these include changes in renal iodine loss, placental storage of iodine and transfer of iodine to the fetus (Bath et al., 2015). This in turn may help explain the lack of consistency in these trend changes across studies. Furthermore, differences in methodologies is a likely explanation for the observed findings (Fuse et al., 2011). Firstly, sample sizes among the studies vary considerably, specifically from 15-1322 participants for studies measuring UIC and from 15-701 participants in studies also measuring iodine-to-creatinine ratio. As suggested by the WHO, the minimum sample size required to assess iodine status of populations is 300 (WHO/UNICEF/ICCIDD, 2007). Only three studies overall, including just one measuring UIC/Cr, had sample sizes larger than 300 participants.

Interestingly, outcomes of these larger studies were inconsistent, with increasing (Aguayo et al., 2013), decreasing (De Zoysa et al., 2016) and no change (Fuse et al., 2011) trends in UIC reported by authors. Whether this WHO sample size recommendation also applies to longitudinal studies is not stated in the literature.

Secondly, the degree to which studies report truly longitudinal results is highly variable and a likely contributing factor for the inconsistencies observed. Specifically, some studies report results based only on complete data sets for participants, that is, the same number of women provide urine samples at each time point so there is no apparent attrition (Ainy et al., 2007; Brander et al., 2003; Elnagar et al., 1998; Fuse et al., 2013; Mehdi et al., 2009; Menon et al., 2011; Smyth et al., 2005). In theory, this should improve the reliability of outcomes by reducing potential bias that could be caused by uneven numbers of data points in each group. However, only two studies with complete data sets report results as UIC/Cr. One such small study (n=15) showed a decreasing trend with advancing gestation (Brander et al., 2003) while a second study (n=65) showed no significant change in UIC/Cr across gestation (Fuse et al., 2013). The remaining studies describe trend results based on incomplete data sets whereby not all participants provided a urine sample at each time point.

In addition, other study design factors likely to contribute to the inconsistencies in results obtained include variability in the number and gestational timing of urine samples collected and the inclusion of multiparous women in some studies. These factors make it difficult to directly compare results, even in areas of similar iodine status. Furthermore, such conflicting results regarding the trend changes in UIC and UIC/Cr with advancing gestation also impede the development of trimester-specific reference values for these measures of iodine status.

2.10.1.2 Thyroid function

In addition to assessing UIC, all except six studies investigating the gestational changes in iodine status in pregnancy also included measures of thyroid function (see Table 2.9). Of those studies, all included maternal serum TSH and FT4. However, their trends across gestation and correlations with urinary measures were not always reported. In those studies that did report trend changes, TSH consistently increased significantly over the course of gestation, irrespective of the iodine status of the women

as judged by median UIC (Aguayo et al., 2013; Bath et al., 2015; De Zoysa et al., 2016; Elnagar et al., 1998; Fuse et al., 2013; Kung et al., 2000; Luton et al., 2011; Mehdi et al., 2009; Menon et al., 2011). In one study, TSH concentration doubled towards term compared with early gestation (Kung et al., 2000). In a second study, TSH increase was greater in the group of iodine-deficient women than iodine-sufficient women. According to the authors, this may indicate thyroid hyperstimulation due to iodine deficiency (Bath et al., 2017). Overall, the increase in TSH during gestation was expected, following first trimester suppression due to hCG (Alexander et al., 2017; Bath et al., 2017), as discussed in Section 2.8.2.1. Despite the increase in TSH across gestation in one study where iodine status was progressively worsening (median UIC was 170.9 µg/L, 123.80 µg/L and 105.95 µg/L in the first, second and third trimesters, respectively), TSH was maintained within the reference range (De Zoysa 2016). This highlights the importance of not using TSH as the sole indicator of iodine status during pregnancy, given the major role of TSH is to drive production of thyroid hormones.

Furthermore, in most studies, FT4 decreased during gestation as anticipated (Aguayo et al., 2013; Bath et al., 2017; De Zoysa et al., 2016; Elnagar et al., 1998; Fuse et al., 2013; Kung et al., 2000; Luton et al., 2011; Mehdi et al., 2009; Vila et al., 2008). However, FT4 increased in one study by 4% ($p=0.034$) for unknown reasons (Menon et al., 2011). Furthermore, just four studies investigated trend changes in FT3, with three finding a significant decrease over gestation as expected (Kung et al., 2000; Luton et al., 2011; Mehdi et al., 2009). In contrast, another study found an increasing trend for FT3, however, only trimesters 1 and 2 were included (Aguayo et al., 2013). Maternal levels of FT4 and FT3 are influenced by the changes in TBG and albumin that occur during gestation. No studies reported trend changes in total T3 or total T4.

The correlation between urinary iodine excretion and thyroid function parameters was also investigated in numerous studies. Interestingly, all studies bar one noted no significant correlation between UIC or UIC/Cr and TSH, FT3 and/or FT4 (Aguayo et al., 2013; Alvarez-Pedrerol et al., 2009; Amouzegar et al., 2014; De Zoysa et al., 2016; Fuse et al., 2011; Luton et al., 2011). The one exception was conducted in an iodine-sufficient area and reported a significant correlation of UIC with thyroid function parameters. Specifically, FT4 values were negatively correlated while TSH values

were positively correlated (Fuse et al., 2013). Despite TSH, FT3 and FT4 being functional biomarkers of iodine status, overall these results highlight the fact that these measures are not sensitive indicators of iodine status (Bath et al., 2017).

Interestingly, only four studies included measures of the functional biomarker Tg in study participants. Possible reasons for the lack of use of Tg in other studies include the invasive nature of Tg measurement (compared with urinary iodine measures) and the additional expense. The recently developed lower-cost method of measuring Tg in dried blood spots may result in increased use of this biomarker in future longitudinal studies involving pregnant women (Bath et al., 2017). Three of the included studies investigated repeated measures of Tg and the trend results across gestation were variable. Specifically, an earlier study in a borderline iodine sufficient area showed no significant change in serum Tg with advancing gestation (Kung et al., 2000). More recently in an iodine-sufficient area of China, Tg levels remained stable from 8 weeks until 36 weeks of gestation, when levels increased by ~30% (C. Li et al., 2016; Zhang et al., 2017). The authors report that this increase may reflect increased thyroid volume and the greater requirement for iodine in the late stages of pregnancy (Zhang et al., 2017).

However, a recent UK study showed that the change in Tg concentration throughout gestation differed with maternal iodine status. In this study, Tg concentration increased significantly with advancing pregnancy in the iodine-deficient group (defined by UIC/Cr <150 μg/g) but there was no significant change in the iodine-sufficient group (≥150 μg/g). The authors suggest that the increase in Tg in the iodine-deficient group is likely due to increased thyroid volume, initially caused by hCG and later as a result of thyroidal adaptation to low dietary iodine intake. The authors also concluded that Tg is a more sensitive indicator of iodine status in pregnancy than TSH, which increased in both iodine-deficient and iodine-sufficient groups, albeit the increase was greater in the former group. In terms of correlation, this study also showed a negative association between iodine status (as measured by UIC/Cr) and serum Tg levels (Bath et al., 2017). This is in contrast to other studies in which whereby maternal Tg was not correlated with thyroid function, UIC or iodine-to-creatinine ratio (De Zoysa et al., 2016; Zhang et al., 2017). Despite Tg showing some promise as a long-term functional indicator of iodine status in pregnant women (Bath et al., 2017), the inconsistent results of the few studies conducted to date suggest further research is required to draw

definitive conclusions about its change throughout gestation and use in assessing iodine status of pregnant women.

2.10.1.3 Thyroid volume

In addition to urinary and serum parameters, thyroid volume (Tvol), as measured by ultrasound, was included in three of the listed studies (see Table 2.9). In all studies, the change in thyroid volume correlated negatively with UIC (De Zoysa et al., 2016; Kung et al., 2000; Vila et al., 2008), as expected. In one study, the mean increase in Tvol over baseline in women with adequate iodine status (n=8) was 17.7%, compared with a mean Tvol increase of 42.5% in women with inadequate iodine status (n=22)(Vila et al., 2008). While consistent results have been obtained in studies for this iodine status biomarker, a limitation of its routine use is that there are currently no guidelines for monitoring in this population subgroup (Skeaff, 2012).

2.10.2 Summary

The changes in maternal iodine status with advancing pregnancy have been investigated in longitudinal studies in both iodine-sufficient (eg. Sri Lanka, Iran, Japan, Hong Kong, Switzerland, Australia and China) and iodine-deficient (eg. India, UK and Spain) regions via a number of assessment measures. Studies involving the most widely used measure, UIC, have produced inconsistent trend results with maternal iodine status shown to increase, decrease and remain unchanged throughout gestation. Similar trend inconsistencies have been shown to exist with UIC/Cr, the considered better urinary measure of iodine status (Knudsen et al., 2000), though fewer studies have included this measure. These trend discrepancies in UIC and UIC/Cr have been observed regardless of the iodine statuses of the general populations and the study cohorts. Suggested reasons to explain the observed trends are 1) physiologic changes associated with pregnancy, of which not all are fully understood (decreasing trend)(Ainy et al., 2007; Brander et al., 2003; Fuse et al., 2011; C. Li et al., 2016; Zhang et al., 2017), and 2) maternal dietary behaviour change (increasing trend)(Aguayo et al., 2013; Bath et al., 2015; Clifton et al., 2013). However, study design/methodology factors are also likely to have influenced the trend results observed, including sample size, the number and timing of urine sample collections, the inclusion of multiparous women and the degree to which truly longitudinal results are reported. These factors make it difficult to directly compare outcomes and draw

definitive conclusions regarding gestational changes in iodine status. Furthermore, such conflicting results regarding the trend changes in UIC and UIC/Cr with advancing gestation also impede the development of trimester-specific reference values for these measures of iodine status.

The most frequently used measures of thyroid function in these studies generally showed consistent and expected trends across gestation (TSH increased and FT4 decreased). However, despite being functional biomarkers of iodine status, their lack of correlation with UIC and/or UIC/Cr supports the fact that these measures are not sensitive indicators of iodine status (Bath et al., 2017). Furthermore, while the functional biomarker Tg is reported to be a more sensitive biomarker of iodine status in pregnancy than is TSH (Bath et al., 2017), inconsistencies in trend results obtained to date suggest further research is required to determine its suitability for use in the assessment of iodine status in pregnant women. Despite its limited use, thyroid volume consistently correlated (negatively) with UIC in these longitudinal studies, as expected. However, its more routine use is hindered by the absence of monitoring guidelines specific for pregnant women (Skeaff, 2012).

Table 2.9 Longitudinal studies related to iodine status during pregnancy and post-partum (1998 to July 2017)

Author and Publication date	Country	Population / Study cohort iodine status	Sample size (n)	Timing of status assessment	UIC	UIC/Cr	TSH	FT3	TT3	FT4	TT4	Tg	Tvol
1. Elnagar et al., 1998	Sudan Sweden	Both mild-moderate deficiency Sufficient/ mild deficiency	25 32	Gest: 10-12, 20-24 & 36-39 wk Gest: 11-13, 24, 32 & 38 wk	24 h ↔ ↔		✓ ↔ ↑		✓ ↔ ↔	✓ ↔ ↓			
2. Kung et al., 2000	China (Hong Kong)	Borderline sufficient / Mild-moderate deficiency	230	Gest: 12-14, 20-24 & 36 wk PP: 6 wk & 3 mo	Spot ↑		✓ ↑	✓ ↓		✓ ↓	✓ ↓	✓ ↔	✓
3. Brander et al., 2003	Switzerland	Borderline sufficient / Sufficient	15	Gest: Trim 1, 2 & 3	Spot ↓	✓ ↓							
4. Smyth et al., 2005	Sri Lanka	Sufficient / Trim 1 sufficient; Trim 2 & 3 mild-moderate deficiency	19	Gest: Trim 1, 2 & 3	Spot ↓								
5. Ainy et al., 2007	Iran	Sufficient / Trim 1 sufficient; Trim 2 & 3 mild deficiency	298	Gest: Trim 1, 2 & 3	Spot ↓								
6. Vila et al., 2008	Spain	Deficient / Deficient	30	Gest: Trim 1 & 3	Spot ↔		✓ ↑			✓ ↓			✓

Author and Publication date	Country	Population / Study cohort iodine status	Sample size (n)	Timing of status assessment	UIC	UIC/Cr	TSH	FT3	TT3	FT4	TT4	Tg	Tvol
7. Mehdi et al., 2009	Bangladesh	Not reported / Mild deficiency	60	Gest: Trim 1, 2 & 3	Spot ↓		✓ ↑	✓ ↓		✓ ↓			
8. Alvarez-Pedrerol et al., 2009	Spain	Not reported / Deficient	239	Gest: Urine Trim 1 & 3; Serum Trim 1	Spot ↔		✓			✓			
9. Menon et al., 2011	India	Deficient / Deficient	183	Gest: 13-22 & 33-37 wk	Spot ↓		✓ ↑			✓ ↑			
10. Fuse et al., 2011	Japan	Sufficient / Sufficient	Gest: 701 PP: 533	Gest: Trim 1, 2 & 3 PP: 34 d	Spot ↔	✓ ↑	✓			✓			
11. Luton et al., 2011	France	Moderate deficiency / Deficiency	108	Gest: 12, 22, 32 wk & birth	Spot or 24 h ↔		✓ ↑	✓ ↓		✓ ↓			
12. Clifton et al., 2013	Australia (South Australia)	Sufficient / Mild deficiency	196	Gest: 12, 18, 30 & 36 wk PP: 6 mo	Spot ↑								
13. Aguayo et al, 2013	Spain	Not reported / Trim 1 deficient; Trim 2 sufficient	1322	Gest: Trim 1 & 2	Spot ↑		✓ ↑	✓ ↑		✓ ↓			

Author and Publication date	Country	Population / Study cohort iodine status	Sample size (n)	Timing of status assessment	UIC	UIC/Cr	TSH	FT3	TT3	FT4	TT4	Tg	Tvol
14. Fuse et al., 2013	Japan	Sufficient / Trim 1 Sufficient; Trim 2 & 3 borderline deficient	65	Gest: Trim 1, 2 & 3	Spot ↓	✓ ↔	✓ ↑			✓ ↓			
15. Lean et al., 2013	India	Deficient / Sufficient	166	Gest: 17 & 34 wk	Spot ↔								
16.Amouzegar et al., 2014	Iran	Sufficient / Sufficient	203	Gest: Trim 1, 2 & 3	Spot ↓		✓		✓	✓	✓		
17. Bath et al., 2015 (& 2017)	United Kingdom	Mild-moderate deficiency/ Mild-moderate deficiency	230	Gest: ~12, 20 & 35 wk	Spot ↑	✓ ↑	✓ ↑			✓ ↓		✓ ↑ & ↔	
18. De Zoysa et al., 2016	Sri Lanka	Sufficient / Trim 1 sufficient; Trim 2 & 3 mild deficiency	425	Gest: Urine Trim 1, 2 & 3; Serum Trim 1 & 3 (Tg Trim 1)	Spot ↓		✓ ↑			✓ ↓		✓	✓
19. Condo et al., 2016	Australia (South Australia)	Sufficient/ Sufficient	738	Gest: <20 and 28 wks	Spot ↓								

Author and Publication date	Country	Population / Study cohort iodine status	Sample size (n)	Timing of status assessment	UIC	UIC/Cr	TSH	FT3	TT3	FT4	TT4	Tg	Tvol
20. Li et al., 2016 (and Zhang et al., 2017)	China	Sufficient / 8-16 wk sufficient; 20-36 wk mild deficiency	143	Gest: Urine 8, 12, 16, 20, 28 &36 wk; Serum 8, 20 & 36 wk PP: Urine 3 & 6 mo; Serum 6 mo	Spot or 24 h ↓	✓ ↑	✓	✓	✓	✓	✓	✓ ↑	

UIC = Urinary iodine concentration (µg/L) UIC/Cr = Iodine-to-creatinine ratio (µg/g) TSH = Thyroid stimulating hormone (mIU/L)
FT3 = Free T3 (pmol/L) TT3 = Total T3 (nmol/L) FT4 = Free T4 (pmol/L) TT4 = Total T4 (nmol/L)
Tg = Thyroglobulin (µg/L) Tvol = Thyroid volume (%) Gest = Gestation Trim = Trimester
PP = Post-partum ✓ = iodine status assessment method used ↑ = significant increase with advancing gestation
↓ = significant decrease with advancing gestation
↔ = no significant change with advancing gestation

Chapter 3 Methods

3.1 Overview of study aims

The primary aims of this study were to investigate gestational changes in iodine status (as measured by urinary iodine excretion); to determine the overall iodine health (based on urinary iodine excretion, thyroid function tests of TSH and free T4, and BMIC); to investigate the use and effect on iodine status of iodine-containing supplements; and to determine the applicability of the national iodine-supplement recommendation in a cohort of pregnant and post-partum women in Perth. For convenience, the study was referred to as the 'Perth Iodine & Pregnancy Study II (PIPS II).

3.2 Study design

The present study's aims were addressed using an observational, longitudinal study design where the same participants were followed throughout pregnancy and into the early post-partum period. Participants provided urine, blood and breast milk samples, together with dietary intake information (including iodine-containing supplement and iodised salt use).

The study was approved by the Curtin University Human Research Ethics Committee (Approval number HR47/2013) and the Western Australian Health Human Research Ethics Committee (Approval Number 2014075EW).

3.3 Subjects

3.3.1 Recruitment of participants

Participant recruitment occurred over an 18-month period from July 2013 – Dec 2014 via a number of methods. Initially, pregnant women attending two private ultrasound practices in Perth for their routine first trimester fetal anomaly screening were invited to volunteer to participate in the study by means of a flyer handed out by practice staff or flyers located in the waiting room (Appendix A). Interested participants provided their contact details to practice staff for passing onto the study coordinator (Anita Jorgensen – AJ) or contacted AJ directly themselves. Several weeks after recruiting

commenced, it became clear that recruiting women as early as 10-12 weeks gestation as planned was difficult and the decision was made (by AJ and supervisors) to include women of 10-14 weeks gestation, resulting in some improved recruitment success.

However, several months later, staff at the practices were no longer willing to assist and recruiting by this method ceased. In-person recruiting by AJ was then trialled one day a week for a month at a pathology centre (targeting women attending for I-Gene genetic testing), however, this method proved time inefficient. After disappointing results from a number of other strategies over the next 8 months (see Table 3.1), the decision was made to further expand the study entry timeframe to 18-22 weeks gestation to enable in-person recruitment (by AJ) to occur at King Edward Memorial Hospital (KEMH), the state's largest public maternity hospital. The KEMH antenatal clinics sampled were conducted weekly and were led by obstetricians and clinic midwives (East Wing Clinic) and midwifery teams (Ruby and Jade clinics). These clinics involved women considered low risk in relation to pregnancy complications. Recruiting could not occur any sooner for this study at these clinics due to a number of other studies undertaking participant recruitment there at the time.

Table 3.1 Participant recruitment activities

Recruitment activity	Dates
Advertising in private women's ultrasound practices (2)	Jul - Oct 2013
Curtin website advertising	Aug 2013
Letters/emails to private gynaecology/obstetric practices (18)	Nov 2013
Community newspaper article (*Canning Times*)	Mar 2014
Pathology centre, in-person (Western Diagnostic)	Mar 2014
Radio advertising (Curtin FM)	Apr 2014
Parent group/association advertising (Playgroup WA, Kids in Perth)	Jun – Jul 2014
Advertising in private women's ultrasound practice (1)	Jun – Dec 2014
King Edward Memorial Hospital clinics, in-person	Sep – Dec 2014

Women recruited by AJ in-person were first identified as being eligible for participating (based on gestational stage/estimated due date) by staff (pathology centre) or by AJ via patient lists (KEMH clinics). These women were approached by AJ and given a verbal explanation of the research. Women who expressed interest in participating were provided with written information about the study (see Appendix

B) and given time to read the information and ask questions prior to providing written informed consent to participate if they desired (see Appendix B). Interested women who became aware of the study via indirect means (eg flyers, advertising) contacted AJ via the study email address or phone. Study information and a consent form were then mailed (together with a reply-paid envelope for return of the completed consent form) or emailed to these women. Efforts were made to ensure that all interested women were aware that participation in the study was voluntary and withdrawal was possible at any time without consequence. An important point to note is that researchers were aware from the outset that given the study design/requirements (see Section 3.4), the study cohort would be unlikely to be representative of the wider population of pregnant women in Perth.

3.3.2 Inclusion criteria

In addition to currently being either 10-14 or 18-22 wk gestation, to be eligible to participate in the study women needed to be aged 18 years or over, not have medically diagnosed thyroid disease/problems, not be taking thyroid medication, not be currently breastfeeding and be English speaking.

3.3.3 Sample size

It was determined that a sample size of 60 women in each group (women taking iodine supplements vs no supplements) would be sufficient to detect a difference in mean urinary iodine excretion of 50 µg/L, across four time points and assuming intra-individual standard deviation of 100 µg/L, with 80% power at a 5% significance level. Therefore, the aim was to recruit 200 participants, anticipating an attrition rate of at least 25% given the study's longitudinal nature.

3.4 Data collection

Data collection occurred at four discrete stages during the study as indicated in Table 3.2. Stage 1 comprised of two groups of women – those who were recruited at 10-14 wk gestation (termed 'Early enrolment') and those recruited at 18-22 wk gestation (termed 'Late enrolment'). Gestational dates were confirmed by first trimester ultrasound details provided by participants. For each stage women were mailed the required instructions and information to complete the stage. Four biomarkers of iodine

status were assessed in the study according to the schedule in Table 3.2. Sources of dietary iodine were also investigated at each study stage. Participants received a voucher valued at $15 (for Myer or the movies) following completion of Stages 2 and 4 as a thank you for their time and willingness to participate.

Table 3.2 Iodine data collection by stage

Study Stage	Urinary iodine	TSH	FT4	Dietary sources	Breast milk iodine
1 10-14 wk or 18-22 wk gestation	✓	✓*	✓*	✓	
2 26-28 wk gestation	✓	✓*	✓*	✓	
3 36-38 wk gestation	✓			✓	
4 4-6 wk post-partum	✓	✓	✓	✓	✓

*either during Stage 1 or Stage 2
TSH – Thyroid stimulating hormone; FT4 – free thyroxine

3.4.1 Urinary iodine, TSH and FT4

Women provided unfasted, spot urine samples in all four study stages during visits to PathWest Pathology collection centres at locations throughout Perth. During these visits in Stage 1 or 2 and Stage 4, women also provided a blood sample for assessment of TSH and FT4. Women were mailed study-specific sample request forms as required (see Appendix C) and handed these to pathology staff at the time of sample provision. This ensured participants were not charged for the testing and that sample results were forwarded to study personnel (AJ).

3.4.2 Dietary iodine

At each stage women completed a paper-based questionnaire and returned it via reply-paid envelope. The initial questionnaire contained 68 items, including a 41-item iodine-specific food frequency questionnaire which included foods considered to have relatively high amounts of iodine per 100 g (based on the New Zealand [NZ] food composition database) or foods previously identified as good iodine sources in the NZ diet. This questionnaire had been developed and used previously in NSW (Charlton et

al., 2013) and NZ (Edmonds, 2013) and was adapted from a FFQ validated in the elderly (Tan, Charlton, Tan, Ma, & Batterham, 2013). However, the FFQ was removed from the present study after two months of use following feedback from women who showed initial interest in participating in the study but did not sign up. Eight of the ten women who were contacted by AJ commented that length of the questionnaire was a significant reason for their non-participation.

Consequently, the FFQ was replaced with a single item relating to current daily consumption of any quantity of six iodine-containing foods, namely cow's milk, cheese, ice cream, yoghurt, bread/bread products and eggs, with tick box (yes) responses required. These foods had been identified as important sources of iodine in a recent study of pregnant Australian women (Charlton et al., 2013). The revised 18-item questionnaire also asked about daily use of an iodine-containing supplement (with common brands listed for easy selection) and use of iodised salt (tick box 'yes' or 'no') (see Table 3.3 for changes to original questionnaire version). Similar to the version used by Edmonds (2013), the Stage 1 questionnaire also confirmed study eligibility with items relating to current breastfeeding status, health status regarding thyroid disease/problems, and current use of thyroid medications. Also consistent with the Edmonds (2013) version, the Stage 1 questionnaire included items about current stage of pregnancy, obstetric history, age, ethnicity, education and income (see Table 3.3 for modifications to some items). In addition, items asking participants to provide their name and contact details (for follow-up purposes) were included in the Stage 1 questionnaire. For women who had already completed the longer version of the Stage 1 questionnaire, information was transferred to the short version by AJ and participants were contacted to clarify details if required.

Subsequent questionnaires for Stage 2-4 continued to ask participants about their current stage of pregnancy (or no. weeks pregnant at birth and baby's birth date for Stage 4), daily use of iodine-containing supplements, daily consumption of the six key iodine foods, use of iodised salt, medication use (including use of any medications for thyroid disease in the past two months for Stages 2 and 3) and for updates to contact details if required. The 12-item questionnaires for Stages 2 and 3 also asked participants if they had recently had a glucose tolerance test and if so, to provide details of any recommended treatment as a result of the test. The Stage 4 questionnaire (7 items) also asked participants if they smoked cigarettes (see Appendix D).

Table 3.3 Modifications made to the original questionnaire by Charlton et al. (2013) and Edmonds (2013) for use in the Perth Iodine and Pregnancy Study II (PIPS II)

Original version	Modification for present study	Justification
41-item iodine-specific FFQ relating to consumption in the last 2 months	Removal of FFQ and inclusion of a single item relating to daily consumption of any quantity of six key foods (cow's milk, cheese, ice cream, yoghurt, bread/bread products and eggs)	Reduce participant burden. Current consumption more relevant to study aims than consumption over previous 2 months.
Item 'Before you became pregnant, did you take any dietary supplements in the preceding year?' Item 'Since you knew you were pregnant, have you taken any dietary supplements?' Possible responses for both items – yes, regularly; yes occasionally or 'no'.	Removal of these items and inclusion of item asking about use of daily dietary supplements with a list of popular iodine-containing varieties for easy selection	Pre-pregnancy use not relevant to study aims. Important to determine if daily consumption or not and quantity of supplemental iodine consumed.
6 items relating to salt/iodised salt use	Removal of these items and addition of the single item 'Do you use iodised salt?' (Yes, No or I don't know)	Reduce participant burden
Item 'What ethnic group do you belong to?' Possible responses – European, Chinese, Indian, Other (Dutch, Japanese, Tokelauan)	Listed ethnic groups changed to Australian, Australian Aboriginal, Torres Strait Islander, Indian, Chinese, British	Consistent with the Australian Standard Classification of Cultural and Ethnic Groups*
Item 'In the last 12 months what did YOU (only you) earn before tax was removed?'	Item changed to 'In the last 12 months what was your household income before tax was removed?'	Household income likely to be a better indicator of socioeconomic status than participant earnings alone
Item "how many weeks pregnant are you?' Possible responses – less than 13 weeks, 14-28 weeks, more than 29 weeks	Item changed to 'Please provide details of your stage of pregnancy.' Participants to provide no. weeks and days pregnant today (with date) and, estimated due date.	Important to know actual gestational weeks for study aims
	Inclusion of items relating to participant's name and contact details	To enable participant follow-up

*(Australian Bureau of Statistics, 2011a)

3.4.3 Breast milk iodine

Participants who were breastfeeding were instructed to provide duplicate 5 mL breast milk samples at home at the start of a morning feed (preferably between 9 am and 12 noon) once their baby was aged 4-6 weeks. Breast milk sample containers were mailed to participants, together with collection instructions (see Appendix E). Participants were asked to write details of baby's age, collection date and time on the sample container labels, to then store the samples in the coldest part of their freezer and to contact AJ to arrange a collection time.

3.5 Sample analysis

All sample analyses were conducted at PathWest Laboratory Medicine, QEII Medical Centre, Sir Charles Gairdner Hospital, Perth, as described below.

3.5.1 Urinary iodine

Urine iodide was assayed by inductively coupled plasma mass spectrometry ICPMS). This is a fast, sensitive, multi-element technique for the determination of trace elements, based on the principles of vaporisation, dissociation and ionisation of chemical elements when introduced into a hot plasma. The ions are separated according to their mass/charge ratio (m/z) by a mass analyser.

Samples

Spot urine samples were collected into a metal free container without preservatives. Samples were stable in the refrigerator for at least 1 week and for longer term storage, they were kept at <-15 °C.

Equipment

- NexION 800D ICPMS (Perkin Elmer, Waltham, Massachusetts, USA),

  The analyser used was a operated according to the manufacturer's instructions.

Reagents

- Deionized water: Millipore MilliQ system (Scientronic Instrument Services, East Victoria Park, Western Australia)
- Nitric acid 65%**,** Merck Suprapur
- Triton X-100 (Iso-octylphenoxypolyethoxyethanol), BDH Product# 30632

- Internal stock standard**:** Var-IS 100 ppm (100 µg/mL) of the following elements Bi, In, Li, Sc, Tb, Y in 5% HCl
- Stock iodide standard: 1000 mg/L iodine/in water
- Di-sodium EDTA **(**Aldrich)
- Ammonia solution 28% Univar UN No. 2672
- Gold calibration standard solution 10: ROWE Scientific standard solution 10,00 ppm Au in 10% HCl
- Gallium stock standard**:** ACR gallium standard solution 1000mg/L in 2% HNO_3
- Propan-2ol (iso-propyl alcohol) BDH – Analar grade
- NexION Diluent (1 L)
- Deionised water 800 mL
- Di-sodium EDTA 1 g
- Triton X-100 1 mL
- NH_3 25 mL
- Iso-propanol 10 mL
- 1000 ppm gold solution 200 µL
- Internal standard intermediate (50 ppm) 400 µL
- Wash solution: 1% ammonia solution: 50 mL ammonia solution in 5 L deionised water.

Quality control reagents

- Internal QC: UTAK Controls (20 and 200 µg/L) and Recipe ClinChek Urine control assayed.
- External QC: The OELM QAP Program has Iodine as one of its elements, and PathWest QE2 subscribes to this scheme.
- Centre of Diseases Control (CDC) run an EQUIP scheme (ensuring the quality of urinary iodine procedures) in which PathWest is enrolled. Samples are distributed quarterly.

Method of analysis

See Breast Milk Iodine Analysis Method (in Section 3.5.5).

Linearity

Recovery

Precision

Accuracy

Detection limits

3.5.2 Urinary creatinine

To determine urinary creatinine the Abbott Architect ci16000 analyser with the Jaffé Creatinine rate method was used (see Appendix F).

3.5.3 Thyroid stimulating hormone (TSH)

Principle of TSH assay

The Abbott Architect i2000SR TSH assay is a two-step non-competitive chemiluminometric (sandwich) immunoassay, using two antibodies. In the first step, sample, assay diluent and mouse monoclonal anti-β TSH antibody coated paramagnetic microparticles are combined and incubated for 18 minutes, during which sample TSH is bound via the antibody to the microparticles. There is then a wash step, which removes all unbound material, including any excessive sample TSH. The second step is the addition of mouse monoclonal anti-α TSH acridinium labelled conjugate which, in turn, binds to the sample TSH, to form a solid phase-TSH-conjugate complex (or sandwich). After a further 4-minute incubation there is another wash, to remove any unbound labelled conjugate. Trigger solutions, consisting of acidification with nitric acid (pH 2.1) and oxidation with hydrogen peroxide, followed by alkalinisation with sodium hydroxide and Triton X-100 are added to produce the light signal. A direct relationship exists between the TSH in the sample and the relative light units (RLUs) detected by the Architect analyser.

Samples

Serum is the recommended specimen and samples can be stored at 4°C for up to two weeks (in house data) but frozen at -20°C for longer periods.

Reference range

From 3 years of age to adults: 0.40 to 4.00 mU/L.

The minimum reportable level for TSH is 0.01 mU/L.

Equipment

- Abbott Architecti2000SR analyzer, Abbott Diagnostics, Illinois, USA.

  TSH assay is performed routinely in PathWest laboratories according to the manufacturer's instructions.

Reagents

Supplier: Abbott Diagnostics Division

- Monoclonal mouse anti-β TSH antibody coated paramagnetic microparticles in TRIS buffer with bovine protein stabilizers and antimicrobial preservative.
- Monoclonal mouse anti-α TSH antibody acridinium-labelled conjugate in MES buffer with bovine protein stabilizers and antimicrobial preservative.
- TSH assay diluent in TRIS buffer with antimicrobial preservative.
- TSH calibrator, 2 levels, 0 and 40 mU/L, prepared by addition of recombinant human TSH of known concentration to obtain target concentration, referenced against WHO TSH 2nd IRP 80/558. In TRIS buffer with protein (bovine) stabilizer and sodium azide preservative.
- Multi assay diluent containing phosphate buffer and antimicrobial preservative.

Quality control reagents

Commercially available quality control material Levels 1, 2 and 3 are run daily.

Linearity

TSH calibrator, 2 levels, 0 and 40 mU/L, prepared by addition of recombinant human TSH of known concentration to obtain target concentration, referenced against WHO TSH 80/558 in TRIS buffer with protein (bovine serum albumin) stabilizer and sodium azide preservative.

Precision

Mean	SD	%CV	n
0.010	0.001	10.6	37
0.084	0.008	9.5	37
3.888	0.357	9.2	37
18.88	1.440	7.6	34

3.5.4 Free thyroxine (FT4)

Principle of FT4 assay

The Abbott Architect i2000SR FT4 assay is a two-step competitive chemiluminescent immunoassay where FT4 in the sample competes with T3 acridinium labelled conjugate for a limited amount of polyclonal anti-T4 antibody. In the first step, sample and polyclonal anti-T4 antibody coated paramagnetic microparticles are combined. FT4 (unbound) present in the sample binds to the anti-T4 coated microparticles. After an 18-minute incubation and washing, T3 acridinium labelled conjugate is added in the second step, followed by a further 4-minute incubation and washing. Trigger solutions (consisting of acidification with nitric acid (pH 2.1) and oxidation with hydrogen peroxide, followed by alkalinisation with sodium hydroxide and Triton X-100) are added to produce the light signal. An indirect relationship exists between FT4 in the sample and the relative light units (RLUs) detected by the Architect analyser.

Samples

Serum is the recommended specimen and samples can be stored at 4°C for up to two weeks (in house data) but frozen at -20°C for longer periods.

Reference range

From 3 years of age to adults: 9 – 19 pmol/L.

Specimens with results below 5 pmol/L are reported as <5 pmol/L.

Equipment

- Abbott Architecti2000SR analyzer, Abbott Diagnostics, Illinois, USA.

  FT4 assay is performed routinely in PathWest laboratories according to the manufacturer's instructions.

Reagents

Supplier: Abbott Diagnostics Division

- Polyclonal sheep anti-FT4 coated paramagnetic microparticles in TRIS buffer with sheep IgG stabilizers and antimicrobial preservative.
- T3 acridinium-labelled conjugate in MES buffer with NaCl and Triton X-100 stabilizers and ProClin preservative.

- Architect *i* Pre-Trigger solution (Cat No 6E23-65) 4 x 975 mL containing 1.32% hydrogen peroxide.
- Architect *i* Trigger solution (Cat No 6C55-60), 4 x 975 mL containing 0.35 N NaOH.
- Architect *i* Wash Buffer (Cat No 6C54-88), 1 x 10 L containing phosphate buffered saline.

Quality control reagents

Commercially available quality control material Levels 1, 2 and 3 are run daily.

Linearity

The reagents include 6 levels of calibrators (Cal A to Cal F) covering a range of 0 to 77.2 pmol/L. The calibrators are prepared in human serum with sodium azide preservative. Free-T4 Calibrators are matched to an Abbott internal reference standard, which is manufactured by gravimetric methods based on the Free Thyroxine calculation using L-Thyroxine and sodium salt pentahydrate (HPLC grade) at each concentration level.

Precision

Between run imprecision using patient serum pools.

n	Mean pmol/L	SD	CV%
51	10.2	0.884	8.7
51	17.0	1.182	7.0
51	53.9	3.566	6.6

3.5.5 Breast milk iodine

A number of analytical methods have been used to determine iodine concentration in complex matrices such as breast milk including the classic Sandell and Kolthoff kinetic–catalytic method (Hedayati, Ordookhani, Daneshpour, & Azizi, 2007), ion chromatography (Malongo et al., 2008), inductively coupled plasma mass spectrometry (ICPMS)(Hammer, 2008), flame atomic absorbance spectrometry (Yebra & Bollaín, 2010), high performance liquid chromatography and ion-specific electrodes (Melichercik, Szijarto, & Hill, 2006). Of these methods, ICPMS is

considered to be the gold standard, due to its high level of accuracy, precision and low detection limit, and is the most widely used approach for iodine quantification in foods. The ICPMS analysis following extraction by tetramethyl-ammonium hydroxide (TMAH) has been adopted by the European Committee for Standards as the official method for the quantification of iodine concentrations in foodstuffs (European Committee for Standards, 2007). ICPMS is a fast, sensitive, multi-element technique for the determination of trace elements. ICPMS is based on the principles of vaporisation, dissociation and ionisation of chemical elements when introduced into a hot plasma. The ions are separated according to their mass/charge ratio (m/z) by a mass analyser. An optimised ICPMS method for breast milk has been published recently (Huynh, Zhou, Gibson, Palmer, & Muhlhausler, 2015) and was adapted for this study. In brief, sonicated breast milk samples are diluted in mild alkali solution, ionized with inductively coupled plasma and the ions are separated and quantified in a mass spectrometer.

Samples

After collection, the breast milk samples were frozen at −20◦C until analysis. After thawing one aliquot, it was homogenized at 20,000/min for 30 seconds, and the iodine concentration was measured according to the method described below.

Internal quality control material

Urine Toxicology Controls (20 and 200 ug/L); Utak Laboratories, INC; Valencia, CA, USA. Liquid stable aqueous quality control material prepared from normal human urine used as certified reference for determining the accuracy of analysis. (https://www.aacb.asn.au/documents/item/747)

Reagents and equipment

Certified reference material (CRM) produced by the National Institute of Standard and Technology (NIST), NIST 1549 non-fat milk powder (Maryland, USA) with a certified iodine level of 3.38 ± 0.02 mg/kg was used to assess the accuracy of iodine determination.

Reagents

- Ammonia solution 28-30% w/w UNIVAR 500 mL.
- Commercial stock iodine standard. Calibrators made from 1000 µg/mL iodide stock (Inorganic Ventures, USA) and diluted in deionised water to make 4 calibrators with the following concentrations: 23.5, 47, 94 and 188 µg/L.
- High purity water (18 mOhm) generated by a Sartorius Water Purification System (Sartorius Stedim Australia Pty. Ltd, Ibis Technology, Osborne Park, Western Australia) was used for the preparation of all reagents, standards and samples.
- Internal Standard made from Stock Standard (Var-IS 100ppm (100 mg/mL) of the following elements Bismuth, Indium, Lithium, Scandium, Terbium, Yttrium in 5% HCl).
- Sample diluent: To 800 mL deionised water add 1 g $EDTANa_2$ (Sigma–Aldrich (New South Wales, Australia), 1 mL Triton X-100 (Sigma–Aldrich (New South Wales, Australia), 25 mL NH_3, 10 mL isopropanol (Sigma–Aldrich (New South Wales, Australia), and 400 mL of Internal Working Standard (50 ppm). The final volume was made to 1000 mL with deionised water.

Equipment

- Ultrasonic Cleaner Model FXP12D (Unisonics Australia, Brookvale, NSW, Australia).

  Sonic energy is provided to the chamber by piezoelectric transducers bonded to the tank bottom with a frequency of 40 kHz http://www.unisonics.com.au/pdf/FXP12D.pdf).

- The Perkin Elmer NexION 300 ICP-MS (ICPMS system)

  This was operated according to the manufacturer's instructions. Operating conditions for the systems are shown in Appendix G. All raw concentration data from the ICPMS was exported to Microsoft Excel. Blank subtraction, drift correction and other data processing (mass and volume adjustments) were performed off- line, using custom-written macro programs operated within Excel.

Sample preparation

Aliquots of well-mixed breast milk (50 µL) were added to 1950 µL of sample diluent, in 5 mL polypropylene tubes (Thermo-Fisher Scientific Australia, Scoresby, Victoria, Australia), mixed by vortex vigorously for several seconds (PV-1, Grant-Bio), then sonicated for 5 mins, mixed again immediately prior to analysis.

Reagents and standard solution preparation

The sample diluent was stored at room temperature.

Internal standard (Indium) stock solutions

Var-IS 100ppm (100 µg/mL) of the following elements Bi, In, Li, Sc, Tb, Y in 5% HCl. A working standard is prepared by adding 5.0 mL of this stock solution to 5.0 mL of deionised water.

Iodide spiked solutions

To prepare iodide spiked solutions, the 1000mg/L iodide stock solution was diluted with high purity water to yield 50mg/L iodide solution. This solution was further diluted in 1% sample diluent to produce final concentrations of 0.2, 0.4 and 0.8mg/L. These solutions in turn were used as spiked solutions in recovery tests, producing concentrations of 750, 375 and 188 µg/L in the final analysed samples.

Blanks

Two types of blanks were used.

- a calibration blank,
- a method blank processed in exactly the same way as the samples and containing all the reagents used in the assay,

All blanks were prepared in sample diluent and also contained the internal standard mix. The calibration blank was used to establish the analytical calibration curve, while the method digestion blank was used to account for batch to batch variation

Wash solutions

Three types of wash solutions were used. Two of the solutions, the autosampler wash station rinse solution and an extra clean wash solution, were sample diluent. Another pre-wash solution was prepared with 1% ammonia (NH4) in high purity water.

Method validation

Linearity

The iodine standards (23.5, 47, 94 and 188 µg/L) were used to establish the calibration curve. In order to ensure accuracy, the iodine concentrations of all samples analysed are required to fall within the range of the calibration curve, otherwise the samples need to be diluted.

The NeXion ICPMS is equipped with a discrete dynode detector which has a wide linear dynamic range. The discrete dynode type detector can also be run in two modes, pulse-counting and analog, which further extends the instrument's linear range and can be used to protect the detector from excessively high signals.

Recovery

A breast milk sample, NIST1549 milk powder and a method digestion blank were used to determine the percent recovery of various levels of added iodine in the samples. The breast milk samples were spiked with 188, 375 and 750 µg/L of iodide solution. The measured iodine concentration was divided by the expected value in order to determine the percent recovery.

Precision

The intra-assay and inter-assay variation were determined by analysing 2 breast milk samples 5 times in a single run (intra-assay coefficient of variation (CV).

Accuracy

The method was validated for accuracy by comparing the iodine concentration obtained for replicates of NIST CRM 1549 milk powder analysis. The CV of repeatability and 95% confidence interval (CI) were calculated.

See Appendix G for results.

3.6 Data entry, preparation and calculations

An Excel database was established to record participant details (such as gestational stage and contact details), track participant progress and record study results (from samples and questionnaires). Data stored in the database was in a coded, re-identifiable form. In terms of data preparation, several variables were recoded to assist with data analysis (see Table 3.4).

Table 3.4 Recoded variables

Variable	Explanation of recoding
Ethnic group	Due to low numbers in some ethnic groups (as reported by participants), this variable was collapsed into two categories – Caucasian and Other*
Highest level of education	Six possible options were collapsed into three categories – Tertiary or professional (Bachelor degree, Post-graduate university degree and professional qualification); Diploma, trade or technical certificate; and Secondary school.
Iodine-containing supplement use	At each stage, participants were classified into one of three categories – Yes, iodine supplement with <150 µg used daily; Yes, iodine supplement with ≥150 µg used daily; or No, iodine supplement not used daily.

* based on the definition for Caucasian as 'relating to a person of white-skinned or European appearance' (Macquarie Dictionary, 2017). This reference is used by the Australian Bureau of Statistics to provide an Australian context for the meanings of other related words.

One calculation was performed to determine the iodine-to-creatinine ratio (UIC/Cr; µg/g), according to the following formula:

$$\text{UIC/Cr (µg/g)} = \frac{\text{iodine (µg/L)}}{\text{[creatinine (mmol/L) x 113] / 1000}}$$

where 113 = molecular weight of creatinine

3.7 Data analysis

Descriptive statistics were obtained for variables of interest. Normally distributed continuous variables (eg. TSH and FT4) were reported as means and standard deviation (± SD) while median and interquartile range (IQR) were used to report skewed data (eg. UIC, UIC/Cr and BMIC). Frequencies and relative percentages were determined for categorical variables. For skewed data, the Kruskal-Wallis test was used to assess differences between 2 groups. Independent sample *t* test and paired samples t tests were used to determine differences between 2 groups of normally distributed data. The variables UIC/Cr and BMIC were transformed (log base 10) to better approximate a normal distribution. Correlation between measures of iodine status (namely UIC/Cr and BMIC) was determined on logged (base 10) data using Pearson correlation.

Iodine status (as urinary iodine excretion) was reported in 2 ways:

1. as the iodine concentration (µg) to describe the iodine status of the group by comparing the median UIC value to the WHO UIC cut-offs for iodine adequacy in pregnancy and lactation (WHO/UNICEF/ICCIDD, 2007)
2. as the iodine-to-creatinine ratio (µg/g), which corrects UIC for variable dilution among spot urine samples, adjusts for GFR and muscle mass (as a marker for body size) .

Analyses to determine gestational changes in iodine status (via urinary iodine) were carried out via linear mixed models for repeated measures longitudinal data, and linear models for associations at a single time. Associations between dietary and demographic factors and the (log-transformed) UIC/Cr were explored. Trend analyses were based on the calculated gestational week of the urine sample, as determined from gestational week at recruitment, rather than on the basis of study stage (ie 1, 2 or 3). All analyses were conducted using IBM Statistical Package for Social Sciences (version 24; SPSS), except for the linear models which were conducted in TIBCO Spotfire S+ (version 8.2). Significance was set at $P < 0.05$.

Chapter 4 Results

4.1 Participants

From July 2013 – March 2015, 121 pregnant women signed consent forms to participate in the study (Figure 4.1). At recruitment, no women reported having thyroid disease/thyroid problems or diabetes, nor taking thyroid medication nor currently breastfeeding. The criterion used to determine completion of each stage and to remain in the study was provision of successive urine samples, with the main focus being on Stages 1-3 (gestation). Approximately two-thirds of recruited women completed Stage 1, and by Stage 3, half the recruited number remained in the study. Stage 4 (post-partum) was completed by 40% of recruited participants. Despite adequate attempts for follow-up, only 79% of participants who completed Stage 3 also completed Stage 4 (48/61 women). 'Missed urine sample' accounted for the highest proportion of non-completing participants (58/73; 79%). Shortly after recruitment two women were deemed ineligible to participate due to thyroid dysfunction and during the course of the study four women were lost due to miscarriage or early delivery.

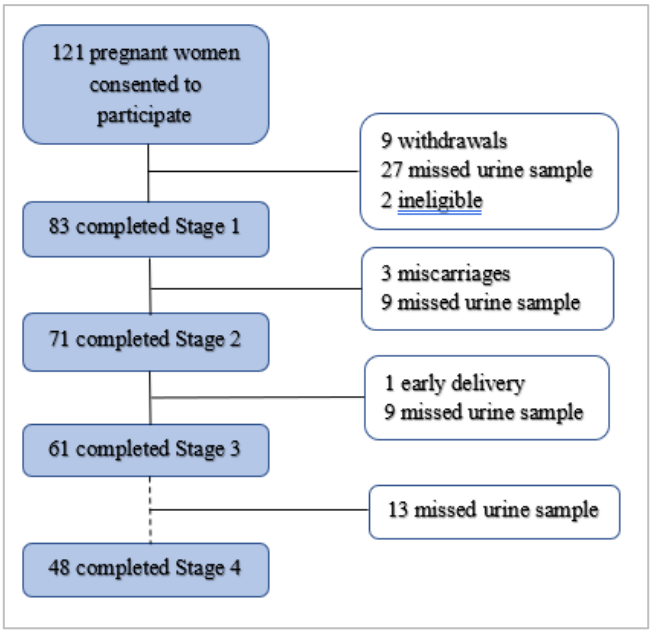

Figure 4.1 Recruitment flow-chart

At Stage 1, participants were grouped as either 'Early enrolment' or 'Late enrolment' depending on the number of weeks gestation at recruitment. 'Early enrolment' were recruited at 10-14 weeks gestation while 'Late enrolment' were 18-22 weeks gestation

at recruitment. Of the 61 participants who completed Stage 3, 30% (n=18) were 'Early enrolment' and 70% (n=43) were 'Late enrolment'.

Table 4.1 indicates the methods used to recruit the 61 women who completed Stages 1-3 of the study. The largest proportion of the participants (47%) were recruited in person from KEMH by the study coordinator (AJ), followed by a quarter from women's health private clinics. Word of mouth (either by participants or study personnel) recruited 13% of participants, similarly for newspaper, radio and website advertising combined.

Table 4.1 Results of recruitment methods

Recruitment method	n (n=61)	%
Advertising		
• Flyer in private practice eg. women's ultrasound, gynaecologist/obstetrician	15	25
• Community newspaper, radio and websites eg. parent organisations, Curtin University	8	13
In-person by study coordinator (AJ)		
• Kind Edward Memorial Hospital (KEMH)	29	47
• Pathology clinic (Western Diagnostic)	1	2
Word of mouth	8	13

4.2 Sociodemographic characteristics

The sociodemographic characteristics of the 61 study participants are summarised in Table 4.2. The mean age (± standard deviation [SD]) of the study women was 31.6 (± 4.2) years (range 20-42 years) and just under half of women were in their first pregnancy (48%). Eleven different ethnicities were reported by participants but due to low numbers in some groups, ethnicity was recoded into two groups, namely Caucasian and Other (see Appendix H for original and recoded ethnic groups). Based on the recoding, 84% of participants were Caucasian, with 78% of these participants being of 'Australian' ethnicity. No women reported being of 'Australian Aboriginal' or 'Torres Strait Islander' backgrounds.

In terms of highest education level completed, almost three-quarters of the participants reported tertiary or professional qualifications (72%), while 12% had diploma, trade

or technical certificate qualifications and 16% of women had completed secondary school only. Despite women given the option of not answering the sensitive question relating to self-reported household income in the last 12 months, only one participant declined to provide this information. The majority of those who answered (64%) reported a household income more than $100 000, followed by 21% who earned $50 000-$100 000 and the smallest group of women earned less than $50 000 (13%).

Table 4.2 Participant sociodemographic characteristics

Participant Variable	n	%
Age (y) (n=61) • Mean (SD) • Range	 31.6 (4.2) 20-42	
Gravidity (n=61) • First pregnancy • Not first pregnancy	 29 32	 48 52
Ethnicity (n=61) • Caucasian • Other	 51 10	 84 16
Highest education level (n=61) • Tertiary or professional • Diploma, trade or technical certificate • Secondary school	 44 7 10	 72 12 16
Household reported income ($AUS)(n=61) • <$50 000 • $50 000-$100 000 • <$100 000 • Do not wish to answer	 8 13 39 1	 13 21 64 2

4.3 Iodine-containing supplement use

Throughout the study, 15 different iodine-containing supplements were used by participants. The majority of these (73%) would meet the NHMRC supplement recommendation by providing at least 150 µg iodine/day, if taken according to manufacturer instructions available at the time. The vast majority of participants (85%; n=52/61) reported using an iodine-containing supplement during at least one stage of the study. For participants with known daily iodine-containing supplement use

behaviour for all four stages (n=48), reported behaviour was consistent across the stages for 73% of participants. Specifically, 54% (n=26/48) used a daily iodine-containing supplement during every study stage, while 19% (n=9/48) of participants reported not using a daily iodine-containing supplement at any stage. For the remaining 27% of participants (n=13/48), daily iodine-containing supplement use was inconsistent throughout the study. Interestingly, all inconsistent users except one (n=12/13) reported using a daily iodine-containing supplement during Stage 1. Another common pattern of inconsistency (n=8/13) was the use of a daily iodine-containing supplement during all three pregnancy stages but not during the post-partum Stage 4.

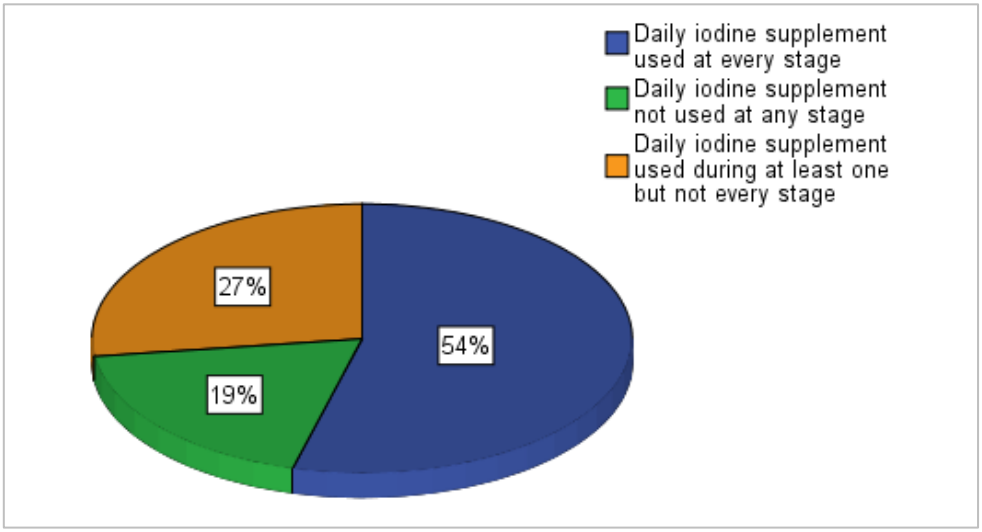

Figure 4.2 Overall reported daily iodine-containing supplement use
Based on participants with known reported daily iodine-containing supplement use behaviour for all four study stages (n=48). Stage 1: 10-14 or 18-22; Stage 2: 26-28; Stage 3: 36-38 wk gestation; Stage 4: 4-6 wk post-partum.

Table 4.3 describes the sociodemographic and pregnancy-related characteristics of daily iodine-containing supplement users (n=52/61), defined as participants who used an iodine-containing supplement daily during at least one stage during pregnancy (Stages 1-3). Due to small numbers in some subcategory groups, the validity of associations (except for Age) is questionable (see Appendix I). A summary of findings is shown in Table 4.4.

Table 4.3 A comparison of demographic and pregnancy-related characteristics of iodine supplement and non-supplement users

Characteristic	Supplement users (n=52)	Non-supplement users (n=9)	*P*-value
Age (n=61) mean; years (SD)	32.1 (4.1)	29.0 (3.6)	0.040
Gravidity (n=61) • First pregnancy • Not first pregnancy	 25 27	 4 5	0.840*
Ethnicity (n=61) • Caucasian • Non-Caucasian	 44 8	 7 2	0.609*
Highest education level (n=61) • Tertiary or professional • Diploma, trade, technical certificate • Secondary school	 40 4 8	 4 3 2	0.057*
Household reported income ($AUS)(n=61) • <$50 000 • $50 000-$100 000 • <$100 000 • Do not wish to answer	 4 9 38 1	 4 4 1 0	0.002*

*****Validity of p-values is questionable due to small numbers of participants in some subcategory groups.

Table 4.4 Summary of sociodemographic and pregnancy-related characteristics of iodine-containing supplement users (n=61)

Sociodemographic/Pregnancy-related variable	Outcome
Age	• supplement users were significantly older than non-users (mean age 32 vs 29 years, respectively; p=0.040).
Gravidity	• 48% (n=25/52) of supplement users were in their first pregnancy • 86% (n=25/29) of women in their first pregnancy used iodine-containing supplements.
Ethnicity	• supplement use was reported by 83% (n=44/51) of Caucasians and 80% (n=8/10) of non-Caucasians.
Highest education level	• 91% (n=40/44) of tertiary qualified/professional participants were supplement users while the values for diploma / trade / technical certificate and secondary school only participants were 57% (n=4/7) and 80% (n=8/10), respectively.
Household reported income	• 50% (n=4/8) of participants with household incomes <$50 000 used supplements, 70% (n=9/13) used supplements in the $50 000-$100 000 category while 97% (n=38/39) of participants with household incomes >$100 000 used supplements. • the sole participant who did not wish to provide household income details was a supplement user.

In terms of daily iodine-containing supplement use by study stage, Figure 4.3 indicates that the number of participants using iodine-containing supplements daily remained relatively constant during Stages 1-3. During these gestation stages, 70-75% (n=43-46) of participants reported daily use of supplements containing ≥150 µg of iodine and a further 7% (n=4) reported daily use of supplements containing <150 ug of iodine during each stage. This equates to 82% (n=50/61), 77% (n=47/61) and 80% (n=48/60) of participants in Stages 1-3, respectively, using some form of supplemental iodine daily. As Figure 4.3 indicates, information on daily iodine-containing supplement use was not available for one participant in Stage 3. This participant has been excluded in the denominator for percentage calculations for Stage 3 (n=60).

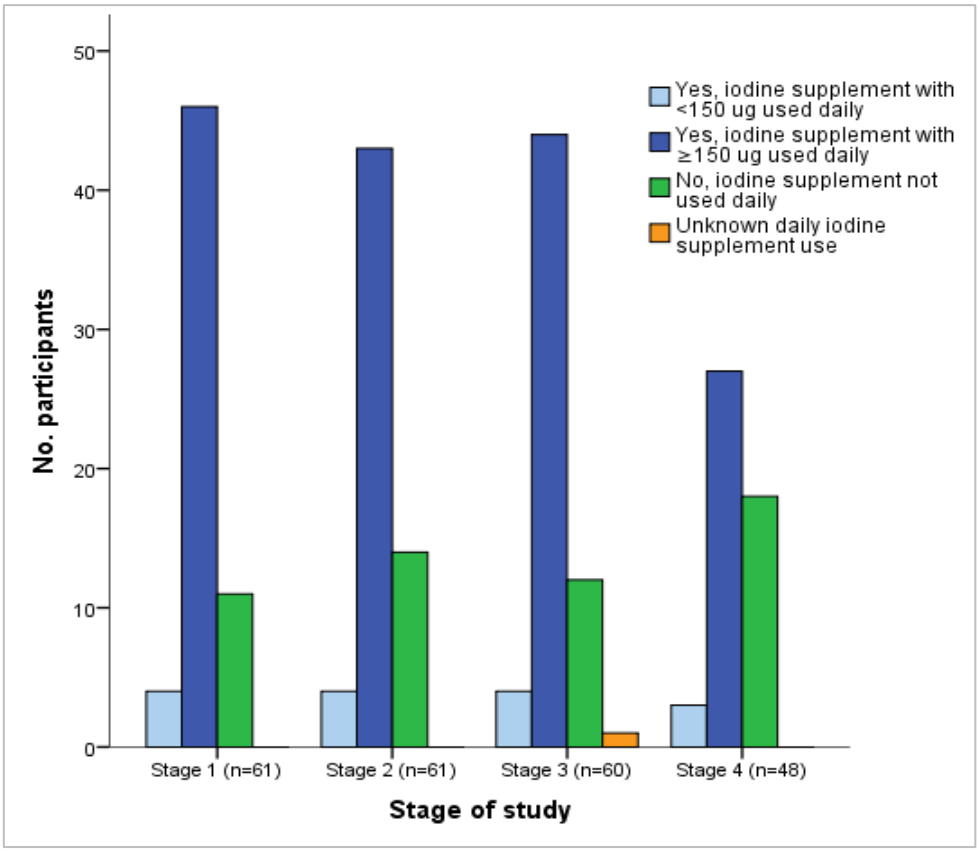

Figure 4.3 Daily iodine-containing supplement use by study stage
Numbers reported in each study stage indicate the number of participants with known supplement use behaviour. Stage 1: 10-14 or 18-22; Stage 2: 26-28; Stage 3: 36-38 wk gestation; Stage 4: 4-6 wk post-partum.

Daily consumption of iodine-containing supplements decreased during the postpartum period (Stage 4) compared to use during Stages 1-3. This finding was observed for participants overall (see Figure 4.3) and for just those participants who completed all four stages of the study (n=48; see Figure 4.4). The majority of women in Stage 4 were breastfeeding (96%; n=46/48), however, only 65% (n=30/46) of breastfeeding participants reported using some form of supplemental iodine daily during Stage 4. While 90% (n=27/30) of breastfeeding women who used an iodine-containing supplement daily used a brand with ≥150 µg of iodine, the overall usage rate of these recommended supplements was just 59% (n=27/46). Furthermore, 35% of lactating Stage 4 participants (n=16/46) did not take any type of iodine-containing supplement daily. The two non-breastfeeding women were also not using an iodine-containing supplement.

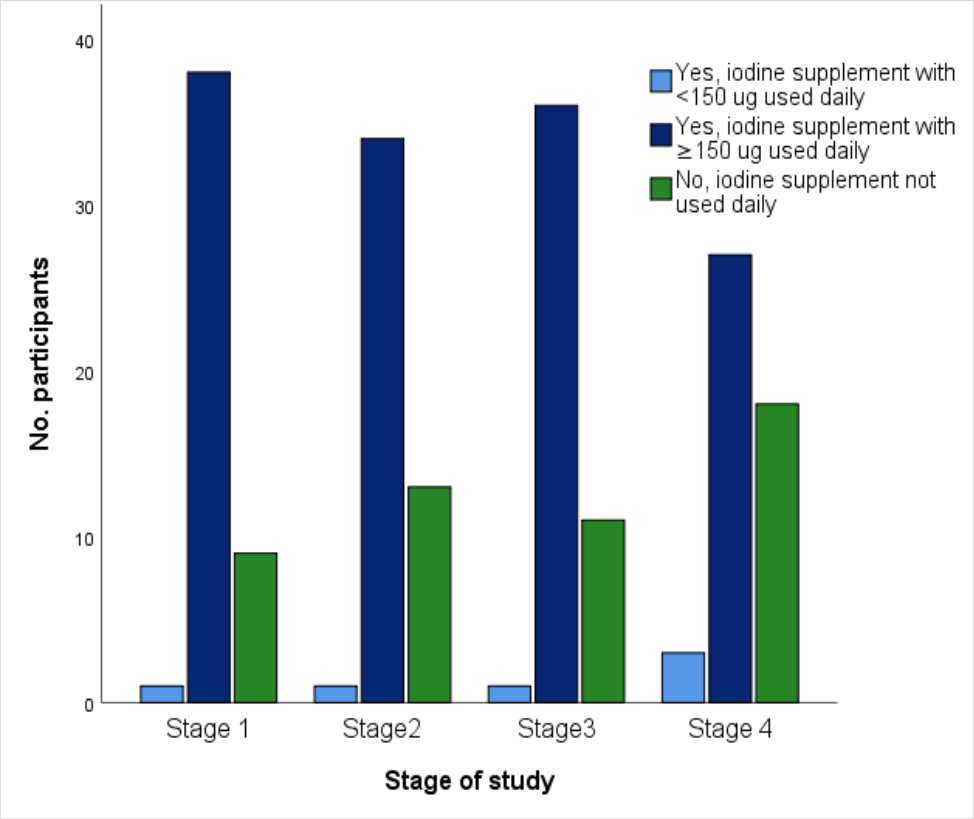

Figure 4.4 Daily iodine-containing supplement use of participants who completed all study stages (n=48)
Numbers reported in each study stage indicate the number of participants with known supplement use behaviour. Stage 1: 10-14 or 18-22; Stage 2: 26-28; Stage 3: 36-38 wk gestation; Stage 4: 4-6 wk post-partum.

4.4 Daily food iodine consumption

The following section describes participant's reported daily consumption (Yes, No or Unknown) of any quantity of the six key iodine-containing foods included in the study. This data was collected to determine if a change in consumption of these foods over gestation could help explain any observed change in iodine status (as measured by UIC/Cr). The data set is complete for all 61 participants for Stages 1 and 2, while daily consumption behaviour was not available for two participants in Stage 3 (n=59). Dietary information was available for all 48 Stage 4 participants. Percentage calculations have again excluded those participants with 'unknown' intake behaviour.

4.4.1 Daily bread/bread products consumption

As Figure 4.5 shows, daily consumption of bread/bread products was high compared to other foods investigated. There was a decreasing trend for daily consumption of these fortified and therefore reliable sources of iodine across gestation (82%, 74% and 64% for Stages 1-3, respectively), while 83% (n=40/48) of participants consumed these products daily postpartum.

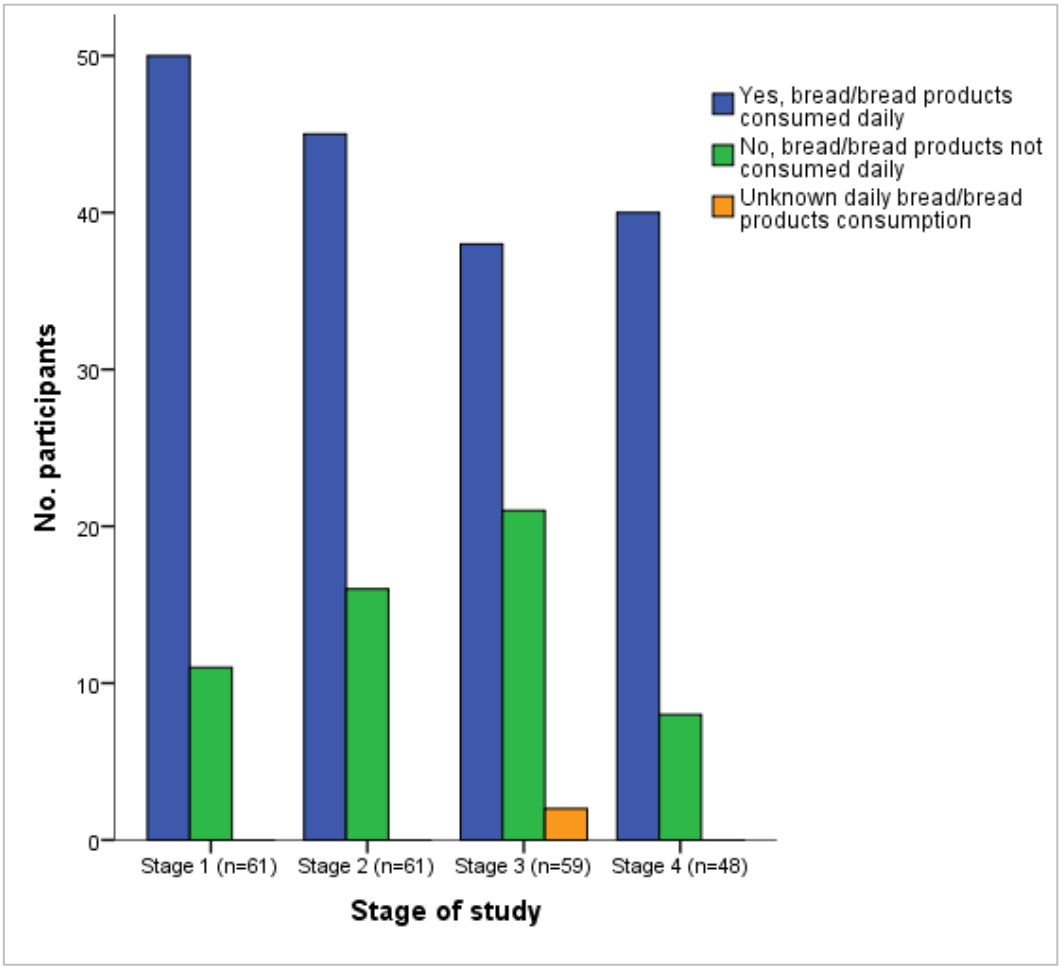

Figure 4.5 Daily bread/bread products consumption by study stage
Responses are based on daily consumption of any quantity of bread/bread products. Numbers reported in each study stage indicate the number of participants with known daily bread/bread products consumption behaviour. Stage 1: 10-14 or 18-22; Stage 2: 26-28; Stage 3: 36-38 wk gestation; Stage 4: 4-6 wk post-partum.

4.4.2 Daily cow's milk consumption

As Figure 4.6 indicates, at each stage of the study, most participants reported consuming some cow's milk each day (regardless of quantity). In addition, the proportion of daily consumers remained relatively constant throughout pregnancy and postpartum (80%, 87%, 86% and 81% for Stages 1-4, respectively).

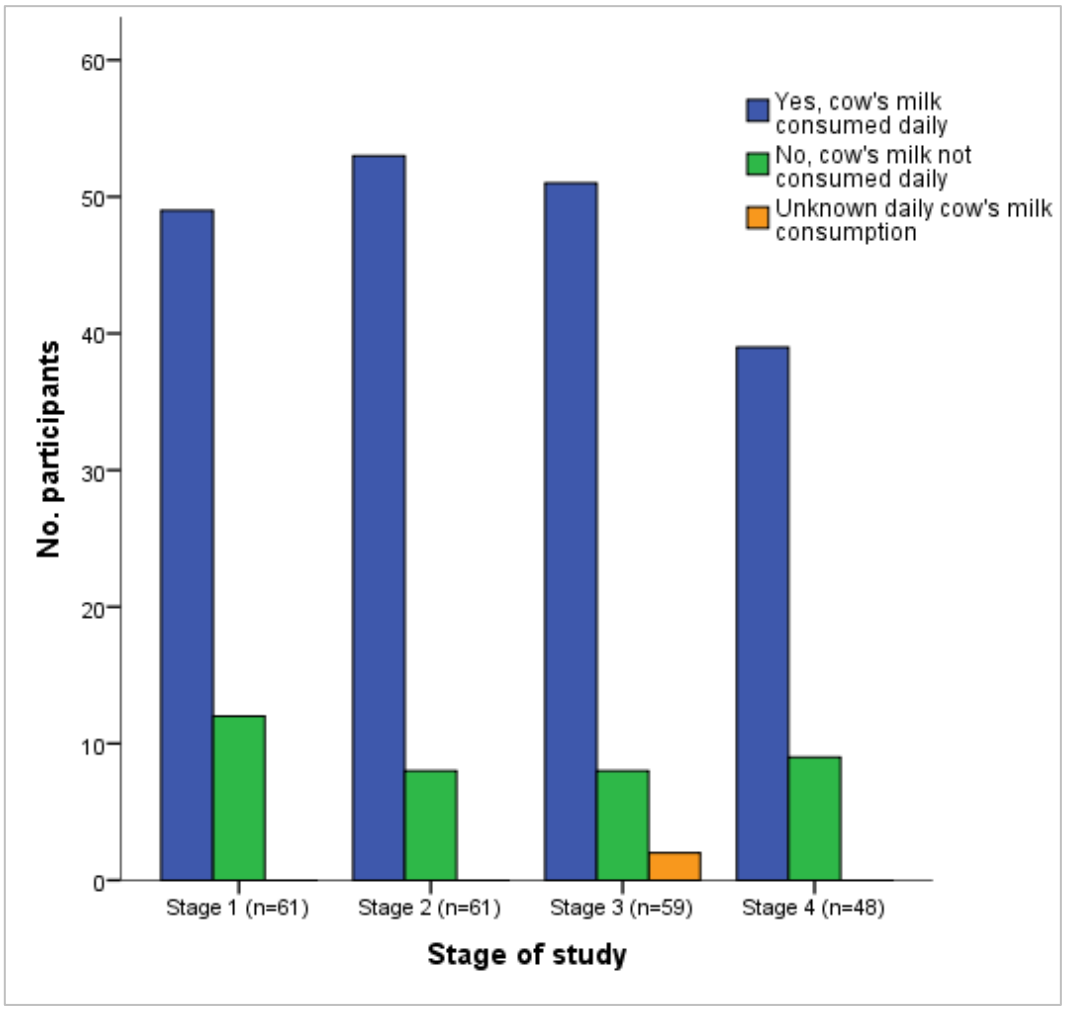

Figure 4.6 Daily cow's milk consumption by study stage
Responses are based on daily consumption of any quantity of cow's milk. Numbers reported in each study stage indicate the number of participants with known daily cow's milk consumption behaviour. Stage 1: 10-14 or 18-22; Stage 2: 26-28; Stage 3: 36-38 wk gestation; Stage 4: 4-6 wk post-partum.

4.4.3 Daily cheese consumption

For participants whose daily cheese consumption behaviour was known, there was only minor variation in the proportion of participants consuming some cheese daily across the four study stages (see Figure 4.7). Specifically, these values ranged from 48% (n=29/61 participants) during Stage 2 to 51% (n=30/59 participants) during Stage 3.

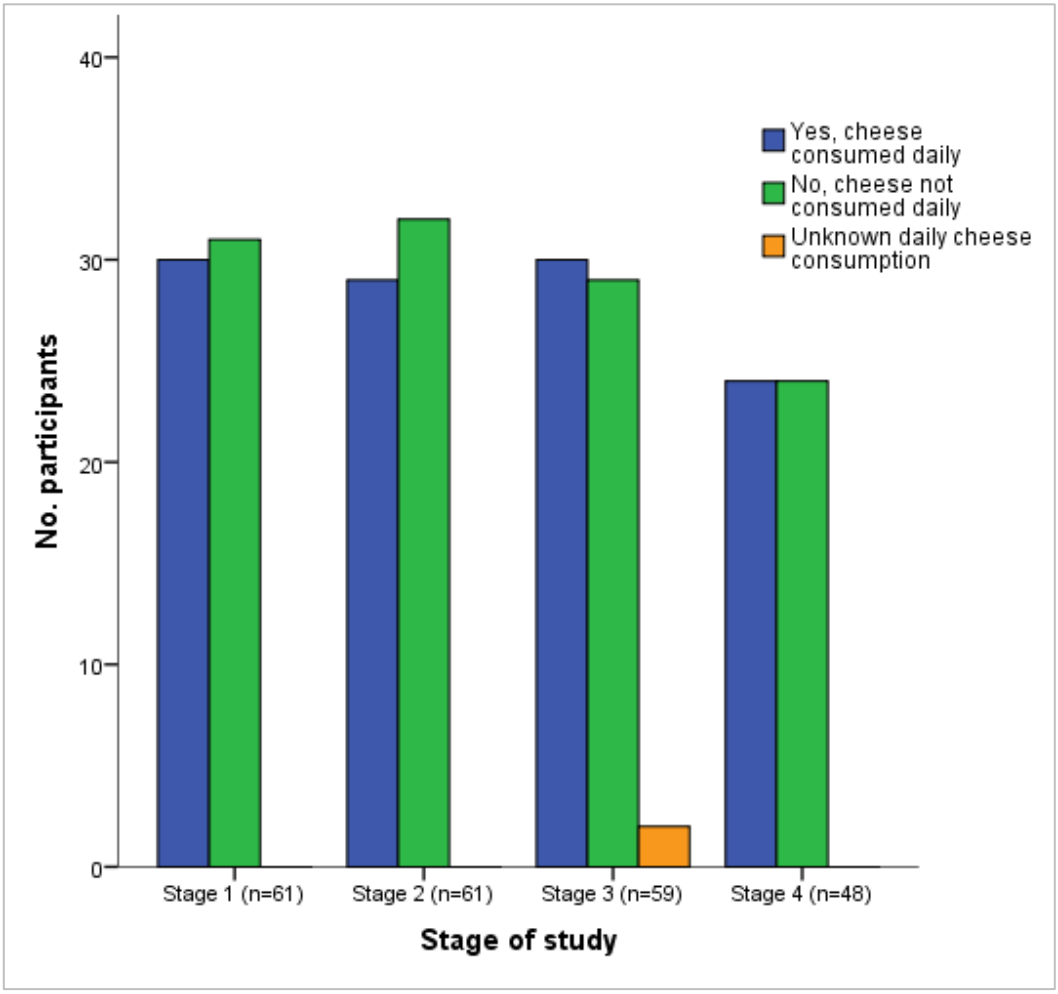

Figure 4.7 Daily cheese consumption by study stage
Responses are based on daily consumption of any quantity of cheese. Numbers reported in each study stage indicate the number of participants with known daily cow's milk consumption behaviour. Stage 1: 10-14 or 18-22; Stage 2: 26-28; Stage 3: 36-38 wk gestation; Stage 4: 4-6 wk post-partum.

4.4.4 Daily yoghurt consumption

The majority of participants at each study stage did not consume yoghurt daily (see Figure 4.8). There was some variation in the proportion of daily consumers across the stages, ranging from 29% (n=14/48) for Stage 4 to 38% (n=23/61) for Stage 2, based on participants with known daily yoghurt consumption behaviour.

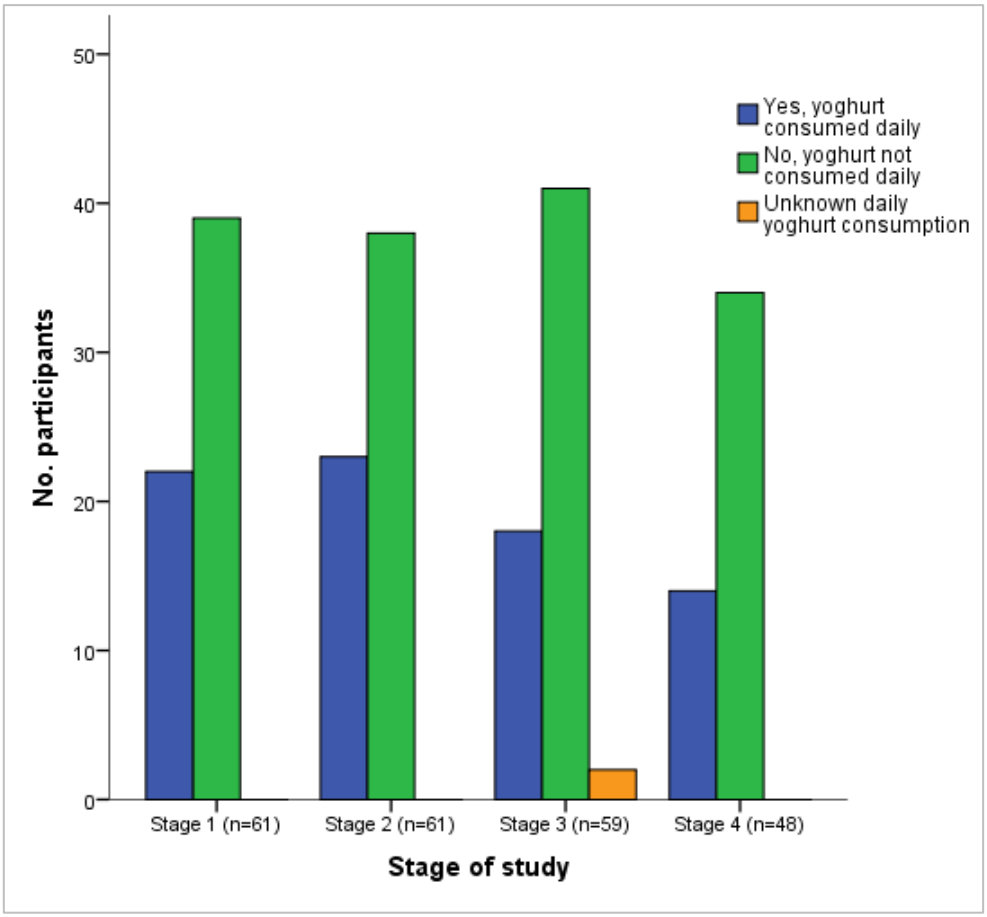

Figure 4.8 Daily yoghurt consumption by study stage

Responses are based on daily consumption of any quantity of yoghurt. Numbers reported in each study stage indicate the number of participants with known daily yoghurt consumption behaviour. Stage 1: 10-14 or 18-22; Stage 2: 26-28; Stage 3: 36-38 wk gestation; Stage 4: 4-6 wk post-partum.

4.4.5 Daily egg consumption

At each study stage, most participants did not consume eggs daily (see Figure 4.9). Daily egg consumption remained relatively constant across the four stages (28%, 26%, 24% and 31% of participants with known egg consumption behaviour for Stages 1-4, respectively).

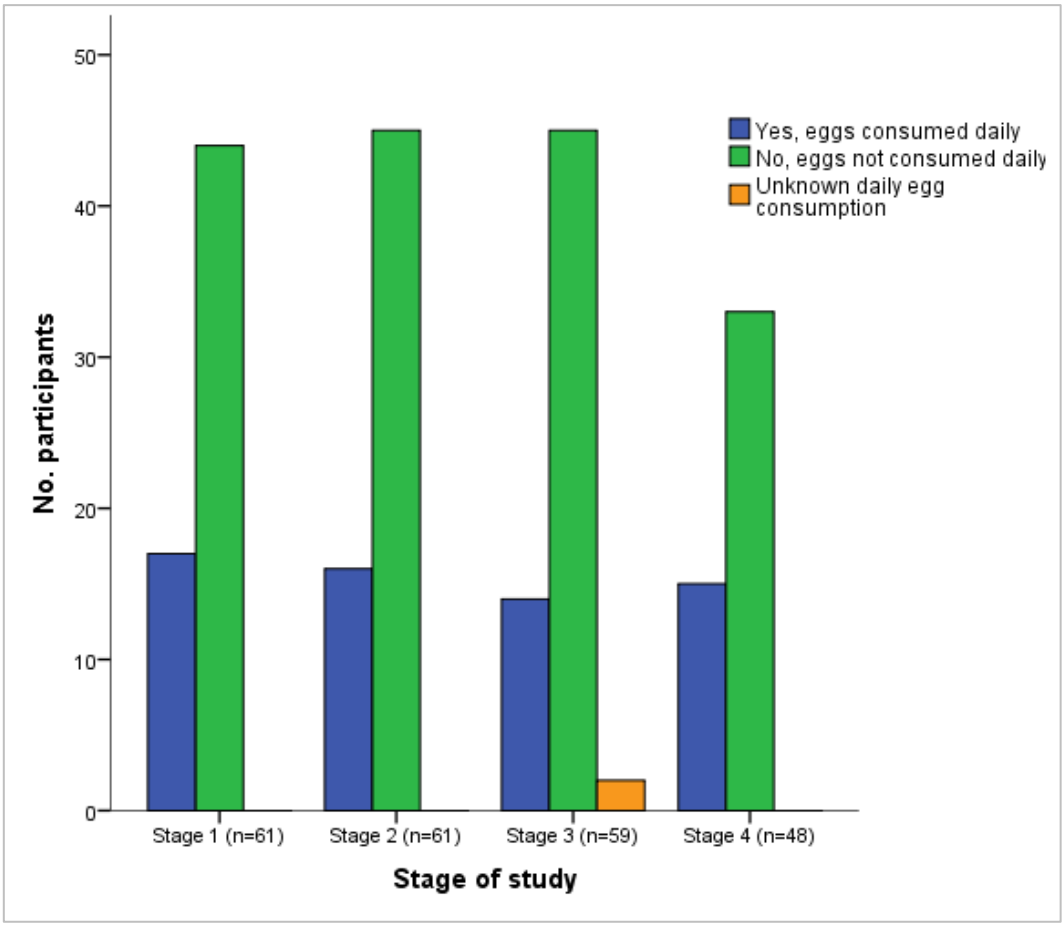

Figure 4.9 Daily egg consumption by study stage
Responses are based on daily consumption of any quantity of eggs. Numbers reported in each study stage indicate the number of participants with known daily egg consumption behaviour. Stage 1: 10-14 or 18-22; Stage 2: 26-28; Stage 3: 36-38 wk gestation; Stage 4: 4-6 wk post-partum.

4.4.6 Daily ice cream consumption

Compared to other foods investigated, daily ice cream consumption was low during all four study stages, ranging from 4% (n=2/48) during postpartum to 10% (n=6/59) during Stage 3, based on known ice cream consumption behaviour (see Figure. 4.10).

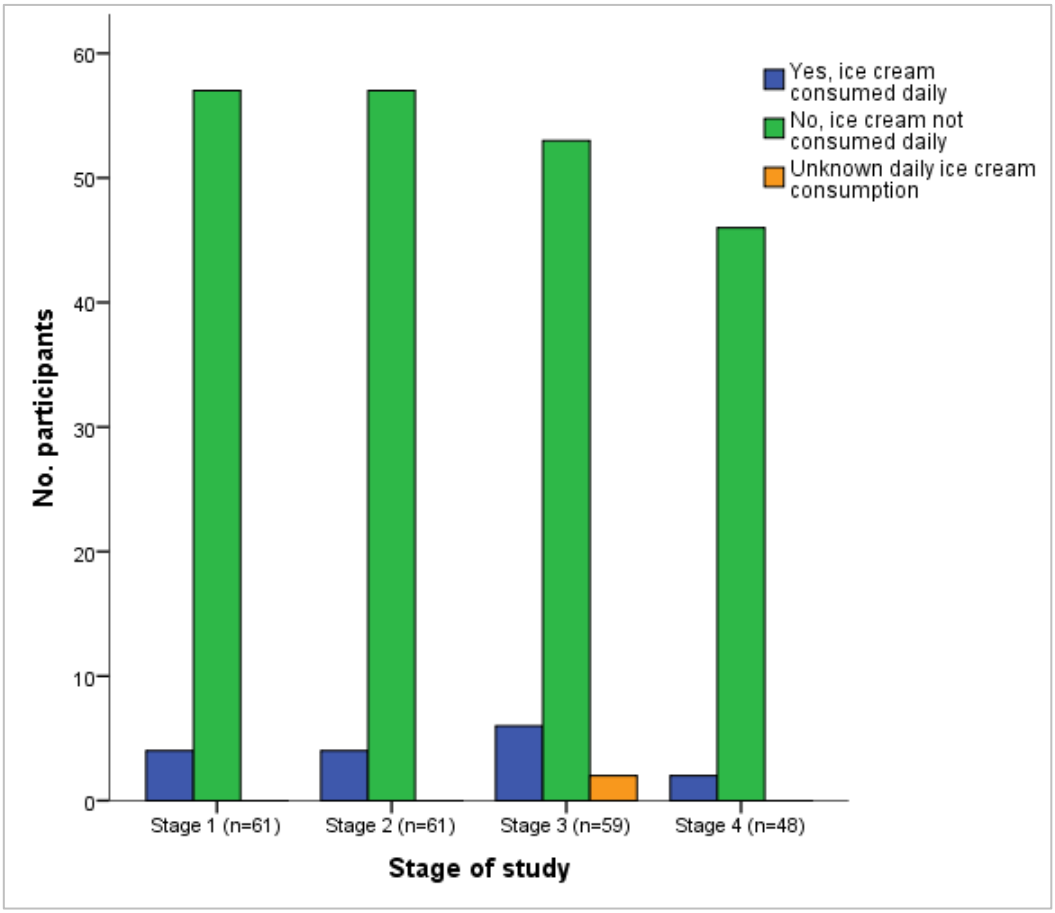

Figure 4.10 Daily ice cream consumption by study stage
Responses are based on daily consumption of any quantity of ice cream. Numbers reported in each study stage indicate the number of participants with known daily ice cream consumption behaviour. Stage 1: 10-14 or 18-22; Stage 2: 26-28; Stage 3: 36-38 wk gestation; Stage 4: 4-6 wk post-partum.

4.5 Iodised salt use

As Figure 4.11 indicates, at each study stage most participants did not use iodised salt (36%, 39%, 37% and 46% reported 'Yes' for Stages 1-4, respectively, based on participants with known iodised salt use behaviour).

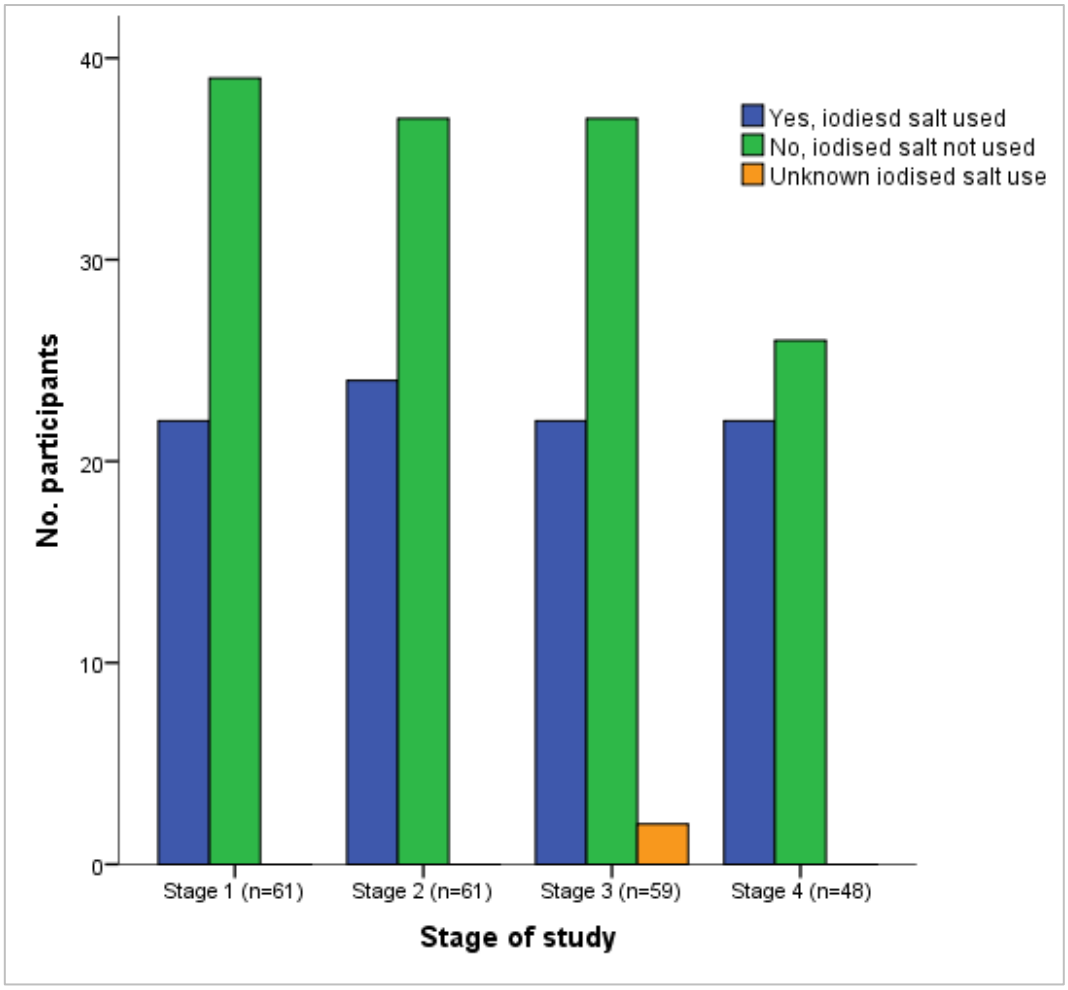

Figure 4.11 Iodised salt use by study stage
Responses are based on daily consumption of any iodised salt. Numbers reported in each study stage indicate the number of participants with known iodised salt use behaviour. Stage 1: 10-14 or 18-22; Stage 2: 26-28; Stage 3: 36-38 wk gestation; Stage 4: 4-6 wk post-partum.

4.6 Urinary iodine

In total, 231 urine samples were collected from participants between July 2013-August 2015. This total consisted of 183 samples during pregnancy (3 samples from 61 participants, one sample in each of Stages 1-3) and 48 single postpartum samples from individual participants during Stage 4. Based on the overall MUIC (IQR) of 182 (106-314) µg/L during pregnancy, the group was classified as having adequate iodine intake, according to WHO criteria (WHO/UNICEF/ICCIDD, 2007) (see Table 2.4 in Section 2.8.1). In addition, Table 4.5 shows that the women were classified as having adequate iodine intakes at each pregnancy study stage, based on MUIC values. Figure 4.12 shows the percentage distribution of UIC during pregnancy, with a wide range of 22-1137 ug/L.

For the post-partum results, a MUIC (IQR) of 106 (63-176) µg/L was indicative of adequate iodine intake for participants who at the time of sampling were breastfeeding (n=46/48) and also those non-breastfeeding (n=2/48) (WHO/UNICEF/ICCIDD, 2007)(see Table 2.4 in Section 2.8.1). The post-partum MUIC value for breastfeeding women only was 105 ug/L, also indicating adequate iodine intake. The range in UIC values for the post-partum samples was 22-359 ug/L (see Figure 4.13).

Table 4.5 UIC results by study stage

	Stage 1 (n=61)	**Stage 2 (n=61)**	**Stage 3 (n=61)**	**Stage 4 (n=61)**
Urinary iodine concentration[1] (µg/L)	186 (105-313)	181 (86-318)	177 (110-322)	106 (63-176)
Adequate iodine intake (µg/L)[2]	150-249	150-249	150-249	≥100

[1]Values are medians; (IQR). Stage 1: 10-14 or 18-22; Stage 2: 26-28; Stage 3: 36-38 wk gestation; Stage 4: 4-6 wk post-partum.
[2]Values are medians. Source: WHO/UNICEF/ICCIDD, 2007.

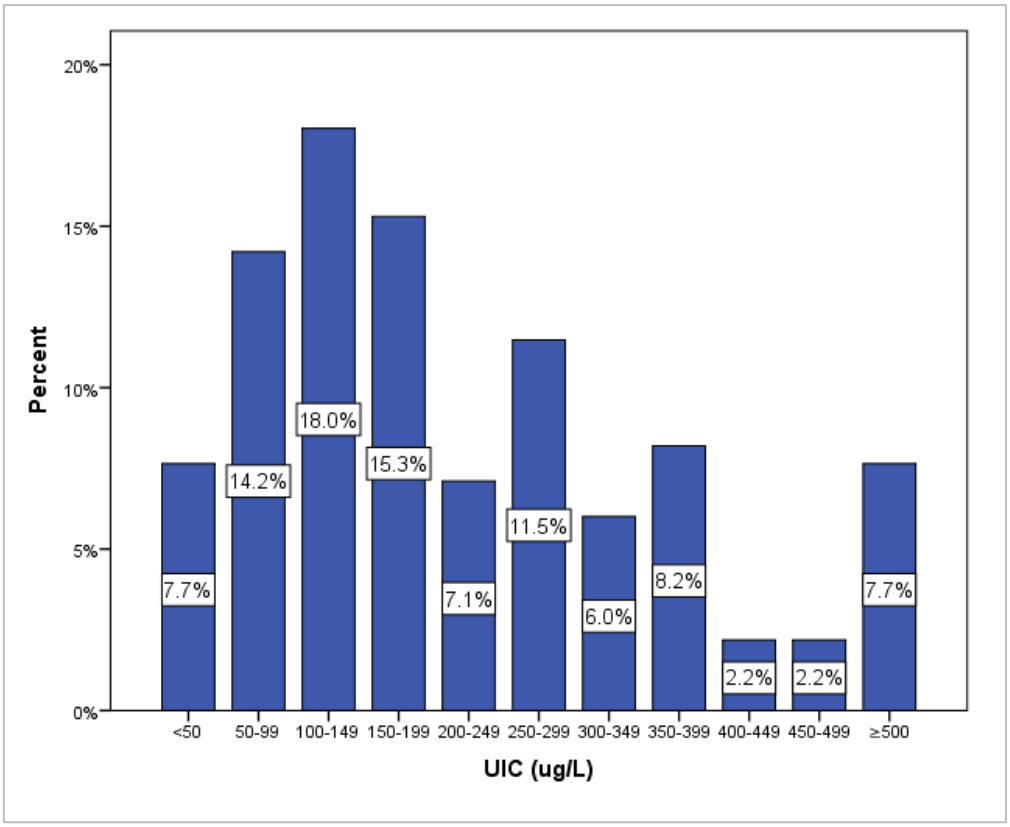

Figure 4.12 Percentage distribution of UIC (ug/L) during pregnancy
UIC: Urinary iodine concentration. Based on 183 urine samples (3 samples from 61 participants provided during pregnancy).

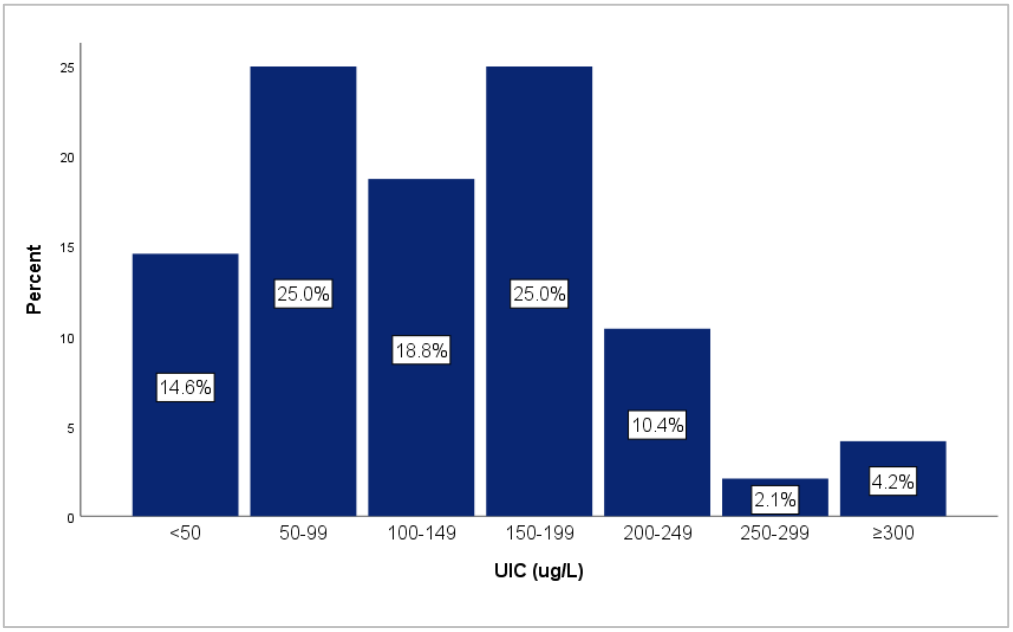

Figure 4.13 Percentage distribution of UIC (ug/L) during post-partum
UIC: Urinary iodine concentration. Based on 48 single post-partum samples from individual participants provided during post-partum.

4.6.1 Iodine-containing supplement use

The effects of reported daily iodine-containing supplement use on urinary iodine excretion during pregnancy are shown in Table 4.6 below. Results are based on three urine samples provided by 61 women over pregnancy (one in each of Stages 1-3), with supplement use unknown for one sample from one woman in Stage 3 (total n=182 samples). Bivariate groupings of 'Yes' for any amount of iodine-containing supplement used daily [includes urine samples from women who used daily supplements with <150 ug/d (n=12 urine samples) and ≥150 µg/d (n=130 urine samples)] and 'No' for samples from women who did not use daily iodine-containing supplements were adopted. No significant difference was found between supplement users and non-users for UIC (p=0.476). In contrast, the difference was significant for users and non-users based on UIC/Cr (p=0.000) (see Appendix I).

Table 4.6 Effect of daily iodine-containing supplement use on urinary iodine excretion over pregnancy

	UIC	**UIC/Cr**
'Yes' daily iodine supplement use[1]	176 (103, 295) n=142	307 (188, 461) n=142
'No' daily iodine supplement use[1]	194 (114, 321) n=40	199 (155, 295) n=40
p-value[2]	0.476	0.000

UIC: Urinary iodine concentration (µg/L): UIC/Cr: Urinary iodine-to-creatinine ratio (µg/g).
Based on 3 urine samples provided by 61 women over pregnancy; supplement use unknown for one sample for one woman (n=182 urine samples)
[1]Values are medians (IQR). 'Yes'/'No' relate to use of any amount of iodine supplement daily.
[2]Significant difference between UIC/Cr 'Yes' and 'No' values (Kruskal-Wallis test).

The outcomes of daily iodine-containing supplement use on urinary iodine excretion by study stage are shown in Tables 4.7 and 4.8 below, using the same 'Yes' and 'No' groupings for supplement use. Analysis showed that there was no significant stage by supplement interaction (p=0.84). Therefore, if there was a supplement effect it was likely to be similar across all stages. Considering each stage separately, the median UIC/Cr 'Yes' values were consistently higher than the corresponding 'No' values (see Table 4.7). The difference was significant for Stage 3 (p=0.010) and marginally significant for Stage 2 (p=0.064) and Stage 4 (p=0.069) (Kruskal-Wallis test)

(Appendix I). Table 4.8 shows that in terms of MUIC, both supplement and non-supplement users had adequate iodine intakes according to WHO criteria. Additionally, there were no significant differences between MUIC values for users and non-users at each stage (See Appendix I).

Table 4.7 Effect of daily iodine-containing supplement use on UIC/Cr by stage

	Stage 1[1]	**Stage 2[1]**	**Stage 3[1]**	**Stage 4[1]**
'Yes' daily iodine supplement use[2]	332 (180, 512) n=49	286 (191, 466) n=46	302 (196, 433) n=47	196 (134, 314) n=30
'No' daily iodine supplement use[2]	216 (175, 314) n=12	212 (160, 337) n=15	161 (131, 256) n=13	129 (91, 230) n=18
p-value[3]	0.128	0.064	0.010	0.069

UIC/Cr: Urinary iodine-to-creatinine ratio (µg/g).
[1]Stage 1: 10-14 or 18-22; Stage 2: 26-28; Stage 3: 36-38 wk gestation; Stage 4: 4-6 wk post-partum.
[2]Values are medians (IQR). 'Yes'/'No' relate to use of any amount of iodine supplement daily.
[3]Significant difference between Stage 3 'Yes' and 'No' values (Kruskal-Wallis test).

Table 4.8 Effect of daily iodine-containing supplement use on UIC by stage

	Stage 1[1]	**Stage 2[1]**	**Stage 3[1]**	**Stage 4[1]**
'Yes' daily iodine supplement use[2]	176 (89, 276) n=49	170 (76, 333) n=46	179 (121, 337) n=47	110 (60, 192) n=30
'No' daily iodine supplement use[2]	264 (154, 377) n=12	181 (106, 314) n=15	167 (105, 294) n=13	106 (64, 164) n=18
p-value[3]	0.102	0.907	0.654	0.798

UIC: Urinary iodine concentration ((µg/L).
[1]Stage 1: 10-14 or 18-22; Stage 2: 26-28; Stage 3: 36-38 wk gestation; Stage 4: 4-6 wk post-partum.
[2]Values are medians (IQR). 'Yes'/'No' relate to use of any amount of iodine supplement daily.
[3]No significant difference between 'Yes' and 'No' values for any stage (Kruskal-Wallis test).

4.6.2 Longitudinal trends in iodine status

As described in Chapter 3, analyses to determine gestational changes in iodine status (via urinary iodine) were carried out via linear mixed models for repeated measures longitudinal data, and linear models for associations as a single time. Trend analyses for (log base 10) UIC/Cr were based on study stages (median UIC/Cr values for Stages

1-3) and on the calculated gestational week of the urine sample (continuous data), as determined from gestational week at recruitment.

4.6.2.1 Analyses by study stage

Table 4.9 presents the median UIC/Cr results for each study stage. Stage 1 has been further divided and analysed according to gestational stage at time of recruitment (as discussed in Section 4.1). There was no significant difference between the median UIC/Cr values of Stage 1 Early v Late enrolment [Kruskal-Wallis test: χ^2(df=1, n=43) =0.315, p=0.575] (see Appendix I). Mixed model analysis on logged (base10) data was used to determine any significant differences between the median UIC/Cr values for Stages 1-4 (see Appendix I). The results are as follows:

- no significant overall difference between the median UIC/Cr values for Stages 1-3 (p=0.15), although there is a slight downward trend with Stage 3 marginally lower than Stage 1 (p=0.054).
- the estimated decline in UIC/Cr over the median range 21 to 37 weeks (16 weeks) would be 13.1%, with the actual median decline from Stage 1 to Stage 3 being 14.0%
- compared with these, the Stage 4 value is significantly lower than any of the Stage 1-3 values separately (p<0.0001) or combined (p<0.0001). (see Figure 4.14).

Table 4.9 UIC/Cr results by study stage

	Stage 1 Early enrolment[1] (n=18/61)	**Stage 1 Late enrolment[1] (n=43/61)**	**Stage 1[1] (n=61)**	**Stage 2[1] (n=61)**	**Stage 3[1] (n=61)**	**Stage 4[1] (n=48)**
Week of sample[2]	12 (10, 13)	21 (19, 23)	21 (10, 23)	27 (26, 29)	37 (36, 39)	6 (4, 7)
UIC/Cr[3] (µg/g)	349 (174-666)	312 (174-419)	314 (180-459)	267 (179-421)	270 (170-424)	183 (113-284)

UIC/Cr: Urinary iodine-to-creatinine ratio.
[1]Stage 1 Early enrolment: 10-14; Stage 1 Late enrolment: 18-22; Stage 1: 10-14 or 18-22; Stage 2: 26-28; Stage 3: 36-38 wk gestation; Stage 4: 4-6 wk post-partum.
[2]Values are medians (minimum, maximum).
[3]Values are medians (IQR). Not significantly different between Stage 1 Early v Late enrolment (Kruskal-Wallis test): *p*=0.57.

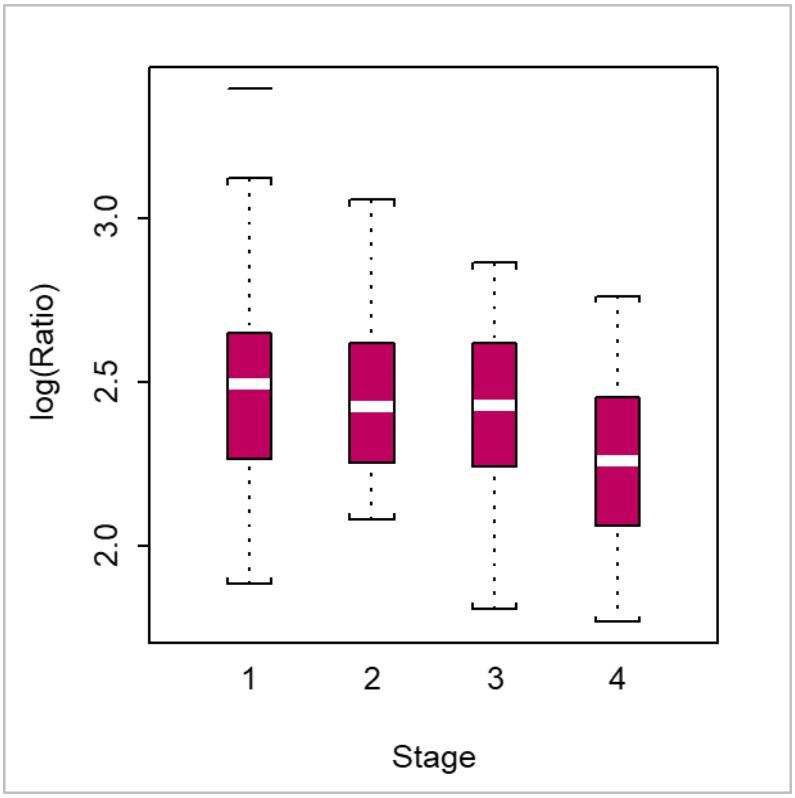

Figure 4.14 Results for UIC/Cr across study stages

UIC/Cr: Urinary iodine-to-creatinine ratio. Stage 1: 10-14 or 18-22; Stage 2: 26-28; Stage 3: 36-38 wk gestation; Stage 4: 4-6 wk post-partum.
Box and whisker plot – outer whiskers represent the data spread (maximum and minimum values); box represents the interquartile range; white horizontal line represents the median.

4.6.2.2 Analyses by gestational week

Analysis of (log) UIC/Cr during pregnancy based on gestational week (n=61) showed the following:

- a non-significant downward trend over the weeks (p=0.073)
- the log10 ratio is estimated to decline by 0.0038 per week on average, corresponding to an estimated decrease per week in UIC/Cr by a factor of 0.99 (95% CI 0.98, 1.08)
- over the range 10 to 39 weeks (29 weeks) the estimated decline of the log10 ratio would be 0.110 (22.4% decline over the period)
- if participants with known or possible gestational diabetes (n=10) are excluded from the analysis (n=61), the non-significant trend in (log) UIC/Cr during pregnancy remains (p=0.37)

With the inclusion of demographic factors (ie age, ethnicity) and dietary variables (ie iodine-containing supplements, iodised salt and six key iodine-containing foods) in the analysis model during pregnancy:

- age was positively associated (p=0.02) with UIC/Cr and iodine supplementation was marginally positive (p=0.06),
- ratio values were marginally significantly lower for Caucasians and those with diploma education (both p=0.07), and
- after adjusting for these factors, the trend over time was downwards but not significant (p=0.11).

4.7 Urine creatinine

Urine creatinine was measured at each study stage for the purpose of calculating the UIC/Cr (see Section 3.6). There was no significant change in creatinine over the weeks of gestation (p=0.15). Additionally, there was no significant difference between pregnancy and post-partum (log base 10) urine creatinine values overall (p=0.690) (See Figure 4.15).

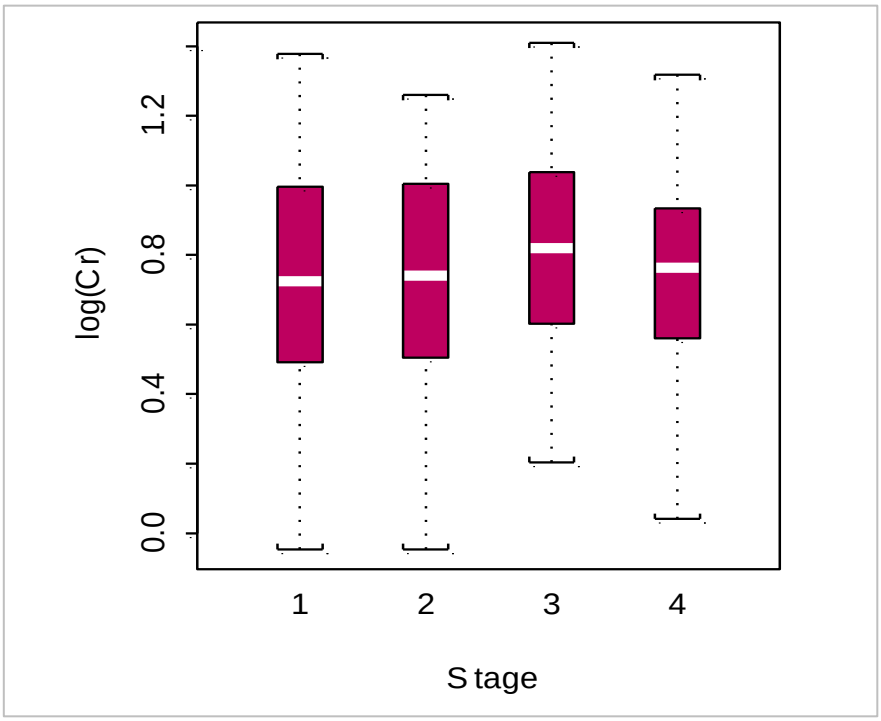

Figure 4.15 Results for urine creatinine
Stage 1: 10-14 or 18-22; Stage 2: 26-28; Stage 3: 36-38 wk gestation; Stage 4: 4-6 wk post-partum. Box and whisker plot – outer whiskers represent the data spread (maximum and minimum values); box represents the interquartile range; white horizontal line represents the median.

4.8 Thyroid stimulating hormone (TSH)

TSH was measured in participants twice during the study, once in Stage 1 or 2 (pregnancy) and once in Stage 4 (post-partum). As Table 4.10 indicates, the mean TSH values during pregnancy (Stage 1 n=18 and Stage 2 n=43) and postpartum (Stage 4 n=48) fell within the respective trimester-specific or non-pregnant reference ranges. Overall, five individual participants had TSH results outside the respective reference ranges as follows – one participant in Stage 1 (high), one participant in Stage 2 (low) and three participants in Stage 4 (all low). There was no significant difference in the mean TSH values for Stages 1 and 2, with individual measures relating to different participants in each stage (t=-0.653, df=59, p=0.516) (see Appendix I). Similarly, there was no significant difference in mean TSH values during pregnancy versus postpartum based on participants (n=48) with repeated TSH measures, that is, in both Stages 1/2 and Stage 4 (1.42 v 1.35 mIU/L, respectively; t=0.490, df=47, p=0.627) (see Appendix I).

Table 4.10 TSH values during pregnancy (Stage 1 or 2) and post-partum

	Stage 1 (n=18/61)	**Stage 2 (n=43/61)**	**Stage 4 (n=48/61)**
Week of sample[1]	12 (10-13)	27 (26-29)	6 (4-7)
TSH (mIU/L)[2,3,4]	1.36 (±0.68) Range: 0.59-3.10	1.47 (±0.59) Range: 0.01-2.90	1.35 (±0.65) Range: 0.3-3.0
Reference range[5]	0.02-2.5 (First trimester)	0.3-3.0 (Second trimester)	0.4-4.0 (Non-pregnant)

TSH: Thyroid stimulating hormone.
[1]Stage 1: 10-14; Stage 2: 26-28 wk gestation; Stage 4: 4-6 wk post-partum. Values are medians; (minimum, maximum).
[2]Values are means; (SD).
[3]Not significantly different between Stages 1 & 2 (Independent samples t-test): p=0.516
[4]Not significantly different between Stages 1/2 & 4 (Paired samples test): p=0.627; n=48
[5]PathWest reference ranges for use in pregnancy and non-pregnancy (See Table 2.5 in Section 2.8.2)

4.9 Free thyroxine (FT4)

FT4 was measured in participants using the same blood sample as TSH, once during pregnancy (Stage 1 or 2) and once postpartum (Stage 4). The FT4 result was not available for one Stage 1 participant. All individual and mean FT4 values for each stage were within the reference range used by PathWest Laboratories (9-19 pmol/L). There was a significant difference in the mean FT4 values for Stages 1 (n=17) and 2

(n=43), again with individual measures corresponding to different participants in each stage (t=3.783, df=58, p< 0.001) (see Table 4.11 and Appendix I). There was also a significant difference in mean FT4 values during pregnancy versus postpartum based on participants (n=47) with repeated FT4 measures, that is, in both Stages 1/2 and Stage 4 (12.9 v 12.1 pmol/L, respectively; t=2.651, df=46, p=0.011) (see Appendix I).

Table 4.11 FT4 values during pregnancy (Stage 1 or 2) and post-partum

	Stage 1 (n=17/61)	**Stage 2 (n=43/61)**	**Stage 4 (n=48/61)**
Week of sample[1]	12 (10-13)	27 (26-29)	6 (4-7)
FT4 (pmol/L)[2,3,4]	14.1 (±1.4) Range: 12-17	12.2 (±1.8) Range: 9-18	12.1(±1.2) Range: 10-15

FT4: Free thyroxine
[1]Stage 1: 10-14; Stage 2: 26-28 wk gestation; Stage 4: 4-6 wk post-partum. Values are medians; (minimum, maximum).
[2]Values are means; (SD).
[3]Significantly different between Stages 1 & 2 (Independent samples t-test): p<0.001
[4]Significantly different between Stages 1/2 & 4 (Paired samples test): p=0.011; n=47

4.10 Breast milk iodine concentration

Breast milk samples were provided by 45 participants (74% of total participants) between 28-56 days postpartum, with a mean (± SD) of 38.3 (± 5.8) days. All samples were collected between 0600 and 1200h. None of the participants were cigarette smokers. The median (IQR) BMIC was 180 (100-279) µg/L, indicating adequate iodine status for the group overall. Despite this, one-quarter of women (n=11/45) had BMIC <100 µg/L, the suggested cut-off level for adequacy (Semba & Delange, 2001) (see Figure 4.16). Of these 11 women, six reported not consuming iodine-containing supplements daily. Overall, there was a significant difference between the median BMIC values for women consuming iodine-containing supplements daily and those who did not [198 (n=28) vs 134 (n=17) µg/L, respectively; Kruskal-Wallis test: χ^2(df=1, n=45) =4.539, p=0.033] (see Appendix I). Outcomes based on all 54 breast milk samples provided in the study, including those from women who did not complete all study stages, are discussed elsewhere (Jorgensen, O'Leary, James, Skeaff, & Sherriff, 2016) (see Appendix J).

In terms of correlation (Pearson) between breast milk iodine and postpartum urinary iodine (Stage 4) as (log base 10) UIC/Cr, Figure 4.17 shows the two measures of iodine status were moderately positively correlated (r=0.43, p=0.004) (see Appendix I).

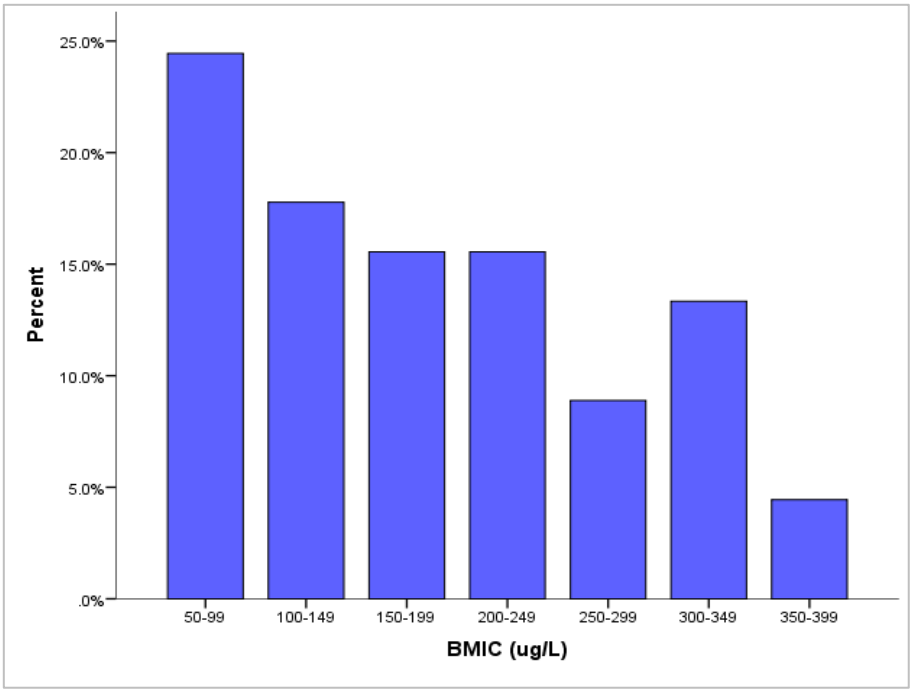

Figure 4.16 Percentage distribution of BMIC (ug/L) (n=45)
Values <100 µg/L indicate BMIC levels below adequacy.

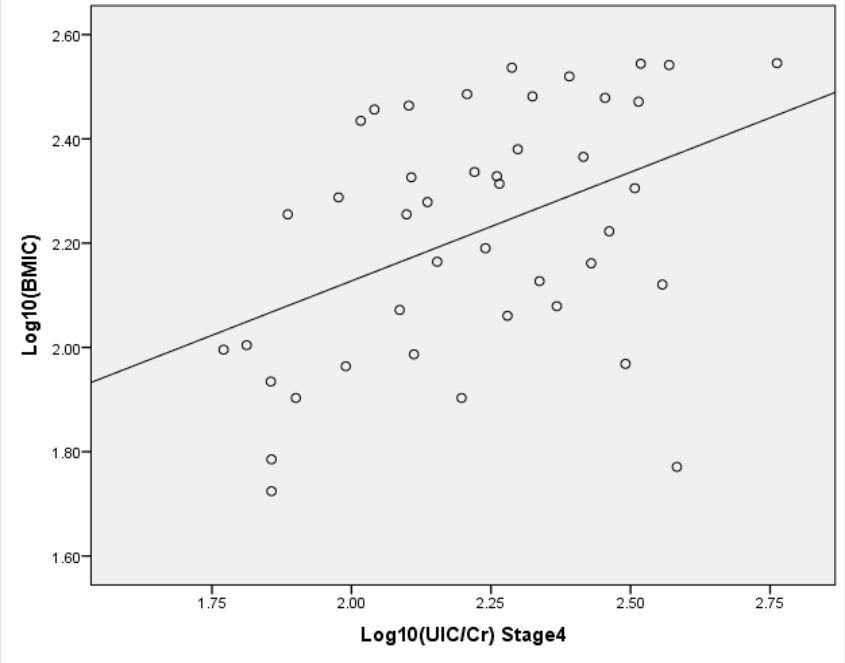

Figure 4.17 Correlation between UIC/Cr and breast milk iodine
Pearson correlations of log-transformed data. UIC/Cr vs Breast milk iodine moderately positively correlated: (r=0.43, p=0.004).

4.11 Summary

Iodine-containing supplement use:

- Overall, 85% of participants reported using an iodine-containing supplement during at least one stage of the study.
- Just over half of the participants (54%) used a daily iodine-containing supplement at every study stage, while 19% reported not using a daily iodine-containing supplement at any study stage.
- The proportion of participants using some form of iodine-containing supplement daily remained relatively constant during pregnancy at 82%, 77% and 80% for Stages 1-3, respectively.
- During pregnancy, 70-75% of participants reported daily use of supplements containing ≥150 µg of iodine and a further 7% of participants in each stage reported daily use of supplements containing <150 µg of iodine.
- Daily consumption of iodine-containing supplements decreased during the post-partum period (Stage 4) compared to use during Stages 1-3, both for participants overall and for those participants who completed all four study stages (n=48). Most women in Stage 4 were breastfeeding (96%; n=46/48) but only 65% (n=30/46) reported using some form of supplemental iodine.
- The overall usage rate of iodine supplements with ≥150 µg of iodine amongst breastfeeding women was 59% (n=27/46).

Daily food iodine consumption (based on participants with known consumption behaviour for each food):

- There was a decreasing trend for daily consumption of bread/bread products across gestation (82%, 74% and 64% for Stages 1-3, respectively) and 83% of post-partum participants consumed these products daily.
- Most participants reported consuming some cow's milk each day, with the proportion of daily consumers remaining relatively constant throughout the study (80%, 87%, 86% % and 81% for Stages 1-4, respectively).
- There was only slight variation in the proportion of participants consuming cheese daily across the four study stages, ranging from 48% (n=29/61) during Stage 2 to 51% (n=30/59) during Stage 3.

- There was some variation in the proportion of daily yoghurt consumers across the stages, ranging from 29% (n=14/48) for Stage 4 to 38% (n=23/61) for Stage 2.
- Daily egg consumption remained low and relatively constant across the four stages (28%, 26%, 24% and 31% for Stages 1-4, respectively).
- Compared to other foods investigated, daily ice cream consumption was the lowest during all four study stages, ranging from 4% (n=2/48) during post-partum to 10% (n=6/59) during Stage 3.

Iodised salt use:

- At each study stage, most participants reported not using use iodised salt (36%, 39%, 37% and 46% reported 'Yes' for Stages 1-4, respectively).

Urinary iodine:

- During pregnancy, the overall median UIC of 182 µg/L (IQR 106, 314) (based on 3 samples from 61 women; total n=183 samples) indicated adequate iodine intake for the group.
- For the post-partum results, a median UIC of 106 µg/L (IQR 63, 176) was indicative of adequate iodine intake for participants both breastfeeding (n=46) and non-breastfeeding (n=2). The post-partum MUIC value for breastfeeding women only was 105 ug/L.
- Participants were classified as having adequate iodine status at each study stage, based on median UIC values (186, 181, 177 and 106 µg/L for Stage 1-4, respectively).
- Overall during pregnancy, no significant difference was found between daily supplement users and non-users for UIC (176 v 194 µg/L, respectively; p=0.476). In contrast, the difference was significant for daily users and non-daily users based on UIC/Cr (307 v 199 µg/L, respectively; p=0.000).
- Considering each study stage separately, the median UIC/Cr values for daily supplement users were consistently higher than the corresponding non-daily user values. The difference was significant for Stage 3 (302 v 161 µg/L; p=0.010) and marginally significant for Stage 2 (286 v 212 µg/L; p=0.064) and Stage 4 (196 v 129 µg/L; p=0.069).
- In terms of MUIC, both supplement and non-supplement users had adequate iodine intakes at each stage, according to WHO criteria. Additionally, there were no significant differences between MUIC values for users and non-users at each stage.

- For longitudinal analysis of UIC/Cr by study stage during pregnancy, there was no significant overall difference between the median values for Stages 1-3 (p=0.15), though there was a slight downward trend with Stage 3 marginally lower than Stage 1 (p=0.054).
- The estimated decline in UIC/Cr over the median range 21 to 37 weeks (16 weeks) would be 13.1%, with the actual median decline from Stage 1 to Stage 3 being 14.0%.
- The Stage 4 value was significantly lower than any of the Stage 1-3 values separately (p<0.0001) or combined (p<0.0001).
- For longitudinal analysis based on actual gestational week rather than study stage, there was a non-significant trend over the weeks (p=0.073).
- The log10 ratio is estimated to decline by 0.0038 per week on average, corresponding to an estimated decrease per week in UIC/Cr by a factor of 0.99 (95% CI 0.98, 1.08).
- Over the range 10 to 39 weeks (29 weeks), the estimated decline of the log10 ratio would be 0.110 (22.4% decline over the period).
- If participants with known or possible gestational diabetes (n=10) are excluded from the analysis (n=61), the non-significant trend in (log) UIC/Cr during pregnancy remains (p=0.37).
- With the inclusion of demographic factors (ie age, ethnicity) and dietary variables (ie iodine-containing supplements, iodised salt and six key iodine-containing foods) in the analysis model during pregnancy, age was positively associated (p=0.02) with UIC/Cr and iodine supplementation was marginally positive (p=0.06).
- Ratio values were marginally significantly lower for Caucasians and those with diploma education (both p=0.07).
- After adjusting for these factors, the trend over time was downwards but not significant (p=0.11).

Urine creatinine:

- There was no significant change in spot urine creatinine over the weeks of gestation (p=0.150).
- Additionally, there was no significant difference between pregnancy and post-partum (log base 10) urine creatinine values overall (p=0.690)

TSH:

- The mean TSH values during pregnancy (Stage 1 n=18 and Stage 2 n=43) and postpartum (Stage 4 n=48) fell within the respective trimester-specific or non-pregnant reference ranges.
- There was no significant difference in the mean TSH values for Stages 1 and 2, with different participants in each stage (t=-0.653, df=59, p=0.516).
- Similarly, there was no significant difference in mean TSH values during pregnancy versus postpartum based on participants (n=48) with repeated TSH measures, that is, in both Stages 1/2 and Stage 4 (1.42 v 1.35 mIU/L, respectively; t=0.490, df=47, p=0.627).

FT4:

- The mean FT4 values for each stage were within the reference range used by PathWest Laboratories (9-19 pmol/L).
- There was a significant difference in the mean FT4 values for Stages 1 (n=17) and 2 (n=43), with different participants in each stage (t=3.783, df=58, p< 0.001).
- There was also a significant difference in mean FT4 values during pregnancy versus postpartum based on participants (n=47) with repeated FT4 measures, that is, in both Stages 1/2 and Stage 4 (12.9 v 12.1 pmol/L, respectively; t=2.651, df=46, p=0.011).

BMIC:

- The median BMIC (IQR; n=45 samples) was 180 (100, 279) µg/L, indicating adequate iodine status for the group overall.
- One-quarter of women had BMIC less than 100 µg/L, the suggested cut-off level for adequacy.
- There was a significant difference between the median BMIC values for women consuming daily iodine-containing supplements and those who did not (198 vs 134 µg/L, respectively).
- There was a moderate positive correlation between post-partum UIC/Cr and BMIC (r=0.43, p=0.004).

Chapter 5 Discussion

The primary objective of this study was to investigate the effect of advancing gestation on iodine status in a cohort of pregnant women in Perth, Western Australia, as determined by urinary iodine excretion. This is the first study to report on the iodine status of pregnant and post-partum/breastfeeding women (same cohort) in WA. It is also among the first to investigate this issue in Australia post-mandatory fortification and NHMRC iodine supplement recommendation. Furthermore, it is one of few studies to assess BMIC in Australian women after the introduction of mandatory iodine fortification and the NHMRC iodine supplement recommendation.

5.1 Iodine status

5.1.1 Urinary iodine excretion

The WHO recommends a median urinary iodine concentration (MUIC) of 150-249 µg/L in pregnant women reflects adequate iodine intake at a population level. For lactating women and children, a MUIC of ≥100 µg/L is considered adequate iodine intake (WHO/UNICEF/ICCIDD, 2007). The present study showed that this cohort of WA pregnant women had sufficient iodine intake, based on the MUIC overall for pregnancy of 182 µg/L and the median UICs at each pregnancy study stage (186, 181 and 177 µg/L for Stages 1-3, respectively). This outcome is consistent with the results of two recent Australian studies investigating iodine status in pregnant women conducted in 2011-2012 post-mandatory fortification and iodine supplement recommendation. Specifically, a cross-sectional study (n=95) conducted in NSW in 2012 involving women in all trimesters recorded a MUIC of 166 µg/L (Charlton et al., 2013). Furthermore, a large longitudinal study conducted in SA recorded MUICs in the same women at <20 wk gestation (189 µg/L; n=781) and at 28 wk gestation (172 µg/L; n=730) (Condo et al., 2016). The results of the present study also compare favourably with those from the most recent national survey of general population iodine status in Australia, the 2011-12 National Health Measures Survey, which showed a MUIC for WA adults (male and female) of 157.4 µg/L and a MUIC of 121.0 µg/L for Australian women of child-bearing age (Australian Bureau of Statistics, 2013). However, as discussed in Section 3.3.1, the present study was not designed to

be representative of the general pregnant population in Perth and therefore the results are unlikely to be generalisable to the wider pregnant population.

For post-partum (Stage 4), the majority of women who provided a urine sample (n=46/48; 96%) were breastfeeding. The MUIC of these samples provided in the first six weeks post-partum was 105.5 µg/L , indicative of adequate iodine intake according to WHO criteria for lactating women of ≥100 µg/L (WHO/UNICEF/ICCIDD, 2007). There is a paucity of data on the iodine status of lactating mothers in Australia. Only two other studies have investigated this issue since the introduction of mandatory fortification, with both MUIC values considerably higher than that in the present study. Specifically, a small study (n=60) conducted in the Illawarra region (Sydney, NSW) involving mothers within their first six months of breastfeeding, found a MUIC of 123 µg/L. This represented an improvement in iodine status in this population group in this location, compared with the pre-fortification period (Axford, Charlton, Yeatman, & Ma, 2011). The second and larger study (n=686) of breast feeding mothers (3 months post-partum), conducted in SA, recorded a similar MUIC of 125 µg/L (Huynh et al., 2017). Possible reasons that may have contributed to the considerable differences between the post-partum MUIC results of the present study and those of the NSW and SA studies include different UIC analysis methods (ICPMS vs Sandell-Kolthoff technique, respectively) and the variability in timing of post-partum sampling. Furthermore, while collectively these studies provide some evidence that Australian breastfeeding women have adequate iodine intakes, based on MUIC levels, similar studies should also be conducted in other states where iodine status has been shown to be more problematic, such as Tasmania.

Despite the present study's impressive MUIC results, UIC results for some urine samples suggested that some women in the study may have been at risk of either inadequate iodine intake or excessive iodine intake. Specifically, the UIC in 40% (n=73/183) of urine samples provided during pregnancy and 44% (n=21/48) of post-partum urine samples indicated insufficient iodine intake, based on the WHO criteria of <150 µg/L for pregnancy and <100 µg/L for lactation and adults in general (WHO/UNICEF/ICCIDD, 2007). In contrast, 7.7% of pregnancy urine samples indicated excessive iodine intake (that is ≥500 µg/L). While such statistics are often reported in the literature, a single spot UIC should not be used to assess iodine status in

individuals due to high day-to-day variability in UIC values. Therefore, it cannot be assumed that all individuals with a spot UIC <150 µg/L (pregnancy) and <100 µg/L (lactation/adults) were iodine deficient (WHO/UNICEF/ICCIDD, 2007; Zimmermann, 2012). While these outcomes may raise concern for individuals, it is for this reason that MUIC is the recommended indicator of iodine status for populations groups.

In terms of longitudinal analyses (unadjusted), there was a small but non-significant decline in iodine status across gestation, as determined by (log) UIC/Cr. This finding was consistent for analyses based on comparisons of median UIC/Cr values for Stages 1-3 ($p=0.15$), for Stage 1 median value compared with Stage 3 median value ($p=0.054$), and for UIC/Cr results based on actual gestational week rather than study stage (ie 10 -39 wks) ($p=0.073$). The impact of demographic and dietary variables on study outcomes for UIC/Cr was inconclusive given the study's small sample size. Nevertheless, after adjusting for factors which had either a significant (age) or marginally significant (iodine supplementation, ethnicity, education) effect on UIC/Cr, the trend over time remained downwards but not statistically significant ($p=0.11$). Interestingly, any changes in the consumption of key-iodine containing foods during gestation did not significantly affect UIC/Cr ratios, including bread/bread products despite the considerable decrease in the number of participants who reported daily consumption of these foods over gestation (see Figure 4.5). The reasons for this decreased consumption are unknown.

Interestingly, gravidity was not a significant factor influencing UIC/Cr in the present study, however, age was significantly positively associated with UIC/Cr during pregnancy and post-partum. Larger, future studies could investigate whether age influenced UIC/Cr values via changes in creatinine excretion or iodine intake. In addition, as concluded by other authors, it is difficult to determine to what extent the underlying physiologic changes that occur during pregnancy (such as changes in renal iodine loss, placental storage of iodine and transfer of iodine to the fetus) affect iodine status during pregnancy (Bath et al., 2015).

Furthermore, a non-significant change in (log) UIC/Cr during pregnancy ($p=0.27$) remained when excluding participants with known or possible gestational diabetes ($n=10$) in the present study. Despite the apparent lack of impact on the change in (log)

UIC/Cr during pregnancy, the potential effects of diabetes on gestational changes in iodine status warrant further investigation in future studies.

As mentioned in Section 2.10.1.1, just one other longitudinal study has shown no significant change in iodine status over gestation, based on UIC/Cr. In this study (n=65), conducted in the iodine-sufficient country of Japan, authors compared median UIC/Cr trimester values (Fuse et al., 2013). However, a significant change was reported by Bath et al. (2015), the only other longitudinal study (n=230) to have used gestational week of sampling to evaluate the change in UIC/Cr over gestation. This UK study found UIC/Cr increased with advancing gestation by a factor of 1.05 (95% CI: 1.02, 1.08) per week in winter and by a factor of 1.04 (95% CI; 1.00, 1.08) per week in summer. The authors stated that this seasonal difference was due to a known marked seasonal change in the iodine content of UK dairy products, the main source of dietary iodine (Bath et al., 2015). Interestingly, dairy foods have also been found to be the main contributor to iodine intake in Australian studies involving pregnant women (Charlton et al., 2013; Condo et al., 2016). Whether there is seasonal variation in the iodine concentration of dairy products influencing gestational change in iodine status in pregnant women in Perth could be explored in future studies.

There are a number of timing-related factors relevant to any investigation of UIC, the present study being no exception. One aspect to consider is the time of the day when sampling occurs. A study in Switzerland of 3 023 spot urine samples collected from 42 individuals (29 adults and 13 children), at any time of the day at the rate of three to five samples per month over a 2-year period, showed a circadian rhythm for UIC. Lowest UIC levels were observed in the morning (8 - 11 h), with increasing concentrations during the afternoon and evening. This observation was independent of the individual subject, age, gender and season (Als et al., 2000). In addition, a Danish study of pregnant women (n=158), their male partners (n=157) and children (n=51) showed this same diurnal variation for both UIC and urinary creatinine, however, adjusting UIC for urinary creatinine concentration levelled out the time-dependent differences in UIC (S. L. Andersen, Sørensen, Krejbjerg, Møller, & Laurberg, 2014).

A second factor of likely greater significance is the timing of spot sampling in relation to intake of iodine-containing food or supplementation. Peaks in UIC have been reported 4-5 hours after main meals, with children's peaks occurring later than that of

adults (Als et al., 2000). Furthermore, Andersen et al., (2014) showed that median UIC was higher in pregnant women (and their male partners) when urine sampling occurred on the same day as iodine supplementation. These results highlight the close relationship between recent iodine intake and the UIC profile. In the present study, timing of spot urine sampling in relation to both time of day and span of time since intake of iodine-containing food and/or supplement was not considered. Participants were instructed to provide non-fasting samples but the timing of sampling was not specified to participants in order to facilitate compliance. This is a topic for further investigation in a larger follow-up study.

5.1.2 Breast Milk Iodine Concentration (BMIC)

The median (IQR) BMIC of 180 (100-279) µg/L for the present study is consistent with the result from the sole other Australian study investigating BMIC post-mandatory iodine fortification and NHMRC supplement recommendation. This South Australian study collected breast milk samples within 7 d of delivery from 653 women in 2012-2013 and reported a median (IQR) BMIC of 187 (130-276) µg/L (Huynh et al., 2016). At follow-up 3 months postpartum, median (IQR) BMIC for the 538 women still breastfeeding in this study was 127 (84-184) µg/L (Huynh et al., 2017). In this study, 13% and 35% of the mothers had a BMIC below the suggested adequate cut-off of 100 µg/L at birth and 3 months, respectively (Huynh et al., 2016; Huynh et al., 2017), compared to 24% in the present study (mean 5.5 wk post-partum). While information regarding iodine-containing supplement use in lactation was not collected in the South Australian study (Huynh et al., 2017), just over half of the women with a BMIC <100 µg/L in the present study reported not consuming daily iodine-containing supplements.

As for urinary iodine excretion, BMIC fluctuates throughout the day (Kirk et al., 2012) and appears to be influenced by recent maternal iodine intake. Studies have shown BMIC to be dependent on the timing of the most recent iodine supplement intake, with a peak median BMIC shown to occur 6 hours after an acute ingestion of high dose supplemental iodine in 16 lactating women in USA, despite MUIC remaining stable over the 8-h study period (Leung, Braverman, He, Heeren, & Pearce, 2012). A second study, conducted in Denmark (n=127), found that BMIC was highest when sampling occurred on the same day as supplement intake (not acute high dose), compared with supplement intake the day before or earlier (S.L. Andersen, Møller, & Laurberg, 2013).

The timing of breast milk sampling in relation to iodine-containing supplement or food intake was not requested in the present study.

The present study is the first Australian study post-mandatory iodine fortification and supplement recommendation to report on the association between BMIC and maternal postpartum urinary iodine excretion (as UIC/Cr). The finding of a moderate, positive correlation ($r=0.43$, $p=0.004$) between these two measures of iodine status is consistent with the result from the only other Australian study investigating this relationship ($r=0.52$, $p<0.001$). This study (n=50) was conducted in Sydney in 2000 before mandatory fortification and samples were collected between 3 to 9 days postpartum (Chan, Hams, Wiley, Wilcken, & McElduff, 2003). A Danish study (n=127) assessing this association also concluded that urinary iodine as UIC/Cr may be a useful predictor of BMIC (S.L. Andersen et al., 2013).

5.1.3 Thyroid Stimulating Hormone (TSH)

As mentioned in Section 2.8.2.1, TSH has been previously assessed in pregnant women in WA by Gilbert et al. (2008) for the purpose of establishing first trimester-specific reference ranges. The present study is the first study to measure TSH concentrations in WA pregnant women in relation to iodine status. As may be expected given the apparent adequate iodine status of the study cohort (as assessed by median urinary iodine excretion), mean TSH concentrations were within the respective reference ranges during both pregnancy and post-partum stages. However, it is important to restate here that while TSH is a good indicator of thyroid function, it is now recognised as being an insensitive marker of iodine status (Bath et al., 2017; Rohner et al., 2014). Nevertheless, this outcome is consistent with the evidence regarding the use of tests to determine iodine status. Against expectations, the observed non-significant differences between mean TSH concentrations during pregnancy (Stage 1 vs Stage 2) and between mean pregnancy vs postpartum concentrations may be explained by the relatively small sample size.

5.1.4 Free Thyroxine (FT4)

The results for individual and mean FT4 concentrations during pregnancy and post-partum also supported the overall finding of adequate iodine status for the study cohort, with the same consideration as for TSH regarding FT4 being an insensitive marker of

iodine status (Bath et al., 2017; Rohner et al., 2014). Despite this limitation, the observed trend in FT4 across the study was as expected, with mean FT4 concentrations being highest in early pregnancy (Stage 1 vs Stage 2) and higher during pregnancy compared to post-partum.

5.2 Iodine-containing supplements

Overall, 87% of participants reported daily use of an iodine-containing supplement (of any quantity of iodine) during at least one stage of the study. The sociodemographic and pregnancy-related characteristics of iodine-containing supplement users are presented in Section 4.3 for interest only and will not be discussed here as this is outside the main focus of the present study.

5.2.1 Pregnancy

Daily iodine supplement use was consistently high during the pregnancy stages of the study, with 82%, 77% and 80% of participants reporting use during Stages 1-3, respectively. These usage rates compare favourably with a large cross-sectional study (n=425) conducted in Perth during 2012-13 involving a different cohort of pregnant women (at any stage of pregnancy) attending the same public tertiary hospital for women and neonates as the present study, where 66% of participants reported use of iodine-containing supplements during pregnancy (Hine, Zhao, Begley, Skeaff, & Sherriff, 2018).

In total, nine published Australian studies involving pregnant women have investigated iodine-containing supplement usage and adherence to the supplement recommendation following its introduction in 2010 (adherence defined as daily iodine supplementation of ≥150 µg/d) (see Appendix K). The present study's iodine-containing supplement use during pregnancy is consistent with these earlier studies, where usage ranged from 47-81% of participants (Charlton et al., 2013; Clifton et al., 2013; Condo et al., 2016; El-mani, Charlton, Flood, & Mullan, 2014; Hine et al., 2018; Lucas, Charlton, Brown, Brock, & Cummins, 2014; Rahman et al., 2011). Five of these studies (71%) were cross-sectional in design, determining iodine-containing supplement use at just one time point. While Clifton et al. (2013) and Condo et al. (2016) determined iodine-containing supplement use four and two times during their respective studies, only

overall supplement use was reported by the authors. Therefore, the present study is the first Australian study to report iodine-containing supplement use on multiple occasions in the same cohort of pregnant women.

Comparatively fewer Australian studies involving pregnant women have investigated compliance with the national daily iodine supplement recommendation, with adherence ranging from 23-62% (Condo et al., 2016; Malek, Umberger, Makrides, & Zhou, 2016; Martin, Savige, & Mitchell, 2014) (see Appendix K). The results of the present study are consistent with these studies, where 70-75% of pregnant participants reported daily use of supplements containing ≥150 µg of iodine. The comparatively high prevalence and adherence rates regarding use of iodine-containing supplements during pregnancy in the present study suggests good awareness of the importance of their use amongst this cohort of women. However, participation in a study of this design likely attracts women with a higher level of overall motivation and commitment than might otherwise be found in the general population of pregnant women, therefore questioning the generalisability of results. The discrepancy with the results of the larger cross-sectional study by Hine et al. (2018), as previously discussed, also supports this line of thinking.

Furthermore, iodine-containing supplement use in the present study was substantially higher than use in all five international studies that have investigated gestational change in iodine status based on UIC/Cr (see Table 2.9). In the UK study, just 3% of participants were taking a supplement that contained iodine. However, the authors reported that most prenatal supplements that contained iodine also contained selenium, the latter being an exclusion criterion as participants in this study were originally recruited for a selenium in pregnancy study (Bath et al., 2015). In addition, women taking iodine-containing supplements were excluded from the Chinese study (C. Li et al., 2016; Zhang et al., 2017). No participants reported iodine-containing supplement use in the small (n=15) Swiss study where iodised salt was the main source of dietary iodine (Brander et al., 2003). Iodine-containing supplement use was 0% in the two studies conducted in Japan, a long-term iodine-sufficient country due to the regular consumption of iodine-rich foods such as seaweeds and kelp and where fortification of table salt was prohibited (Fuse et al., 2011; Fuse et al., 2013). Therefore, these studies were able to observe the changes in iodine excretion across gestation with

minimal or no background of iodine-containing supplement-use. This situation would be more difficult and somewhat undesirable to achieve in WA and Australia as a whole given the national supplement recommendation for this population subgroup.

In terms of the effect of iodine-containing supplement use during pregnancy, no significant differences were found between the MUIC of daily iodine supplement users and non-users overall (for Stages 1-3 combined) and for each pregnancy stage individually. Furthermore, the MUIC values overall during pregnancy and for each pregnancy stage for both supplement users and non-users indicated adequate iodine intake, according to WHO criteria (WHO/UNICEF/ICCIDD, 2007). Other larger Australian longitudinal (Condo et al., 2016) and cross-sectional (Charlton et al., 2013) studies have reported significantly higher MUIC levels in women taking iodine-containing supplements compared with non-supplement users. A smaller sample size may have been the reason this expected outcome was not obtained in the present study. Expressing urinary iodine excretion as UIC/Cr rather than as UIC may be more appropriate under these circumstances. Based on UIC/Cr, the median value of supplement users was significantly higher than that for non-users overall (306.8 vs 199.2 µg/g; p=0.000) and for Stage 3 (302 vs161 µg/g; p=0.010), with marginal significant difference for Stage 2 (286 vs 212 µg/g; p=0.064). These results may indicate the greater utility of the iodine-to-creatinine ratio compared to the UIC alone, given the ratio corrects for GFR, state of hydration and muscle mass.

Furthermore, a point of interest to note was the considerable increase in the numerical values in the 'Yes' daily supplement use group but not in the 'No' daily iodine supplement use group when comparing data expressed as UIC/Cr with the corresponding data expressed as UIC (see Tables 4.7 and 4.8). The most likely reason for this observation is a statistical aberration due to the considerable differences in the number of urine samples between the 'Yes' and 'No' groups.

5.2.2 Lactation

The prevalence of iodine-containing supplement use during lactation in the present study (65%) was within the range reported for the two other Australian studies conducted to date post-supplement recommendation. In the earlier study in NSW (n=60), 45% of breastfeeding women used iodine-containing supplements (Axford et

al., 2011) (see Appendix K). The second and larger study (n=635), conducted in SA, found iodine-containing supplement use was 90% amongst participants (Huynh et al., 2016). However, neither study reported the adherence to the recommendation of daily intake of 150 µg of supplemental iodine during lactation, which was determined as 59% in the present study.

Despite the drop-off in use of iodine-containing supplements in general, and of those that meet the national supplement recommendation, from pregnancy to lactation in the present study, post-partum MUIC levels indicated adequate iodine intake for both iodine-containing supplement users and non-supplements users. Furthermore, there was no significant difference between MUIC values for the two groups. The difference in median values for supplement and non-supplement groups was marginally significant based on UIC/Cr ratio (196 vs 129 µg/g; p=0.069). Conversely, in the study by Axford et al. (2011), women consuming iodine-containing supplements had significantly higher MUIC than those who did not and non-supplement users did not have adequate iodine intake, based on WHO criteria for MUIC (206 vs 97 µg/L, respectively; p=0.029). In addition, in the SA study, MUIC levels were significantly higher in supplement users (195 µg/L) compared with non-users (137 µg/L; p<0.001) and consistent with the present study, both groups had adequate iodine intakes (Huynh et al., 2016).

In terms of BMIC, those women in the present study who reported using iodine-containing supplements daily had a significantly higher median BMIC than those who did not. Despite this, the median BMIC value of 134 µg/L for women who reported not consuming iodine-containing supplements daily indicates it was possible for some women in the study cohort to achieve adequate BMIC without daily supplementation. Nevertheless, it would appear that the simplest way to essentially guarantee adequate BMIC would be for women to adhere to the current supplement recommendation. For this to be achieved, greater promotion of the importance of continuing iodine-containing supplement use during lactation is required.

5.2.3 Evidence update

A point of considerable interest is that the recommendation for routine iodine-containing supplement use in women who are pregnant, breastfeeding or planning a

pregnancy still remains questionable. As an update to discussion in Section 2.7.2, a recent Cochrane review involving over 2700 women from 11 randomised control trials (RCT) failed to draw any significant conclusions regarding the benefits and risks of routine iodine supplementation. Evidence was found for both benefits (eg. decreased likelihood of postpartum hyperthyroidism) and adverse effects (eg. increased likelihood of digestive intolerance) of iodine supplementation, which need to be considered when deciding about routine use or not (Harding et al., 2017). It must be acknowledged, however, that this outcome is based on limited data of low to very low quality of evidence, mostly due to limitations in study design and wide confidence intervals, resulting in most of the findings coming from one or two trials with small numbers of women involved (Harding et al., 2017). In addition, almost all of the trials included in the review were conducted in regions of mild to moderate deficiency, so applicability to areas with severe deficiency is unknown. Two key recommendations of the review were for more high-quality RCTs to provide conclusive evidence on the issue and for trials to investigate outcomes in children beyond the neonatal period (Harding et al., 2017).

One such study published since the review (and mentioned in Section 2.7.2) randomly assigned 832 women in early pregnancy (mean gestational age 10.7 wk) from two mildly iodine-deficient areas (India: n=318 and Thailand: n=514) to receive either 200 µg iodine supplement orally or placebo once daily until term. Supplementation compliance was assessed by monthly tablet counts and was found to be 87% (mean). The results showed no significant effect of daily iodine supplementation on child neurodevelopment at 5-6 years, as measured by IQ and executive function (Gowachirapant et al., 2017). However, according to one reviewer, while this trial contributes to the body of evidence on this topic, it should not be taken as definitive proof as there were some important issues regarding the study design that need to be considered when interpreting the results. Consequently, further RCTs are still required (Bath, 2017).

5.3 Strengths of this study

A considerable strength of the present study in terms of study design was the inclusion of repeated measures of dietary and biochemical factors in the same individuals, thereby

reducing inter-individual variability. Furthermore, the present study was truly longitudinal, with no missing urinary iodine data (the biomarker of most interest in this study) for the 61 participants who completed Stages 1-3. In addition, this study reported urinary iodine excretion in two ways, as UIC and as UIC/Cr which corrects for variation in urine volume/dilution in spot samples. Most other studies exploring gestational change in iodine excretion have been cross-sectional in design, have not included complete urinary data sets for all participants and have reported urinary iodine excretion as UIC only.

Another strength of the present study was the inclusion of women via a variety of recruitment methods and from a number of settings, including both public and private health care systems. While this eventuated by necessity due to early recruitment difficulties, it most likely contributed to the diversity of the study cohort. Also, recruitment and data collection occurred year-round, thereby limiting seasonal bias (in relation to iodine intake) which has been reported in some UK studies (Bath et al., 2015; Bath, Walter, Taylor, Wright, & Rayman, 2014).

5.4 Limitations of this study

A limitation of this study, and many other similar studies, was that women were recruited by self-selection, thereby introducing selection bias. This factor, combined with the ongoing and high degree of participant involvement required, likely contributed to a study cohort with an over-representation of women with a higher reported education level and higher reported household incomes compared with available Western Australian data from the Australian Bureau of Statistics Census 2011 (Australian Bureau of Statistics, 2011b). In addition, women in the study were slightly older than the average woman who gave birth in WA in 2013 (mean 31.6 vs mean 29.8 yr, respectively) and a higher proportion were pregnant for the first time compared with WA figures at the time (48% vs 43%, respectively) (Hutchinson & Joyce, 2016). For these reasons, the results of this study may not be generalisable to the wider WA pregnant and post-partum population. However, it is also important to restate here that from the outset, the study was unlikely to consist of a representative sample of this subpopulation.

A second key limitation of the present study was the small sample size. In spite of early recruitment difficulties (as described in Section 3.3.1), a tight overall study timeframe with lengthy participant follow-up meant that recruitment had to cease when scheduled to ensure study completion. Furthermore, the attrition rate of 50% (from recruitment/consent to Stage 3) was twice that expected. The limited sample size, together with the study being conducted only in Perth, challenges the generalisability of results to the WA pregnant and post-partum population and may also have contributed to the lack of significant change in iodine status observed during pregnancy.

Although the study reports the change in iodine excretion across gestation based on the gestational week of the urine sample (as opposed to trimester as in most other studies), due to the study design, there was a cluster of samples around certain weeks of gestation (namely 10-14, 18-22, 26-28 and 36-38 wk). There are no data before 10 wk nor for every week of gestation. These limitations are acknowledged by Bath and colleagues in their longitudinal study of pregnant UK women of similar design to the present study (Bath et al., 2015). In addition, for practical reasons, there were no repeat urine samples within each stage. However, this would have provided a better estimate of usual intake for the group by correcting for intraindividual variation (Bath et al., 2015). Similarly, repeat breast milk samples may have improved measurement of daily iodine output or maternal iodine sufficiency, for the reasons discussed in Section 5.1.2.

The study asked participants to report intake of iodine-containing supplements and key foods as ‘yes’ or ‘no’, based on daily consumption. However, it was possible that participants consumed the foods/supplement less frequently than daily (and so reported ‘no’ for daily intake) but then consumed the foods and/or supplement on the day of urine or breast milk sample provision, thereby distorting UIC/BMIC results. In addition, as previously mentioned, the time span between intake of iodine-containing food and/or supplements and spot urine and breast milk sampling was unknown in the present study. A better way to investigate this may have been to also ask participants about supplement use on the actual days of sample provision, and perhaps even to ask participants to record the time of supplement taking on these days. Despite being useful, it would have been too burdensome to have asked participants to record timing and content of last meal/food consumed in relation to sample provision.

The present study did not measure maternal thyroid peroxidase antibodies (TPO-Ab), which are known to influence TSH concentrations. As previously mentioned in

Section 2.8.2.1, increased TPO-Ab concentrations are associated with higher TSH concentrations (Pearce et al., 2008). For this reason, similar studies excluded women who were positive for TPO-Ab (Bath et al., 2015; C. Li et al., 2016), however, limited financial resources prevented measurement of this factor in the present study. It is difficult to predict what effect this had on TSH outcomes in this study. Importantly, a Japanese study reported no significant difference in median UIC and UIC/Cr between TPO-Ab-positive (n=77) and TPO-Ab-negative (n=653) pregnant women (Fuse et al., 2011).

Furthermore, participants were asked to report on their cigarette smoking behaviour during Stage 4 (post-partum) only. It was an oversight to not ask women to report their smoking status in all stages of the study, as the thiocyanate in cigarette smoke is a known potent inhibitor of iodide transport into the thyroid (see Section 2.4.2) and the lactating mammary gland (see Section 2.4.3). For this reason, current smokers were excluded in other similar studies (eg Bath et al., 2015). Conversely, other authors reported that smoking was not associated with changes in UIC, although no supporting data were available (Clifton et al., 2013). Interestingly, no participants reported smoking during Stage 4 in the present study, making it unlikely yet unconfirmed that any participants were smokers during Stages 1-3.

Chapter 6 Conclusions and Recommendations

This was the first study to assess the iodine status of WA pregnant and post-partum women using biochemical measures (namely UIC, TSH, FT4 and BMIC) and to conduct a longitudinal investigation of these biomarkers in the same cohort of WA women. It was also among the first in WA to examine the use of iodine-containing supplements in these subgroups and to determine the appropriateness of the national iodine supplement recommendation in this state.

The present study's finding of no significant change in (log) UIC/Cr over gestation adds to the limited longitudinal evidence to date based on this measure of iodine status. This analysis was based on the actual gestational week of urine sampling rather than trimester as this provided more precise information. Interestingly, the considerable reduction in the number of participants who reported daily consumption of bread/bread products (Australia's only mandatory iodine-fortified foods) over gestation did not significantly influence UIC/Cr outcome. The sole factor found to have a significant (positive) effect on UIC/Cr during pregnancy, and also during post-partum, was maternal age. It is unknown whether age influenced UIC/Cr values via changes in creatinine excretion or iodine intake.

Pregnant and post-partum/breastfeeding women (n=61 and n=48, respectively) in the present study had adequate iodine intake, based on all biomarkers of iodine status assessed. This outcome is consistent with the results of other published Australian studies of pregnant and post-partum women (based on MUIC and median BMIC) conducted since the introduction of mandatory fortification and iodine supplement recommendation in NSW (Axford et al., 2011; Charlton et al., 2013) and SA (Condo et al., 2016; Huynh et al., 2016; Huynh et al., 2017). The outcome of the present study is also consistent with results from recent investigations of iodine status, as measured by MUIC, in other population subgroups in WA, namely adults and school-aged children (Australian Bureau of Statistics, 2013). This suggests that these groups may be reasonable proxies for pregnant and post-partum (including breastfeeding) women in relation to iodine status in this Australian state.

A key outcome of the study was the consistently high use of daily iodine-containing supplements during gestation, including those with ≥150 µg iodine as per the NHMRC recommendation. However, for the unadjusted analysis, there was no significant difference between the MUIC of daily iodine-containing supplement users compared with non-users during pregnancy, both overall and for each study stage individually. In addition, the MUIC of pregnant women who did not use iodine-containing supplements daily was still indicative of adequate iodine intake. Furthermore, there was only marginal evidence of an increase in UIC/Cr with supplement usage, based on the adjusted analysis using actual gestational week. For post-partum, the differences in MUIC and median UIC/Cr between supplement users and non-users were not significant. In contrast, the median BMIC of supplement users was significantly higher than that for non-supplement users. Despite the considerable drop-off in use of iodine-containing supplements from pregnancy to post-partum, the post-partum MUIC and median BMIC of both supplement users and non-users indicated adequate iodine intake.

Overall, these outcomes challenge the applicability of the NHMRC routine recommendation of daily iodine supplementation for all pregnant and breastfeeding women in Perth. However, it is acknowledged that results of the present study may not be generalisable to the wider pregnant and post-partum populations in Perth and WA. This is due to a small sample size and the fact that women in the study were self-selected, highly motivated and on average slightly older and better educated than the general pregnant and post-partum WA populations. Therefore, in the absence of more representative research suggesting otherwise, there is reasonable argument in support of maintaining the current iodine supplement recommendation for pregnant and post-partum women in Perth.

Based on results of the present study and learnings from the current literature in this area, the following recommendations are proposed:

1. Conduct a larger longitudinal study on this population subgroup in Perth and/or WA to more extensively explore the gestational changes in iodine status. Consider excluding TSH and FT4 measures from this study due to their insensitivity in relation to iodine status and additional expense. The absence of blood samples may in turn encourage increased participation/reduced attrition.

2. Conduct regular monitoring of the iodine status of these population subgroups in WA via large representative samples. This will be especially important if there is increased use of iodised salt in foods by the food industry leading to increased consumption of iodine.
3. Increased promotion on the importance of maintaining iodine-containing supplement use during pregnancy and breastfeeding.
4. Explore the influence of gestational diabetes on urinary iodine excretion in a larger study.
5. Research should continue to focus on a reliable means to determine iodine status in individuals. This will address a major limitation in all studies of iodine status. It is likely that this current inability contributes to inconsistencies in results observed in iodine status studies conducted to date.

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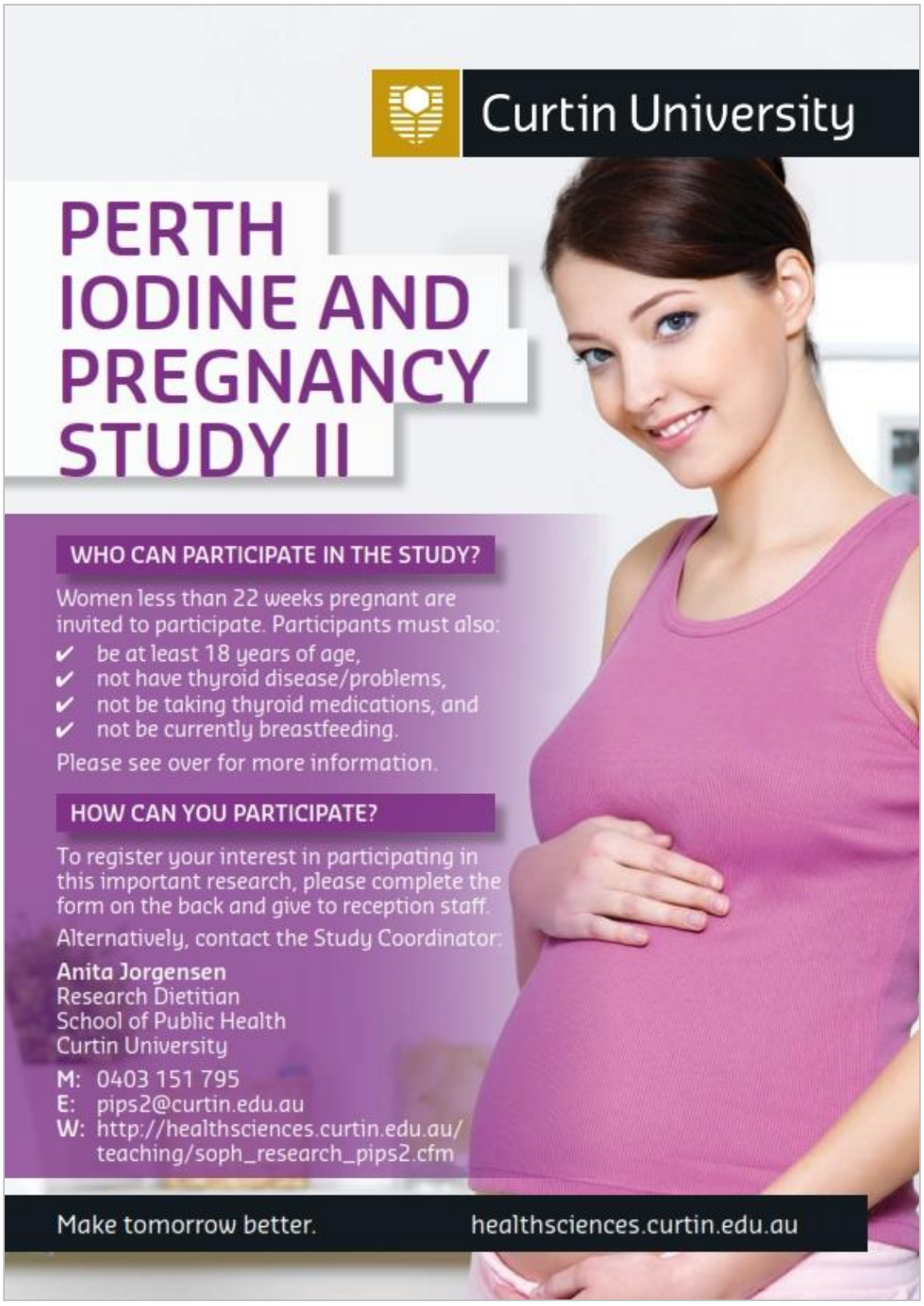
Appendix A Study Flyer



PERTH IODINE AND PREGNANCY STUDY II

WHAT IS THE STUDY ABOUT?

Our research will investigate the iodine health of Perth women during pregnancy and soon after giving birth. Iodine is essential for the normal growth and development of the baby's brain and central nervous system. Women's iodine health will be measured by four methods – urine analysis, dietary intake, blood analysis and breast milk analysis.

WHY IS THE STUDY IMPORTANT?

Pregnant women living in the eastern states of Australia have been shown to have mild iodine deficiency. However, there is no information on the iodine status of pregnant women in WA. Your participation will help us to provide recommendations and guidelines for all pregnant women in WA.

WHAT DOES THE STUDY INVOLVE?

Participants will be involved in the study during pregnancy and until 6 weeks after the baby is born. Participants will be required to:

- ✔ provide four (4) random urine samples,
- ✔ have two (2) blood tests,
- ✔ complete four (4) brief dietary questionnaires, and
- ✔ provide one (1) breast milk sample, if breastfeeding.

For their time, participants will receive 2 gift vouchers during the study (total value $30).

This study has been approved by the Curtin University Human Research Ethics Committee (Approval Number HR 47/2013).

PERTH IODINE AND PREGNANCY STUDY II
Name:
Email:
Phone:
No. weeks pregnant:

Appendix B Participant Information Sheet and Consent Form

B.1 Participant Information Sheet

Curtin University
School of Public Health

Participant Information Sheet

'Perth Iodine and Pregnancy Study II (PIPS II): Longitudinal investigation of the biomarkers of iodine status in pregnant and post-partum women in Perth'

Introduction
The aim of this research project is to investigate the iodine health of Perth women during pregnancy and in the first few weeks after giving birth. Iodine is required by the body for healthy thyroid function which plays a vital role in promoting normal brain development of your baby, also its growth and energy production. This study will involve collecting urine samples to measure iodine excretion, measuring thyroid function via the blood, using a questionnaire to determine dietary intakes of iodine and measuring the iodine content in a breast milk sample.

To participate in the research you must - be aged 18 years and over; be in the first trimester of pregnancy (ie less than 13 weeks pregnant); not have medically diagnosed thyroid disease/problems; not be taking thyroid medication; not be currently breastfeeding; and be English speaking.

Your Role
Your involvement in the research will be over approximately 10 months, starting during your first trimester of pregnancy (10-12 weeks) and ending 4-6 weeks after giving birth (post-partum). As a thank you for your time, you will receive 2 gift vouchers (total value $30) during the course of the study. To participate, you will be required to:

1) **provide four (4) urine samples** – one random urine sample to be collected in each trimester of pregnancy and one in the post-partum period. You will need to provide your samples during visits to any PathWest pathology collection centre located throughout Perth. There will be no charge to you for analysis of urine samples.

2) **have blood collected for two (2) thyroid function tests (TSH and free T4)** – have a blood test for thyroid function on the same day as providing your random urine sample at PathWest in the first trimester and 4-6 weeks after birth. The cost of these blood tests will be covered by the researchers.

3) **complete four (4) questionnaires** – the questionnaire has approximately 16 items, is self-administered and is anticipated to take you 5 minutes to complete. The questionnaire records your current intake of key iodine food sources and also includes some questions on your personal details. The remaining three questionnaires are follow-ups based on the initial questionnaire and are to be completed in the second and third trimesters and post-partum period.

Page **1** of **2**

4) **provide a breast milk sample** - if you choose to breastfeed, we would like you to provide an expressed milk sample (~5 mL) at the beginning of a morning feed at 4-6 weeks after birth. Further instructions for storage and collection of breast milk will be provided to you soon after your baby is born. There will be no cost to you for the analysis of the iodine content of your breast milk.

Consent to Participate

Your involvement in this research is entirely voluntary. Non-participation will not affect your rights or access to normal clinical care. You have the right to withdraw from the research at any time without prejudice or negative consequences and doing so will not affect your normal clinical management.

Confidentiality

Only my supervisors and I will have access to the information you provide. Your consent and completed questionnaires will be filed and kept in a locked filing cabinet at Curtin University. Your name as identification will be used only for the purpose of laboratory analysis of your samples and for follow-up contact with you eg. reminders to complete study components. However, all data recorded and stored in the study database will be in a coded form without your name or other personal details and held in a secure electronic environment within Curtin University. Password protection will be used for the computer containing study data. All information will be kept securely for at least five years, before a decision is made as to whether it should be destroyed. It is intended that aggregated data from this research be published in national and international peer-reviewed journals. Only combined data will be published and you will not be personally identified in any publication.

Risks and Benefits

There are no anticipated risks to you or your fetus/baby as a result of participating. The care and well-being of you and your fetus/baby have been given the highest priority in the design of this research. The procedures used in the research (such as the collection of urine, blood and breast milk) are considered part of normal clinical management. The research does not involve physical pain beyond possible mild discomfort. While you will not directly benefit financially or clinically from the research in your current pregnancy, your participation will help to provide valuable information for future pregnant Perth women relating to their iodine health. A summary of group data will be available to you at the end of the study.

Further Information

This research will form part of the requirements for a Master of Philosophy (Public Health) degree at Curtin University. This study has been approved by the Curtin University Human Research Ethics Committee (Approval Number HR 47/2013). The Committee is comprised of members of the public, lawyers, doctors and pastoral carers. If needed, verification of approval can be obtained either by writing to the Curtin University Human Research Ethics Committee, c/- Office of Research and Development, Curtin University, GPO Box U1987, Perth, 6845 or by telephoning 9266 2784 or by emailing hrec@curtin.edu.au. For further information about the research, please feel free to contact me.

Anita Jorgensen, Student Researcher and Study Coordinator
Ph: 0403 151 795 or Email: pips2@curtin.edu.au

Alternatively, you can contact my Supervisor.
Associate Professor Jill Sherriff
Ph: 9266 7948 or Email: j.sherriff@curtin.edu.au

B.2 Consent Form

CONSENT FORM

Perth Iodine and Pregnancy Study II (PIPS II): Longitudinal investigation of the biomarkers of iodine status in pregnancy and post-partum women in Perth.

- I have been provided with the participant information sheet for the above study.
- I have read and understand the information provided to me.
- I have been given the opportunity to ask any questions about this research and any questions I have asked have been answered to my satisfaction.
- I understand I may withdraw from the study at any stage and withdrawal will not interfere with routine care.
- I understand that the procedure itself may not benefit me directly.
- I agree that research data gathered from the results of this study may be published, however, no personal identifying information such as my name and address will be used.
- I understand that all information will be securely stored for 5 years after the study is completed and then it will be destroyed.
- I agree to participate in the study.

Name: _____

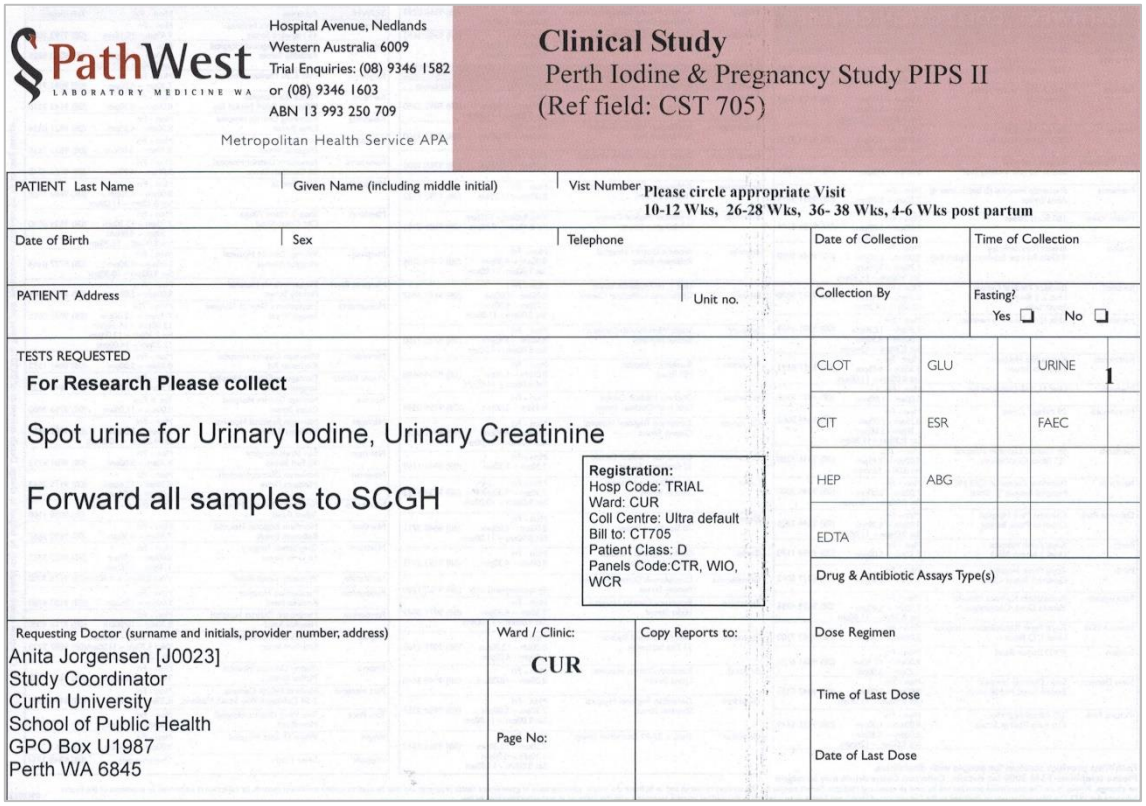
Signature: _____

Date: _____

Witness: _____

Date: _____

Appendix C Sample Request Forms



PathWest LABORATORY MEDICINE WA
Hospital Avenue, Nedlands
Western Australia 6009
Trial Enquiries: (08) 9346 1582
or (08) 9346 1603
ABN 13 993 250 709
Metropolitan Health Service APA

Clinical Study
Perth Iodine & Pregnancy Study PIPS II
(Ref field: CST 705)

PATIENT Last Name | Given Name (including middle initial) | Vist Number
Please circle appropriate Visit
10-12 Wks, 26-28 Wks, 36- 38 Wks, 4-6 Wks post partum

Date of Birth | Sex | Telephone | Date of Collection | Time of Collection

PATIENT Address | Unit no. | Collection By | Fasting? Yes ❑ No ❑

TESTS REQUESTED
For Research Please collect

Spot urine for Urinary Iodine, Urinary Creatinine

Forward all samples to SCGH

Registration:
Hosp Code: TRIAL
Ward: CUR
Coll Centre: Ultra default
Bill to: CT705
Patient Class: D
Panels Code:CTR, WIO, WCR

CLOT	GLU	URINE 1
CIT	ESR	FAEC
HEP	ABG	
EDTA		

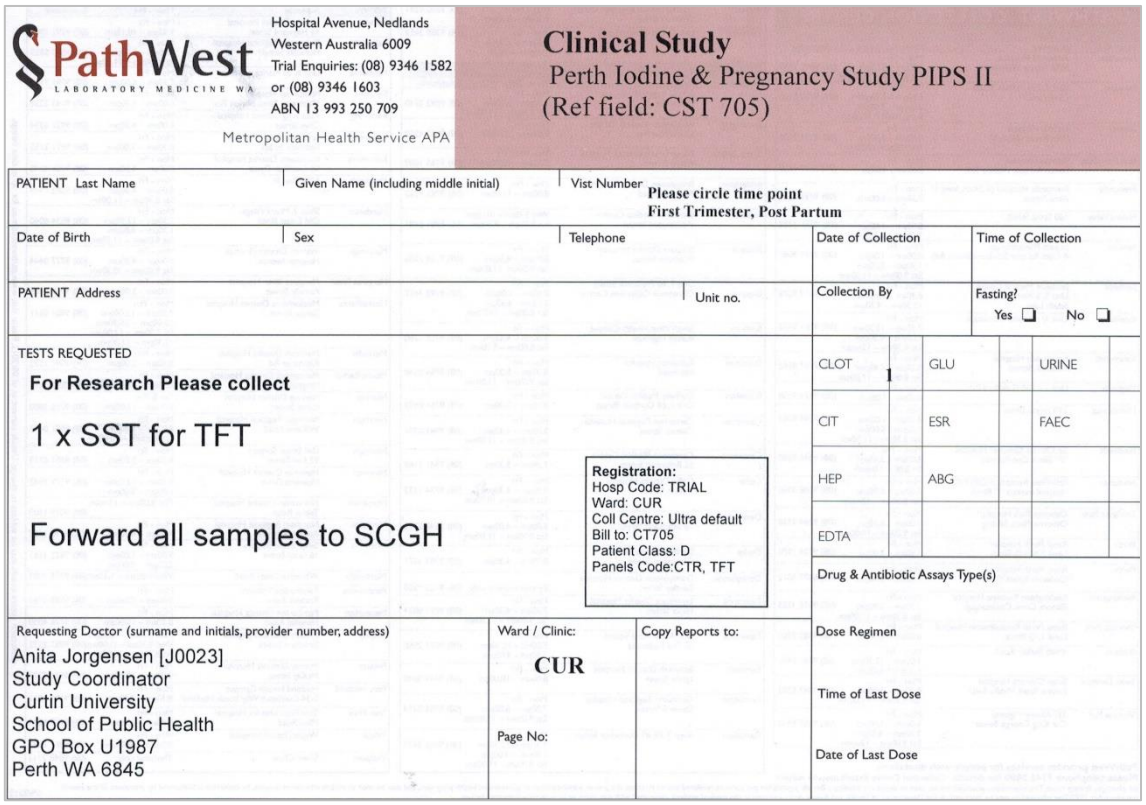
Drug & Antibiotic Assays Type(s)

Requesting Doctor (surname and initials, provider number, address)
Anita Jorgensen [J0023]
Study Coordinator
Curtin University
School of Public Health
GPO Box U1987
Perth WA 6845

Ward / Clinic: **CUR**
Page No:

Copy Reports to:

Dose Regimen
Time of Last Dose
Date of Last Dose



PathWest LABORATORY MEDICINE WA
Hospital Avenue, Nedlands
Western Australia 6009
Trial Enquiries: (08) 9346 1582
or (08) 9346 1603
ABN 13 993 250 709
Metropolitan Health Service APA

Clinical Study
Perth Iodine & Pregnancy Study PIPS II
(Ref field: CST 705)

PATIENT Last Name | Given Name (including middle initial) | Vist Number
Please circle time point
First Trimester, Post Partum

Date of Birth | Sex | Telephone | Date of Collection | Time of Collection

PATIENT Address | Unit no. | Collection By | Fasting? Yes ❑ No ❑

TESTS REQUESTED
For Research Please collect

1 x SST for TFT

Forward all samples to SCGH

Registration:
Hosp Code: TRIAL
Ward: CUR
Coll Centre: Ultra default
Bill to: CT705
Patient Class: D
Panels Code:CTR, TFT

CLOT 1	GLU	URINE
CIT	ESR	FAEC
HEP	ABG	
EDTA		

Drug & Antibiotic Assays Type(s)

Requesting Doctor (surname and initials, provider number, address)
Anita Jorgensen [J0023]
Study Coordinator
Curtin University
School of Public Health
GPO Box U1987
Perth WA 6845

Ward / Clinic: **CUR**
Page No:

Copy Reports to:

Dose Regimen
Time of Last Dose
Date of Last Dose

Appendix D PIPS II Questionnaires

D.1 Questionnaire No. 1

Perth Iodine and Pregnancy Study II (PIPS II) Questionnaire No. 1

1. For Study Staff Only.

Study ID Number:

2. Please provide details of your stage of pregnancy.

No. weeks and days pregnant today:

Estimated due date:

Today's date:

3. Is this your first pregnancy?

○ Yes ○ No

4. Are you currently breastfeeding?

○ Yes ○ No

5. Have you ever been told by a doctor that you have thyroid disease or thyroid problems?

○ Yes ○ No

6. Are you currently taking any thyroid medications?

○ Yes ○ No

7. Have you ever been told by a doctor that you have diabetes?

○ Yes ○ No

8. Please tick if you are taking any of the following dietary supplements daily.

☐ Blackmores Conceive Well Gold
☐ Blackmores I-Folic
☐ Blackmores Pregnancy and Breastfeeding Gold
☐ Cenovis Pregnancy and Breastfeeding Formula
☐ Elevit Women's Multi
☐ Elevit with Iodine
☐ Fefol Multi Pregnancy
☐ Fabfol Plus
☐ Swisse Pregnancy Ultivite

Please list any other dietary supplements you are currently taking daily.

9. Which of the following foods do you currently eat daily? You can tick more than one food.

☐ Cow's Milk
☐ Cheese
☐ Ice Cream
☐ Yoghurt
☐ Bread/Bread Products
☐ Eggs

10. Do you use iodised salt?

◯ Yes ◯ No ◯ I don't know

11. Please provide your name.

Surname:

Given name(s):

12. Please provide your contact details.

Home phone:

Work phone:

Mobile:

Email:

13. What is your current age in years?

14. What is your postcode?

15. What ethnic group do you belong to?

☐ Australian ☐ Torres Strait Islander ☐ Chinese

☐ Australian Aboriginal ☐ Indian ☐ British

Other (please specify)

16. In the last 12 months what was your household income BEFORE tax was removed?

◯ Less than $50 000 ◯ More than $100 000

◯ $50 000 - $100 000 ◯ Do not wish to answer this question

17. What is your HIGHEST level of education?

☐ Secondary School Qualification ☐ Professional Qualification (eg Teacher, Nurse)

☐ Bachelors degree (eg, BA BSc) ☐ Diploma

☐ Post-graduate University degree (eg, MSc, PhD) ☐ Trade or Technical Certificate

Other (please specify)

End of questionnaire. Thank you for your participation.

D.2 Questionnaire No. 2

Perth Iodine and Pregnancy Study II (PIPS II) Questionnaire No. 2

1. For Study Staff Only.

Study ID Number:

2. How many weeks pregnant are you? Please also write today's date.

No. weeks pregnant:

Today's date:

3. Have you started taking any medication(s) for thyroid disease in the past two months (ie since you completed the previous questionnaire)?

- ◯ Yes
- ◯ No

4. If 'Yes' to Q. 3, when (no. weeks pregnant) did you start taking the medication(s)?

5. Please tick if you are taking any of the following dietary supplements daily.

- ☐ Blackmores Conceive Well Gold
- ☐ Blackmores I-Folic
- ☐ Blackmores Pregnancy and Breastfeeding Gold
- ☐ Cenovis Pregnancy and Breastfeeding Formula
- ☐ Elevit Women's Multi
- ☐ Elevit with Iodine
- ☐ Fefol Multi Pregnancy
- ☐ Fabfol Plus
- ☐ Swisse Pregnancy Ultivite

Please list any other dietary supplements you are currently taking daily.

6. Have you recently had a Glucose Tolerance Test? (This usually happens around 28 weeks pregnancy).

- ◯ Yes
- ◯ No

7. If 'Yes' to Q.6, what treatment (if any) has been recommended for you for this?

Perth Iodine and Pregnancy Study II (PIPS II) Questionnaire No. 2

8. Which of the following foods do you currently eat daily? You can tick more than one food.

- ☐ Cow's Milk
- ☐ Ice Cream
- ☐ Bread/Bread Products
- ☐ Cheese
- ☐ Yoghurt
- ☐ Eggs

9. Do you use iodised salt?

- ◯ Yes
- ◯ No
- ◯ I don't know

10. Please provide your name.

Surname: _____

Given name(s): _____

11. Please update your contact details if required (ie any changes in past 2 months).

Home phone: _____

Work phone: _____

Mobile: _____

Email: _____

End of questionnaire. Please return completed questionnaire in reply-paid envelope enclosed.

Thank you for your participation.

D.3 Questionnaire No. 3

Perth Iodine and Pregnancy Study II (PIPS II) Questionnaire No. 3

1. For Study Staff Only.

Study ID Number:

2. How many weeks pregnant are you? Please also write today's date.

No. weeks pregnant:

Today's date:

3. Have you started taking any medication(s) for thyroid disease in the past two months (ie since you completed the previous questionnaire)?

- ◯ Yes
- ◯ No

4. If 'Yes' to Q. 3, when (no. weeks pregnant) did you start taking the medication(s)?

5. Please tick if you are taking any of the following dietary supplements daily.

- ☐ Blackmores Conceive Well Gold
- ☐ Blackmores I-Folic
- ☐ Blackmores Pregnancy and Breastfeeding Gold
- ☐ Cenovis Pregnancy and Breastfeeding Formula
- ☐ Elevit Women's Multi
- ☐ Elevit with Iodine
- ☐ Fefol Multi Pregnancy
- ☐ Fabfol Plus
- ☐ Swisse Pregnancy Ultivite

Please list any other dietary supplements you are currently taking daily.

6. Have you recently had a Glucose Tolerance Test? (This usually happens around 28 weeks pregnancy).

- ◯ Yes
- ◯ No

7. If 'Yes' to Q.6, what treatment (if any) has been recommended for you for this?

Perth Iodine and Pregnancy Study II (PIPS II) Questionnaire No. 3

8. Which of the following foods do you currently eat daily? You can tick more than one food.

- ☐ Cow's Milk
- ☐ Ice Cream
- ☐ Bread/Bread Products
- ☐ Cheese
- ☐ Yoghurt
- ☐ Eggs

9. Do you use iodised salt?

- ◯ Yes
- ◯ No
- ◯ I don't know

10. Please provide your name.

Surname: _____

Given name(s): _____

11. Please update your contact details if required (ie any changes in past 2 months).

Home phone: _____

Work phone: _____

Mobile: _____

Email: _____

End of questionnaire. Please return completed questionnaire in reply-paid envelope enclosed.

Thank you for your participation.

D.4 Questionnaire No. 4

Perth Iodine and Pregnancy Study II (PIPS II) Questionnaire No. 4

1. For Study Staff Only.

Study ID Number:

2. Please provide details of your baby's birth.

No. weeks and days pregnant:

Birth date:

3. Please tick if you are taking any of the following dietary supplements daily.

- ☐ Blackmores Conceive Well Gold
- ☐ Blackmores I-Folic
- ☐ Blackmores Pregnancy and Breastfeeding Gold
- ☐ Cenovis Pregnancy and Breastfeeding Formula
- ☐ Elevit Women's Multi
- ☐ Elevit with Iodine
- ☐ Fefol Multi Pregnancy
- ☐ Fabfol Plus
- ☐ Swisse Pregnancy Ultivite

Please list any other dietary supplements you are currently taking daily.

4. Which of the following foods do you currently eat daily? You can tick more than one food.

- ☐ Cow's Milk
- ☐ Cheese
- ☐ Ice Cream
- ☐ Yoghurt
- ☐ Bread/Bread Products
- ☐ Eggs

5. Do you use iodised salt?

- ◯ Yes
- ◯ No
- ◯ I don't know

6. Do you smoke cigarettes?

- ◯ Yes
- ◯ No

Please provide your name and today's date.

Surname:

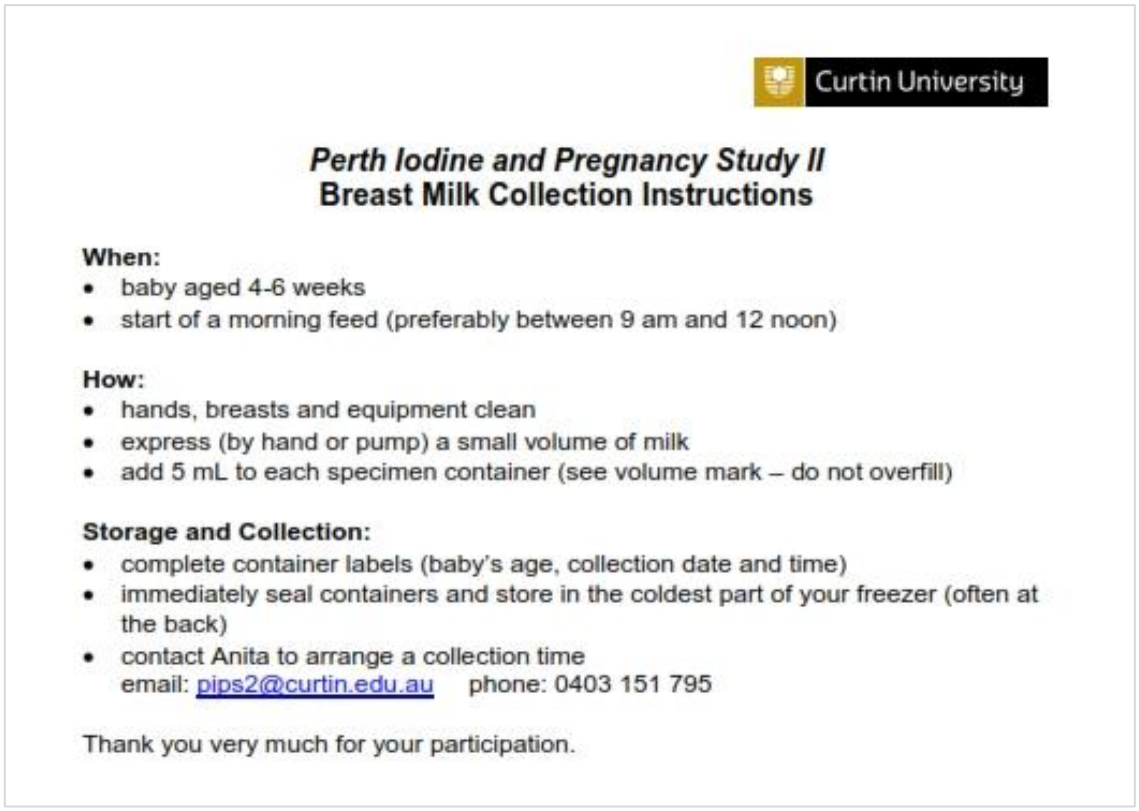
Given name(s):

Today's date:

End of questionnaire. Please return completed questionnaire in reply-paid envelope enclosed.

Thank you for your participation.

Appendix E Breast Milk Collection Instructions



Curtin University

Perth Iodine and Pregnancy Study II
Breast Milk Collection Instructions

When:
- baby aged 4-6 weeks
- start of a morning feed (preferably between 9 am and 12 noon)

How:
- hands, breasts and equipment clean
- express (by hand or pump) a small volume of milk
- add 5 mL to each specimen container (see volume mark – do not overfill)

Storage and Collection:
- complete container labels (baby's age, collection date and time)
- immediately seal containers and store in the coldest part of your freezer (often at the back)
- contact Anita to arrange a collection time
  email: pips2@curtin.edu.au phone: 0403 151 795

Thank you very much for your participation.

Appendix F Urine Creatinine Assay Procedure

URINE CREATININE

ABBOTT ARCHITECT C16000

Note: Refer to the document AUM093 for description of the Architect Enzymatic Creatinine method. This method is used for plasma creatinine and miscellaneous fluid creatinine measurement.

LIS PANEL CODE	24 hour urine **UCR** Spot urine **WCR** Creatinine Clearance **CCR** Creatinine Clearance (non surface area corrected) **CCN**
OUTSTANDING WORK LIST	Outstanding requests are listed on a WJUA worklist.
ASSAY FREQUENCY	Available 24/7
SPECIMEN	• Non-preserved spot urine specimens are acceptable. • 24 hr urine specimens may be collected with 10g Sulphamic Acid (H_3NSO_3), 20ml of 50% acetic acid preservative. Unpreserved urine is also suitable for urine creatinine analysis. • Urine specimen aliquots must be centrifuged for 8 minutes at 3000g prior to analysis.
SPECIMEN STABILITY/ ADDED TEST	• Sample stability – 6 days at 2-8°C.
UNITS	**24 hour urine** millimole/day (mmol/day) **Spot urine** millimole/litre (mmol/L) **Creatinine Clearance** millilitres/second (ml/s)
ANALYTICAL RANGE	0.4 -65.4 mmol/L
REPEAT CHECK	1.0< mmol/L will automatically be rerun to check >65.4 mmol/L will rerun automatically with onboard dilution
DILUTION PROTOCOL	Onboard dilution 1/40 for samples >65.4 mmol/L (Note: Urine creatinine original run at 1/20).
REAGENT KIT INSERT	Abbott Package Inserts - c16000

PRINCIPLE

Jaffé Creatinine method

Creatinine reacts with picric acid in an alkaline medium to produce a coloured creatinine-picrate complex. The rate of formation of this complex, measured over a selected time interval at a primary wavelength of 500nm and secondary wavelength of 572 nm, is proportional to the concentration of creatinine in the sample.

INSTRUMENT

Abbott Architect ci16000

.

For detailed information on assay file installation and for instructions on instrument operation, refer to the ARCHITECT System Operations Manual.

SPECIMEN

Urine

- Non-preserved spot urine specimens are acceptable.
- 24 hr urine specimens may be collected with 10g Sulphamic Acid (H_3NSO_3), 20ml of 50% acetic acid preservative. Unpreserved urine is also suitable for urine creatinine analysis.
- Urine specimen aliquots must be centrifuged for 8 minutes at 3000g prior to analysis.
- Sample stability – 6 days at 2-8°C.

Creatinine Clearance

A 24 hr urine sample is collected with a blood specimen taken during the collection time of the urine If no other specimens are available, a blood specimen taken within 1 collection interval either side of the urine collection time is acceptable.

REAGENT

Abbott Architect Creatinine reagent

Catalogue No.3L81-32

Estimated tests 7500

Available from Abbott Diagnostics

Store reagent at 15-30°C.

R1 – 10 x 55ml, 0.8 mol/L Sodium Hydroxide.

Use reagent as supplied.

Reagent stable for 5 days on the instrument.

R2 – 10 x 32ml 24 mmol/L Picric acid

Use reagent as supplied.

Reagent stable for 5 days on the instrument.

Precautions: R1 contains sodium hydroxide solution – corrosive. R2 contains picric acid – hazardous, toxic. Avoid contact with skin. Wash thoroughly if contact with skin occurs. Wear eye protection when preparing and loading reagents. Seek immediate medical advice if the reagent comes in contact with eyes.

CALIBRATION

Urine

Abbott Multiconstituent Calibrator MC Cal (MCC)

Catalogue No 1E65-05

Available from Abbott Diagnostics

Store at 2-8°C

Calibrator traceability: Calibrator IDMS traceable, Reference material NIST SRM 967 and 914. Information from manufacturer.

Cal 1 and Cal 2 3 x 5 ml

Lot specific calibrator values are listed in the MCC Value Sheet (2). Ensure the correct values are installed on the analyser prior to use.

The MCC Cal 1 and 2 require no preparation prior to use. Mix the vial gently before aliquoting for use.

Opened calibrator is stable for 7 days from opening when stored capped at 2-8°C.

Calibration is performed by running a water blank using on board water and the MCC Cal 1 and 2 set.

Calibrate with every lot change of reagent and as indicated by the analyser. Calibration may also be required when indicated by quality control. Quality control must be assayed after calibration.

QUALITY CONTROL

Urine

Bio-Rad Liquichek Urine Chemistry control levels 1 and 2

Catalogue No. 397 Level 1, 12 x 10 ml

Catalogue No. 398 Level 2, 12 x 10 ml

Store at 2-8°C. Opened QC is stable for 30 days when stored capped at 2-8°C.

2 levels of QC are run routinely at approximately 8 hour intervals during operation. QC must also be assayed when there has been a calibration, reagent change, maintenance or troubleshooting performed on the instrument. The operator must use barcoded QC, or program the QC on the analyser when required.

See AUIO016 for instructions on assaying QC material and rules governing the acceptability of quality controls.

REFERENCE INTERVALS

24 hour urine

AGE	*SEX*	*Ref. Range (mmol/D)*
3 – 8 yr	M and F	1.0 – 6.0
9 – 12 yr	M and F	2.0 – 13.0
13 – 17 yr	M and F	3.0 – 17.0
>18 yr	M	9.0 – 18.0
>18 yr	F	5.0 – 16.0

CREATININE CLEARANCE

Adult:

Surface area corrected 1.25 - 2.08 ml/s/1.73m^2

Derived from DS Young. Implemenation of SI Units for Clinical Laboratory Data. Annals of Internal Medicine (1987) 106: 114-129.

Not body surface area corrected >1.3 ml/s

Child <9 years:

Surface area corrected >0.70 ml/s/1.73m^2

CALCULATIONS

24 hr Urine Creatinine

UCR = urine creatinine (mmol/L) x 24 hr urine volume (L) = mmol/day.

Creatinine Clearance

$$CCR = \frac{\text{urine creat } (\mu mol/l) \times \text{urine flow rate (ml/s)}}{\text{pl.creat } (\mu mol/l)}$$

The above formula reduces to

$$CCR = \frac{U \times UV \times 11.6}{P}$$

Where:

P = plasma creatinine (µmol/l)
U = urine creatinine (mmol/l)
UV = urine volume (litres)
CCR = creatinine clearance (ml/s)
11.6 = conversion factor for P from umol/l to mmol/l; UV from litres to millilitres and time from 24 hrs to seconds.

Correction for Body Surface Area (for Creatinine Clearance calculation)
The result of the above calculation may be corrected for the patients body

surface area by multiplication by the following factor:

$$\frac{1.73}{A}$$

where A is the body surface area derived from height (cm) and weight (kg) of the patient according to reference (9).

REPORTING

Urine

When the results are produced check the quality control results and check for any flags against the results. See AUIO016 for instructions regarding acceptability of quality control. Release the results if appropriate from the instrument interface. See AUIO021 for instructions on use of the interface.

See AUSP009 for instructions on validation of results in the laboratory computer.

For 24 hour urine creatinine the 24 hour urine volume in litres is entered into the computer at the time of request registration. The 24 hour urine volume is measured in the CRA of PathWest by weight.

24 hour urine creatinine is reported with all 24 hour urine requests as an indicator of completeness of collection. Appropriate comments are reported automatically if the 24 hour urine creatinine is outside the following limits for adults (≥18 years).

IF 24 hour urine creatinine <4.0 mmol/day for females or <6.0 mmol/day for males

THEN the comment *"Possibly less than 24 hr collection"* is reported automatically.

IF 24 hour urine creatinine >18.0 mmol/day for females or >20.0 mmol/day for males

THEN the comment *"Possibly more than 24 hr collection"* is reported automatically.

Should the automatically reported comment be inappropriate for the particular case, it can be removed by adding a coded comment UCX and report BUCX.

See AUSP009 for instructions on validation of results in the laboratory computer.

Automatic Calculation of Creatinine Clearance

When the following parameters are resulted in the computer (ULTRA) the creatinine clearance will calculated automatically: plasma creatinine (μmol/L), spot urine creatinine (mmol/L), urine volume (L), patient height (cm), patient weight (kg).

If height and weight are not available (contact the hospital ward or requestor first), the following comment will be reported automatically

"Creatinine clearance corrected for body surface area gives a more accurate indication of renal function. Please submit patients height and weight for calculation of body surface area."

SAMPLE STORAGE

Urine

All specimens are stored in the routine Core Biochemistry sample storage system. see AUSP001 for instructions on storage system.

Appendix G Breast Milk Sample Analysis (Operating Conditions and Results)

Table 1. ICPMS operating conditions.

RF power (W)	1600
Auxiliary Gas Flow(L/min)	1.2
Plasma Gas Flow (L/min)	16
Nebulizer gas (L/min)	1.09
Makeup gas (L/min)	0.20
Nebulizer	PFA
Spray chamber	Cyclonic
Nebulizer pump (rps)	2
Lens settings	Optimised with each run
Iodine (I) – mass	127
Indium (Te) – mass	115
Scanning mode	Peak hoping
Points/peak	254
Number of replicates	5
Mode	Standard**

**Standard mode: The collision cell is actively vented (no gas) which enables the instrument to be run with the cell conditions optimized for maximum ion transmission.

Results

1. Linearity

The standard curve was linear up to 200 µg/L iodine:

Table 2. Linearity of iodine assay

Standard Iodine µg/L	Nett Intensity (cps)
0	5751
23.5	418525
47	847181
94	1616880
188	3547258

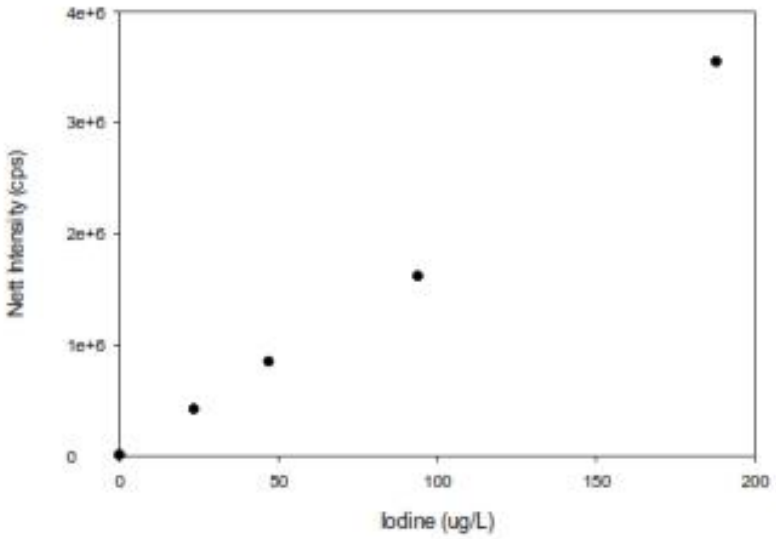
Intensity (cps) = 18778*Iodine (µg/L) - 36757

Parameter	Coefficient	Std. Error	95% CI	t	P
Intercept	-36757	49541	-194421to 120906	-0.7419	0.5119
Slope	18778	511	17151to 20405	36.7265	<0.0001

Least squares regression

Sample size	5
Coefficient of determination R^2	0.7679
Residual standard deviation	812931

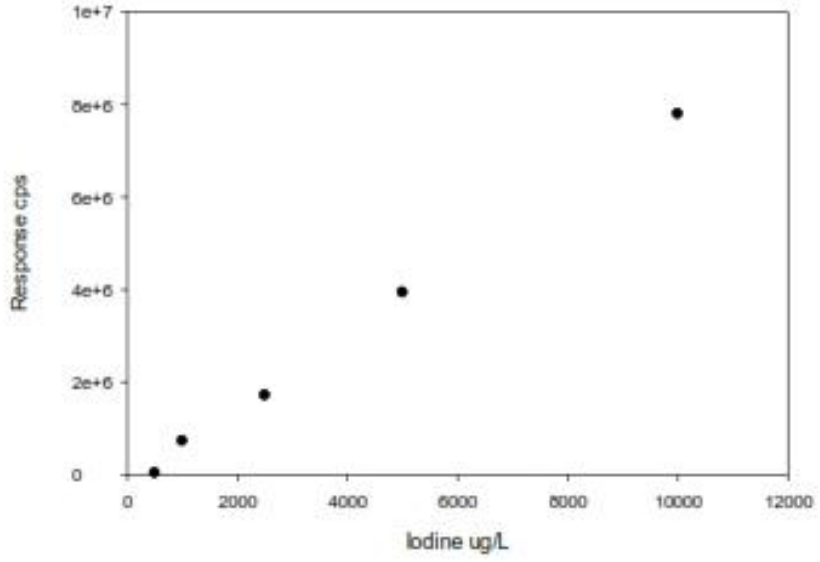
Figure 1. Relationship between iodine concentration and NeXion ICPMS response



The standard curve was linear up to 10000 µg/L iodine:

Table 3. Extended linearity of iodine assay

Standard Iodine µg/L	Nett Intensity (cps)
500	37004
1000	727242
2500	1713635
5000	3934406
10000	7799793



y = -226187.1924 + 807.5272 x					
Parameter	Coefficient	Std. Error	95% CI	t	P
Intercept	-226187	97457	-536339 to 83964	-2.3209	0.1030
Slope	807	18	747 to 867	42.6547	<0.0001

Least squares regression

Sample size	5
Coefficient of determination R^2	0.9984
Residual standard deviation	147010

2. *Recovery*

For the breast milk samples (n=6), recoveries between 95.6% and 114 % were achieved for solutions spiked with low (188 µg/L), medium (375 µg/L) and high (750 µg/L) concentrations of iodine (Table 4).

Table 4. Recovery of iodine in spiked samples of breast milk

Base Sample Iodine µg/L	Spike concentration Iodine µg/L	Measured concentration Iodine µg/L	Recovery %
202	375	563	102
134	750	924	95.6%
93	188	293	95.9
331	188	526	98.7
286	375	628	105.2
98	286	250	114

3. *Precision*

The intra-assay CVs for iodine concentration of 5 replicates of 2 breast milk samples were 1.3% and 0.9%, respectively.

4. *Accuracy*

The results obtained for the NIST milk standard using this method was 3.70 ± 0.02 mg/kg was in a close agreement with the certified value of 3.38 ±0 .02 mg/kg.

5. *Detection limits* (Minimum Quantifiable Limit)

The IDL for iodine in human milk was 0.3 µg/L. Assuming a dilution factor of 40, the MQL was 0.8 µg/L.

Appendix H Ethnic Groups (Original and Recoded)

Original ethnic group	**Frequency (n)**	**Recoded ethnic group [Caucasian (C), Other (O)]**
Australian	40	C
British	5	C
Chinese	3	O
South African	3	C
Indian	3	O
Irish	2	C
Middle East/Iran	1	O
American	1	C
Maori/New Zealand	1	O
Liberian	1	O
Malay	1	O
TOTAL	61	

Appendix I Selected Analysis Outputs

I.1 Comparison of demographic and pregnancy-related characteristics of iodine supplement and non-supplement users (Table 4.3)

Age

Group Statistics

	Any supplement use during study	N	Mean	Std. Deviation	Std. Error Mean
Participant age	yes, supplement used	52	32.08	4.110	.570
	no, supplement not used	9	29.00	3.640	1.213

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Participant age	Equal variances assumed	.044	.834	2.104	59	.040	3.077	1.462	.151	6.003
	Equal variances not assumed			2.295	11.831	.041	3.077	1.341	.151	6.002

Gravidity

Any supplement use during study * First pregnancy Crosstabulation

Count

		First pregnancy		
		yes	no	Total
Any supplement use during study	yes, supplement used	25	27	52
	no, supplement not used	4	5	9
Total		29	32	61

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.041[a]	1	.840		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.041	1	.840		
Fisher's Exact Test				1.000	.565
Linear-by-Linear Association	.040	1	.842		
N of Valid Cases	61				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 4.28.

b. Computed only for a 2x2 table

Ethnicity

Any supplement use during study * Ethnicity Crosstabulation

Count

		Ethnicity		
		Caucasian	Non-caucasian	Total
Any supplement use during study	yes, supplement used	44	8	52
	no, supplement not used	7	2	9
Total		51	10	61

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.262[a]	1	.609		
Continuity Correction[b]	.001	1	.981		
Likelihood Ratio	.244	1	.621		
Fisher's Exact Test				.633	.457
Linear-by-Linear Association	.257	1	.612		
N of Valid Cases	61				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 1.48.

b. Computed only for a 2x2 table

Highest education

Any supplement use during study * Highest education Crosstabulation

Count

		Highest education			
		tertiary/professional	diploma/trade/technical	secondary school only	Total
Any supplement use during study	yes, supplement used	41	4	8	53
	no, supplement not used	3	3	2	8
Total		44	7	10	61

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	5.736[a]	2	.057
Likelihood Ratio	4.670	2	.097
Linear-by-Linear Association	2.030	1	.154
N of Valid Cases	61		

a. 2 cells (33.3%) have expected count less than 5. The minimum expected count is 1.03.

Household income

Any supplement use during study * Household income Crosstabulation

Count

		Household income				
		<$50 000	$50-100 000	>$100 000	4	Total
Any supplement use during study	yes, supplement used	4	9	38	1	52
	no, supplement not used	4	4	1	0	9
Total		8	13	39	1	61

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	15.334[a]	3	.002
Likelihood Ratio	14.607	3	.002
Linear-by-Linear Association	14.609	1	.000
N of Valid Cases	61		

a. 4 cells (50.0%) have expected count less than 5. The minimum expected count is .15.

I.2 Effect of daily iodine-containing supplement use on urinary iodine excretion over pregnancy (Table 4.6)

UIC

Statistics

All supps Stages 1-3

N	Valid	142
	Missing	102
Median		176.50
Percentiles	25	103.25
	50	176.50
	75	295.25

Statistics

No supps Stages 1-3

N	Valid	40
	Missing	204
Median		194.50
Percentiles	25	114.00
	50	194.50
	75	320.75

Ranks

	Use of supps yes or no Stage 1-3	N	Mean Rank
UIC for Stages 1-3 combined	yes	142	90.02
	no	40	96.75
	Total	182	

Test Statistics[a,b]

	UIC for Stages 1-3 combined
Chi-Square	.509
df	1
Asymp. Sig.	.476

a. Kruskal Wallis Test

b. Grouping Variable: Use of supps yes or no Stage 1-3

UIC/Cr

Statistics		
UIC/Cr All Supps for Stages 1-3		
N	Valid	142
	Missing	102
Median		306.800
Percentiles	25	188.250
	50	306.800
	75	461.175

Statistics		
UIC/Cr No supps for Stages 1-3		
N	Valid	40
	Missing	204
Median		199.2500
Percentiles	25	155.0500
	50	199.2500
	75	294.9000

Ranks

	Use of supps yes or no Stage 1-3	N	Mean Rank
UIC/CR for Stages 1-3	yes	142	98.73
	no	40	65.84
	Total	182	

Test Statistics[a,b]

	UIC/CR for Stages 1-3
Chi-Square	12.165
df	1
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: Use of supps yes or no Stage 1-3

I.3 Effect of daily iodine-containing supplement use on UIC/Cr (Table 4.7) and UIC (Table 4.8) by stage

UIC/Cr - Stage 1

Percentiles

		Recoded supp Stage 1 Yes or No	Percentiles						
			5	10	25	50	75	90	95
Weighted Average (Definition 1)	UIC/Cr Stage 1	yes	112.6500	139.1000	179.7000	331.6000	512.1500	778.8000	1165.8000
		no	119.1000	133.5900	175.4250	215.7500	313.6000	382.9000	.
Tukey's Hinges	UIC/Cr Stage 1	yes			185.3000	331.6000	489.5000		
		no			179.7500	215.7500	292.4000		

Ranks

	Recoded supp Stage 1 Yes or No	N	Mean Rank
UIC/Cr Stage 1	yes	49	32.71
	no	12	24.00
	Total	61	

Test Statistics[a,b]

	UIC/Cr Stage 1
Chi-Square	2.323
df	1
Asymp. Sig.	.128

a. Kruskal Wallis Test

b. Grouping Variable: Recoded supp Stage 1 Yes or No

Stage 2

Percentiles

		Recoded supp Stage 2 Yes or No	Percentiles						
			5	10	25	50	75	90	95
Weighted Average (Definition 1)	UIC/Cr Stage 2	yes	124.6400	144.2900	190.8500	286.2500	466.3000	630.4500	698.3700
		no	125.1000	137.3400	160.3000	211.7000	336.9000	405.2400	.
Tukey's Hinges	UIC/Cr Stage 2	yes			193.8000	286.2500	461.4000		
		no			166.9500	211.7000	309.4500		

Ranks

	Recoded supp Stage 2 Yes or No	N	Mean Rank
UIC/Cr Stage 2	yes	46	33.40
	no	15	23.63
	Total	61	

Test Statistics[a,b]

	UIC/Cr Stage 2
Chi-Square	3.425
df	1
Asymp. Sig.	.064

a. Kruskal Wallis Test

b. Grouping Variable: Recoded supp Stage 2 Yes or No

Stage 3

Percentiles

		Recoded supp Stage 3 Yes or No	Percentiles						
			5	10	25	50	75	90	95
Weighted Average (Definition 1)	UIC/Cr Stage 3	yes	92.2200	152.8000	196.3000	302.0000	433.3000	522.4200	565.2800
		no	66.5000	87.8600	130.8000	160.9000	256.2000	613.6200	.
Tukey's Hinges	UIC/Cr Stage 3	yes			199.6500	302.0000	432.0500		
		no			132.0000	160.9000	213.2000		

Ranks

	Recoded supp Stage 3 Yes or No	N	Mean Rank
UIC/Cr Stage 3	yes	47	33.55
	no	13	19.46
	Total	60	

Test Statistics[a,b]

	UIC/Cr Stage 3
Chi-Square	6.630
df	1
Asymp. Sig.	.010

a. Kruskal Wallis Test

b. Grouping Variable: Recoded supp Stage 3 Yes or No

Stage 4

Percentiles[a]

		Recoded supp Stage 4 Yes or No	Percentiles						
			5	10	25	50	75	90	95
Weighted Average (Definition 1)	UIC/Cr Stage 4	Yes	66.0950	79.6000	134.2750	196.2000	313.9750	369.8600	470.9100
		No	64.9000	71.0200	90.9750	128.7000	230.1000	334.6200	.
Tukey's Hinges	UIC/Cr Stage 4	Yes			136.8000	196.2000	309.7000		
		No			94.8000	128.7000	217.2000		

a. There are no valid cases for UIC/Cr Stage 4 when Recoded supp Stage 4 Yes or No = 3.000. Statistics cannot be computed for this level.

Ranks

	Recoded supp Stage 4 Yes or No	N	Mean Rank
UIC/Cr Stage 4	Yes	30	27.35
	No	18	19.75
	Total	48	

Test Statistics[a,b]

	UIC/Cr Stage 4
Chi-Square	3.315
df	1
Asymp. Sig.	.069

a. Kruskal Wallis Test

b. Grouping Variable: Recoded supp Stage 4 Yes or No

UIC – Stage 1

Percentiles

		Recoded supp Stage 1 Yes or No	Percentiles						
			5	10	25	50	75	90	95
Weighted Average (Definition 1)	UIC Stage 1	yes	46.00	49.00	89.00	176.00	276.00	556.00	838.50
		no	87.00	94.80	153.75	264.50	377.25	710.80	.
Tukey's Hinges	UIC Stage 1	yes			101.00	176.00	274.00		
		no			164.50	264.50	372.50		

Ranks

	Recoded supp Stage 1 Yes or No	N	Mean Rank
UIC Stage 1	yes	49	29.16
	no	12	38.50
	Total	61	

Test Statistics[a,b]

	UIC Stage 1
Chi-Square	2.666
df	1
Asymp. Sig.	.102

a. Kruskal Wallis Test

b. Grouping Variable: Recoded supp Stage 1 Yes or No

Stage 2

Percentiles

		Recoded supp Stage 2 Yes or No	Percentiles						
			5	10	25	50	75	90	95
Weighted Average (Definition 1)	UIC Stage 2	yes	36.30	49.70	76.25	169.50	332.75	475.70	695.50
		no	22.00	53.20	106.00	181.00	314.00	357.40	.
Tukey's Hinges	UIC Stage 2	yes			78.00	169.50	332.00		
		no			120.50	181.00	296.50		

Ranks

	Recoded supp Stage 2 Yes or No	N	Mean Rank
UIC Stage 2	yes	46	30.85
	no	15	31.47
	Total	61	

Test Statistics[a,b]

	UIC Stage 2
Chi-Square	.014
df	1
Asymp. Sig.	.907

a. Kruskal Wallis Test

b. Grouping Variable: Recoded supp Stage 2 Yes or No

Stage 3

Percentiles

		Recoded supp Stage 3 Yes or No	Percentiles						
			5	10	25	50	75	90	95
Weighted Average (Definition 1)	UIC Stage 3	yes	52.20	64.80	121.00	179.00	337.00	491.60	620.40
		no	38.00	64.00	105.00	167.00	294.00	824.20	.
Tukey's Hinges	UIC Stage 3	yes			121.50	179.00	314.50		
		no			106.00	167.00	280.00		

Ranks

	Recoded supp Stage 3 Yes or No	N	Mean Rank
UIC Stage 3	yes	47	31.03
	no	13	28.58
	Total	60	

Test Statistics[a,b]

	UIC Stage 3
Chi-Square	.201
df	1
Asymp. Sig.	.654

a. Kruskal Wallis Test

b. Grouping Variable: Recoded supp Stage 3 Yes or No

Stage 4

Percentiles[a]

		Recoded supp Stage 4 Yes or No	Percentiles						
			5	10	25	50	75	90	95
Weighted Average (Definition 1)	UIC Stage 4	Yes	32.85	43.30	60.25	110.50	191.75	221.80	319.40
		No	22.00	38.20	64.50	106.50	163.75	219.00	.
Tukey's Hinges	UIC Stage 4	Yes			62.00	110.50	190.00		
		No			68.00	106.50	162.00		

a. There are no valid cases for UIC Stage 4 when Recoded supp Stage 4 Yes or No = 3.000. Statistics cannot be computed for this level.

Ranks

	Recoded supp Stage 4 Yes or No	N	Mean Rank
UIC Stage 4	Yes	30	24.90
	No	18	23.83
	Total	48	

Test Statistics[a,b]

	UIC Stage 4
Chi-Square	.065
df	1
Asymp. Sig.	.798

a. Kruskal Wallis Test

b. Grouping Variable: Recoded supp Stage 4 Yes or No

I.4 Stage 1 median UIC/Cr Early v Late enrolment

Percentiles

		UIC Stage 1 Early or Late	Percentiles						
			5	10	25	50	75	90	95
Weighted Average (Definition 1)	UltoCrAllStage1	early	77.100	114.900	174.375	348.550	666.225	1434.110	.
		late	121.240	140.100	174.100	312.200	419.100	632.080	903.280
Tukey's Hinges	UltoCrAllStage1	early			185.300	348.550	662.500		
		late			179.750	312.200	416.050		

Ranks

	UIC Stage 1 Early or Late	N	Mean Rank
UltoCrAllStage1	early	18	32.97
	late	43	30.17
	Total	61	

Test Statistics[a,b]

	UltoCrAllStage1
Chi-Square	.315
df	1
Asymp. Sig.	.575

a. Kruskal Wallis Test

b. Grouping Variable: UIC Stage 1 Early or Late

I.5 Longitudinal analysis of UIC/Cr by study stage

- no significant overall difference between the median UIC/Cr values for Stages (p=0.15)

```
> fit1 <- lme(log10(ratio) ~ as.factor(stage), data = datstg, na.action =
    na.exclude, random =  ~ 1 | id, method = "ML")
> fit2 <- lme(log10(ratio) ~ 1, data = datstg, na.action = na.exclude, rar
    =  ~
    1 | id, method = "ML")
> anova(fit1, fit2)
     Model df      AIC      BIC    logLik   Test  L.Ratio p-value
fit1     1  5 7.751631 23.79906  1.124185
fit2     2  3 7.561362 17.18982 -0.780681 1 vs 2 3.809731  0.1488
```

- slight downward trend with Stage 3 marginally lower than Stage 1 (p=0.054).

```
Fixed effects: log10(ratio) ~ as.factor(stage)
                    Value  Std.Error  DF   t-value p-value
      (Intercept)  2.478443 0.03301130 120  75.07862  <.0001
as.factor(stage)2 -0.034159 0.03708555 120  -0.92108  0.3589
as.factor(stage)3 -0.072317 0.03708555 120  -1.95001  0.0535
```

- Stage 4 value is significantly lower than any of the Stage 1-3 values separately (p<0.0001) or combined (p<0.0001).

```
Data: dat[dat$stage == 1 | dat$stage == 4,  ]

Fixed effects: log10(ratio) ~ (stage == 4)
                Value  Std.Error DF   t-value p-value
(Intercept)  2.478443 0.03515146 47  70.50754  <.0001
 stage == 4 -0.222127 0.04426419 47  -5.01821  <.0001

Data: dat[dat$stage == 2 | dat$stage == 4,  ]

Fixed effects: log10(ratio) ~ (stage == 4)
                Value  Std.Error DF   t-value p-value
(Intercept)  2.444284 0.03030516 47  80.65570  <.0001
 stage == 4 -0.199288 0.03762201 47  -5.29711  <.0001

Data: dat[dat$stage == 3 | dat$stage == 4,  ]

Fixed effects: log10(ratio) ~ (stage == 4)
                Value  Std.Error DF   t-value p-value
(Intercept)  2.406126 0.03188688 47  75.45817  <.0001
 stage == 4 -0.160922 0.03540671 47  -4.54497  <.0001

Fixed effects: log10(ratio) ~ (stage == 4)
                Value  Std.Error  DF   t-value p-value
(Intercept)  2.442951 0.02512272 169  97.24070  <.0001
 stage == 4 -0.190522 0.03345512 169  -5.69487  <.0001
```

I.6 Longitudinal analysis of UIC/Cr by gestational week

Analysis of (log) UIC/Cr during pregnancy based on gestational week (n=61) showed the following:

- a non-significant downward trend over the weeks (p=0.073)
- the log10 ratio is estimated to decline by 0.0038 per week on average, corresponding to an estimated decrease per week in UIC/Cr by a factor of 0.99 (95% CI 0.98, 1.08)

```
Fixed effects: log10(ratio) ~ weeks
                Value  Std.Error  DF  t-value p-value
(Intercept)  2.5492727 0.06635023 121 38.42146   0.000
weeks       -0.0038278 0.00211623 121 -1.80878   0.073
```

- if participants with known or possible gestational diabetes (n=10) are excluded from the analysis (n=51), the non-significant trend in (log) UIC/Cr during pregnancy remains (p=0.37)

```
Fixed effects: log10(ratio) ~ weeks
                Value  Std.Error  DF   t-value p-value
(Intercept)  2.506507 0.06867915 101  36.49589  <.0001
      weeks -0.001985 0.00218882 101  -0.90698  0.3666
```

With the inclusion of demographic factors (ie age, ethnicity) and dietary variables (ie iodine-containing supplements, iodised salt and six key iodine-containing foods) in the analysis model during pregnancy:

- age was positively associated (p=0.02) with UIC/Cr and iodine supplementation was marginally positive (p=0.06),
- ratio values were marginally significantly lower for Caucasians and those with diploma education (both p=0.07), and
- after adjusting for these factors, the trend over time was downwards but not significant (p=0.11).

```
             Value Std.Error  DF   t-value p-value
(Intercept)  2.188112 0.1876348 119  11.66155  <.0001
      weeks -0.003426 0.0021346 119  -1.60515  0.1111
        age  0.011973 0.0052093  57   2.29849  0.0252
       cauc -0.106273 0.0568450  57  -1.86953  0.0667
    educdipl -0.185172 0.0999497  57  -1.85265  0.0691
 iodinesupp  0.091090 0.0484090 119   1.88168  0.0623
```

I.7 TSH values during pregnancy (Stage 1 or 2) and post-partum (Stage 4)

Stage 1 Early vs Late enrolment

Group Statistics

	TSH early or late starters	N	Mean	Std. Deviation	Std. Error Mean
TSH All first measures	early	18	1.3583	.67668	.15950
	late	43	1.4714	.59114	.09015

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
TSH All first measures	Equal variances assumed	.122	.728	-.653	59	.516	-.11306	.17321	-.45966	.23354
	Equal variances not assumed			-.617	28.423	.542	-.11306	.18321	-.48810	.26197

Stage 1 or 2 v Post-partum

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	TSH All first measures	1.4194	48	.59888	.08644
	TSH Second measure Stage 4	1.3527	48	.65162	.09405

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	TSH All first measures - TSH Second measure Stage 4	.06667	.94327	.13615	-.20723	.34056	.490	47	.627

I.8 FT4 values during pregnancy (Stage 1 or 2) and post-partum (Stage 4)

Stage 1 Early vs Late enrolment

Group Statistics

	FT4Stage1and2	N	Mean	Std. Deviation	Std. Error Mean
FT4 All first measures	1	17	14.12	1.409	.342
	2	43	12.23	1.850	.282

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
FT4 All first measures	Equal variances assumed	1.123	.294	3.783	58	.000	1.885	.498	.888	2.882
	Equal variances not assumed			4.254	38.430	.000	1.885	.443	.988	2.782

I.9 Breast Milk Iodine Concentration Analysis

BMIC for daily supplement v non-daily supplement users

Ranks

	BMIC and supp use	N	Mean Rank
Breast milk samples	yes	28	26.25
	no	17	17.65
	Total	45	

Test Statistics[a,b]

	Breast milk samples
Chi-Square	4.539
df	1
Asymp. Sig.	.033

a. Kruskal Wallis Test

b. Grouping Variable: BMIC and supp use

Correlation between BMIC and UIC/Cr

Correlations

		LnBMIC	LnAltoCrStage4
LnBMIC	Pearson Correlation	1	.434**
	Sig. (2-tailed)		.004
	N	45	43
LnAltoCrStage4	Pearson Correlation	.434**	1
	Sig. (2-tailed)	.004	
	N	43	43

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

Appendix J Assessment of Breast Milk Iodine Concentrations in Lactating Women in Western Australia

nutrients

MDPI

Article

Assessment of Breast Milk Iodine Concentrations in Lactating Women in Western Australia

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Abstract: Breast-fed infants may depend solely on an adequate supply of iodine in breast milk for the synthesis of thyroid hormones which are essential for optimal growth and cognitive development. This is the first study to measure breast milk iodine concentration (BMIC) among lactating women in Western Australian (n = 55). Breast milk samples were collected between 2014 and 2015 at a mean (±SD) of 38.5 (±5.5) days post-partum. The samples were analysed to determine median BMIC and the percentage of samples with a BMIC < 100 μg/L, a level considered adequate for breast-fed infants. The influence of (a) iodine-containing supplements and iodised salt use and (b) consumption of key iodine-containing foods on BMIC was also examined. The median (p25, p75) BMIC was 167 (99, 248) μg/L and 26% of samples had a BMIC < 100 μg/L. Overall, BMIC tended to be higher with iodine-containing supplement usage (ratio 1.33, 95% confidence interval (CI) (1.04, 1.70), p = 0.030), cow's milk consumption (ratio 1.66, 95% CI (1.23, 2.23), p = 0.002) and lower for Caucasians (ratio 0.61, 95% CI (0.45, 0.83), p = 0.002), and those with secondary school only education (ratio 0.66, 95% CI (0.46, 0.96), p = 0.030). For most women, BMIC was adequate to meet the iodine requirements of their breast-fed infants. However, some women may require the use of iodine-containing supplements or iodised salt to increase BMIC to adequate levels for optimal infant nutrition.

Keywords: iodine; breast milk; supplementation; iodine status

1. Introduction

Iodine, an essential nutrient, is required by humans for the synthesis of thyroid hormones which are vital for normal growth and development [1,2]. A regular and adequate supply of iodine is particularly important during the critical period for brain and central nervous system development, namely, from the second trimester of pregnancy to 3 years of age [1]. Iodine deficiency during this time results in a spectrum of adverse effects known as iodine deficiency disorders, with the most severe outcomes, irreversible mental impairment and cretinism, resulting from severe iodine deficiency during pregnancy. In infants, iodine deficiency leading to inadequate thyroid activity results in delayed growth and physical development, and impaired cognitive function [1,2].

During intrauterine life, iodine is transferred from the mother to the fetus [3]. This results in a pool of iodine stored in the fetal thyroid gland, with the size of the pool strongly reflecting maternal dietary iodine intake. However, even under conditions of maternal iodine sufficiency, this fetal iodine pool is small and turns over rapidly after birth to partly support the iodine demand of newborns [4]. Infants, however, rely solely on dietary sources to meet their iodine needs. Breast-fed infants are particularly vulnerable to iodine deficiency as they may be completely dependent on the iodine

concentration of breast milk for their intake of iodine [5]. Consequently, maternal iodine requirements are increased during breastfeeding to provide sufficient amounts for the mother and to also meet the iodine demands of the developing infant, via breast milk. Given that 40%–45% of the iodine ingested by the mother appears in breast milk [6], a maternal iodine intake during breastfeeding of 190 μg/day (Australian Estimated Average Requirement (EAR)) would provide just under the Australian Adequate Intake (AI) of 90 μg/day for infants aged 0–6 months [7]. This is achieved by a physiological response during breastfeeding whereby iodine is strongly concentrated by the lactating mammary gland due to the increased expression of the sodium iodide symporter, the main iodine transporter in lactating breast cells [8]. This results in human milk having an iodine concentration 20–50 times higher than that of plasma [4].

Breast milk iodine concentration (BMIC) is influenced by, and may be an indicator of, maternal iodine status during breastfeeding [4,5,8–11]. BMIC is also influenced by other factors including recent maternal iodine intake [12] and duration of lactation [5]. While no reference ranges for the adequate iodine concentration of breast milk have been specified, values above 75 μg/L have been suggested to indicate sufficient maternal iodine intake [4]. An iodine balance study of full-term infants found that a positive iodine balance is only achieved when iodine intake is 15 μg/kg per day, which equates to a BMIC of 100–200 μg/L [8].

A wide range of median or mean BMIC values has been reported in several reviews conducted in areas of varying iodine sufficiency [4,8,10]. BMIC typically ranges from <50 μg/L in iodine-deficient areas [5] to 100–150 μg/L in areas of iodine sufficiency [4] and as high as 150–180 μg/L in areas of good iodine supply [8,10]. A BMIC < 100 μg/L has been identified in studies from France, Germany, Belgium, Sweden, Spain, Italy, Denmark, Thailand and Zaire while studies from Iran, China, USA and some parts of Europe have identified above this level [4]. A recent study in Nepal identified a median BMIC of 250 μg/L, and the estimated iodine intake of the infants involved (0–6 months) was 200 μg/day [13]. WHO's recommended maximum iodine intake for infants <2 years old is 180 μg/day [14], therefore some infants in this area may be consuming excessive iodine intakes through breast milk [13]. This can result in subclinical hypothyroidism and permanently affect their neurodevelopment [15].

In recent decades, Australia has been regarded as a country with mild iodine deficiency. Two initiatives introduced in response to the re-emergence of this public health issue are the mandatory fortification of all bread (except organic) with iodised salt in 2009 [16] and the 2010 National Health and Medical Research Council recommendation that all pregnant and breastfeeding women take a daily supplement containing 150 μg of iodine [17]. Despite this recommendation, only two studies have examined the iodine content of breast milk in Australia to assess either iodine provision to breastfed infants or maternal iodine status. The first was a small (n = 50) cross-sectional study of breastfeeding women in Sydney, conducted more than a decade ago and prior to mandatory iodine fortification. This study identified a median BMIC of 84 μg/L [18], indicating inadequate maternal iodine intake based on the adequate cut-off of 100 μg/L. The second larger and more recent study compared the BMIC of lactating women in South Australia pre- (n = 291) and post- (n = 653) mandatory fortification. The median BMIC of samples from both periods were indicative of adequate breast milk iodine levels, however, BMIC was significantly higher in the post-fortification samples compared with the pre-fortification samples (187 vs. 103 μg/L; $p < 0.05$) [19].

To date, there is no information regarding BMIC for lactating mothers in Western Australia (WA), nor for iodine status of WA breastfeeding women. WA has long been considered an iodine-sufficient area of Australia, based on measures of iodine status in studies involving school-children and adults [20,21]. However, this outcome may not reflect the iodine status in breastfeeding women, who have substantially greater requirements for iodine [1]. In the present study we examined BMIC in breastfeeding women in a local cohort to determine adequacy of iodine provision to breastfed infants. We also investigated the influence of iodine-containing supplements and iodised salt use, as well as the consumption of key iodine-containing foods, on this biomarker of iodine status.

2. Materials and Methods

2.1. Subjects and Design

Participants were recruited in 2013–2014 as part of the Perth Iodine and Pregnancy Study II (PIPS II) via advertising (flyer in private women's ultrasound practices n = 15 and newspaper, radio and websites n = 7), in-person by study coordinator (public maternity hospital antenatal clinics n = 21 and pathology centre n = 3) and word of mouth (n = 8). At the time of recruitment, women were aged 18 years and over and were in the first or second trimester of pregnancy (gestation range 5–22 weeks). Other inclusion criteria were no history of thyroid disease, not currently taking thyroid medication, having a singleton birth and not currently breastfeeding but with the intention to breastfeed their baby. Women were excluded from the study if English was not the main language spoken at home. The study was approved by the Curtin University (Approval No. HR 47/2013; 15 April 2013) and Women and Newborn Health Service Human Research Ethics Committees (Approval No. 2014075EW; 4 August 2014) and informed written consent was obtained from each participant.

Breast milk samples were collected between February 2014 and August 2015. Participants were mailed vials for sampling together with instructions to provide (duplicate) 5 mL nonfasted breast milk samples at home at the start of a single morning feed (preferably between 0900 and 1200 h) when their baby was aged 4–6 weeks. Women were asked to record baby's age and time of day of sampling. Participants were also asked to provide information on current medication use, daily use of dietary supplements, daily intake (yes/no) of any amount of six key iodine-containing foods (cow's milk, cheese, ice cream, yoghurt, bread/bread products, eggs), use of iodised salt (yes/no) and whether or not they smoke cigarettes. Sociodemographic characteristics of the women, namely parity, age, postcode, ethnicity, household income and education, had been collected previously.

2.2. Laboratory Procedures

Breast milk samples were stored at −20 °C from time of sampling until collection and then at −80 °C until analysis. After thawing, milk samples were homogenized before analysis by inductively coupled plasma mass spectrometry (ICPMS) in an accredited commercial laboratory (PathWest Laboratory Medicine WA, Nedlands, Australia). ICPMS is considered the gold standard to determine iodine concentration in complex sample matrices such as breast milk [9,22]. An optimised ICPMS method for breast milk has been published recently [22] and was adapted for this study. In brief, sonicated breast milk samples were diluted in mild alkali solution, ionized with inductively coupled plasma and the ions separated and quantified in a Perkin Elmer NexION 300 ICP-MS mass spectrometer (PerkinElmer Inc., Waltham, MA, USA).

2.3. Statistical Analysis

Distributions of BMIC were skewed and descriptive statistics reported as medians and 25th, 75th percentile. The proportion of women with BMIC < 100 μg/L, the suggested cut-off for providing an adequate iodine supply to breast-fed infants, was also determined. Statistical analyses of BMIC were carried out on the log base 10 scale to better approximate normality. Multiple regression analyses were used to assess associations of BMIC with: (a) use of iodine supplements and iodised salt; (b) daily consumption of six key iodine-containing foods (yes/no); and (c) all studied factors simultaneously. Associations of cohort characteristics with use of iodised salt or iodine supplements were assessed via logistic regressions. Data were analysed using IBM SPSS version 20 (IBM Corporation, Tokyo, Japan) and TIBCO Spotfire S+ version 8.2 (TIBCO Software Inc., Boston, MA, USA). A 5% level of significance was chosen.

3. Results

Sociodemographic characteristics of the 55 study participants are shown in Table 1. The mean age (±standard deviation (SD)) of the study women was 31.4 (±4.7) years. This is consistent with

the average age of women who gave birth in WA in 2013 of 29.8 years [23]. The majority of women were pregnant for the first time (52.7%), were tertiary educated (72.3%), had a total household income of >$AUS100K (67.3%) and were Caucasian (80.0%). Compared with available Western Australian data from the Australian Bureau of Statistics Census 2011, our cohort included an over representation of women with a higher reported education level and higher household incomes [24]. All women provided breast milk samples, however one woman was excluded as she reported being a smoker, thus leaving 54 women for breast milk analysis. Breast milk samples were collected at 28–56 days postpartum with a mean (±SD) of 38.5 (±5.5) days. All samples were provided in the morning between 0600 h and 1200 h. The median (p25, p75) BMIC was 167 (99, 248) µg/L indicating adequate maternal iodine intake for the group. However, 26% of women had BMIC less than the suggested cut-off level for adequacy of 100 µg/L (see Figure 1).

Table 1. Sociodemographic characteristics of study participants (n = 55).

	n	%
First pregnancy		
Yes	29	52.7
No	26	47.3
Highest education [1]		
Secondary school	7	12.7
Trade or technical	3	5.5
Diploma	5	9.1
Professional	2	3.6
Bachelor degree	22	40.0
Postgraduate university	16	29.1
Total household income		
<$AUS50K	8	14.5
$AUS50–100K	8	14.5
>$AUS100K	37	67.3
Don't wish to answer	2	3.6
Ethnicity		
Caucasian	44	80.0
Non-Caucasian	11	20.0

[1] Tertiary educated includes Professional, Bachelor degree and Postgraduate university.

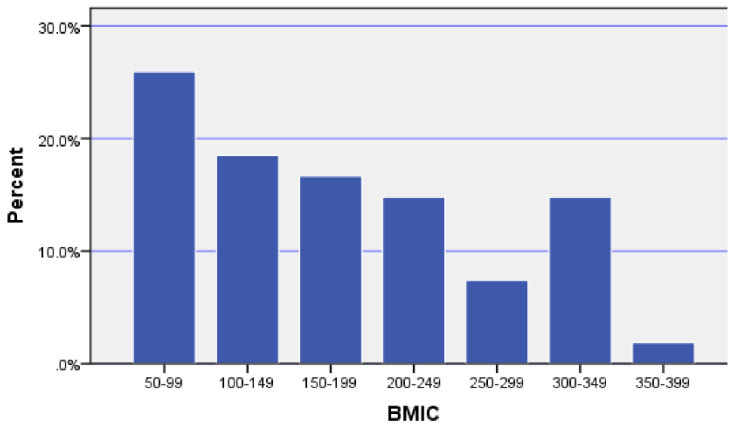

Figure 1. Percentage distribution of breast milk iodine concentration (BMIC) (µg/L).

Thirty-one women (57.4%) reported the daily use of an iodine-containing supplement, some of which contained less than the amount recommended (i.e., 150 μg). Use of iodised salt and iodine-containing supplements were independently associated with increases of similar magnitudes in BMIC (ratio 1.37, 95% CI (1.05, 1.80), p = 0.025 and ratio 1.37, 95% CI (1.04, 1.79), p = 0.029, respectively—see Table 2). There was no significant difference (p = 0.96) between the median BMIC values for the use of either iodised salt or iodine-containing supplements without the other. Among all cohort characteristics jointly considered only low household income (<$AUS50K) remained significantly (negatively) associated with iodine supplement usage (1/8 vs. 30/46, p = 0.010 Fisher test). Exactly half of the women reported using iodised salt, with usage higher among non-Caucasians (10/11 vs. 17/43, p = 0.005).

Table 2. Effect of iodine supplement and iodised salt use on BMIC.

	n	Median BMIC (μg/L)
Yes supplement + Yes salt	15	272 **
Yes supplement + No salt	16	151 *
No supplement + Yes salt	12	156 *
No supplement + No salt	11	98 **

Overall p = 0.028; * There was no difference between the 'Yes supplement + No salt' and 'No Supplement + Yes salt' groups (p = 0.960); ** There was a significant difference between the 'Yes supplement + Yes salt' and 'No supplement + No salt' groups (p = 0.003).

For the six key iodine-containing foods, the majority of women reported daily consumption of bread/bread products (79.6%) and cow's milk (77.8%) and with just less than half of women (46.3%) reporting daily intake of cheese. Furthermore, about a third of women (37.0%) reported daily consumption of yoghurt, around a quarter (27.8%) ate eggs daily and 5.6% of women said they ate ice cream each day. However, only daily cow's milk intake was significantly associated with higher BMIC values after adjusting for the other foods or on its own (ratio 1.44, 95% CI (1.01, 2.06), p = 0.49 and ratio 1.44, 95% CI (1.03, 2.01), p = 0.040, respectively). None of the other foods were significant, jointly or marginally, in influencing BMIC values. Furthermore, cow's milk remained the only food positively associated with BMIC after adjustment for iodine-containing supplements and iodised salt use (ratio 1.50, 95% CI (1.10, 2.04), p = 0.013). Overall in the joint model, BMIC tended to be higher with iodine-containing supplement usage and cow's milk consumption and lower for Caucasians and those with secondary school only education (see Table 3).

Table 3. Significant joint explanatory variables for BMIC *.

Variable	Ratio ** (95% CI)	*p*-Value
Caucasian ethnicity	0.61 (0.45, 0.83)	0.002
School only education	0.66 (0.46, 0.96)	0.030
Iodine supplement use	1.33 (1.04, 1.70)	0.030
Cow's milk consumption	1.66 (1.23, 2.23)	0.002

* Analyses carried out on the log BMIC scale with non-significant terms (sociodemographic and dietary factors) removed by backwards elimination; ** Exponentiated coefficient from the joint model for log (BMIC) predicts the ratio of BMIC for the listed category relative to those not in the category, given fixed values of the other variables.

4. Discussion

This is the first study to report BMIC values for breastfeeding women in Western Australia. The median BMIC value of women would provide an adequate iodine supply for breastfed infants. However, BMIC levels were below the suggested adequate cut-off (100 μg/L) for 26% of women, indicating some infants may be at risk for iodine deficiency, especially if exclusively breast-fed as is recommended. These findings are consistent with results for the post-fortification cohort of the recent South Australian study. However, compared to our study, the proportion of women with BMIC below

the adequate cut-off level was considerably lower in the study by Huynh et al. (26% vs. 13%) [19]. The one participant who reported being a smoker in the present study was excluded from breast milk analysis as the chemical thiocyanate found in cigarettes competitively inhibits the sodium iodide transporter in the lactating breast and impairs iodine transport into breast milk [25], thereby distorting BMIC values.

Despite the NHMRC recommendation for all breastfeeding women to use a daily 150 μg iodine supplement, only about half of the women (54%) in the present study reported behaviour consistent with this (an additional two women reported use of a daily iodine supplement containing less than the recommended iodine amount). In a recent study of breastfeeding women conducted in regional New South Wales (n = 60), iodine-containing supplements were being taken by 45% of women, although frequency of use and iodine content were not documented [26]. These results suggest a low level of awareness and/or compliance amongst Australian breastfeeding women regarding the national iodine supplement recommendations. In contrast, 90% of South Australian women in the post-fortification cohort reported use of supplements containing any iodine [19], although again details of frequency of use and iodine content were not documented. Furthermore, given the low use of iodine-containing supplements in low income cases compared to higher income participants (12.5% vs. 65.2%, respectively) in the present study, perhaps the availability of government subsidized iodine supplements is warranted in Australia, as is the case in New Zealand. Interestingly, in the Perth Infant Feeding Study Mark II conducted in 2002–2003 prior to the supplement recommendation, no breastfeeding women reported taking iodine supplements [27].

In addition, 50% of women in the present study reported using iodised salt. This is similar to the 45% of lactating women using iodised salt in the regional New South Wales study by Charlton et al. [26]. In the present study, use of iodised salt was significantly higher among non-Caucasians (p = 0.013), possibly explaining why BMIC tended to be higher in non-Caucasian mothers compared with Caucasian mothers (p = 0.002). This later finding is consistent with the results of the South Australian study by Huynh et al. [19].

As shown in Table 2, use of both iodine-containing supplements and iodised salt together resulted in the highest median BMIC value (272 μg/L). The use of either iodine-containing supplements or iodised salt had similar positive effects on median BMIC values, suggesting both methods are equally effective in improving the iodine content of breast milk. Our results are consistent with other recent studies that have examined the effect of supplementation and/or iodised salt use on breast milk iodine content [28,29]. The lowest median BMIC was recorded for those women using neither iodine-containing supplements nor iodised salt. This median BMIC value of 98 μg/L is borderline for inadequate BMIC using the cut-off of 100 μg/L. This suggests that for women in our study, food sources alone may not provide the amounts of iodine required during breastfeeding to meet maternal and infant needs. Furthermore, given breast milk samples in the present study were provided in the early post-partum period and BMIC of iodine-deficient lactating women has been shown to decrease in the first 6 months postpartum [5], the use of some form of iodine supplementation by these women is important.

Of the six key iodine-containing foods examined in the study, only daily cow's milk consumption was significantly associated with higher BMIC values, independent of other foods and supplement and iodised salt use. Some cow's milk was consumed daily by more than three-quarters of women in the study. Despite quantity not being examined in the present study, this suggests the importance of cow's milk consumption in terms of iodine intake for breastfeeding women. Interestingly, milk and dairy foods were the highest contributors to iodine intake in the study by Charlton et al. which used a self-administered validated iodine-specific food frequency questionnaire to assess dietary iodine intake of Australian breastfeeding women [26]. Conversely, daily consumption of bread/bread products was not associated with higher BMIC values, despite the fact that a very high proportion of women reported consumption of these foods daily and their known fortification with iodine. This finding

therefore questions the impact of the bread fortification initiative for lactating women in relation to BMIC.

There are some limitations to the interpretation of our study findings. Firstly, the impact of time of supplement intake, iodised salt use and consumption of key iodine-containing foods relative to breast milk sampling were not examined. Leung et al. [12] reported a rise in BMIC following acute oral ingestion of 600 µg potassium iodide, with peak levels at 6 h post-ingestion, and concluded that recent maternal iodine intake would influence the interpretation of BMIC values. Furthermore, as BMIC values fluctuate throughout the day, single breast milk samples provide an imprecise measurement of daily iodine output or maternal iodine sufficiency [30]. In addition, actual compliance with reported supplement use, use of iodised salt or intake of foods examined in the 24-h prior to breast milk sampling could not be confirmed with participants. Finally, while the study included a cross-section of breastfeeding women from both public and private health care systems, the sample size is relatively small and all women who participated (bar one) lived in the Perth metropolitan area, so generalisability of results to the wider breastfeeding population is made with qualifications.

5. Conclusions

Despite these limitations, for the majority of women in the present study, BMIC was adequate to meet the iodine requirement of their breast-fed infants. However, the study also indicates that some breast-fed infants may be at risk of iodine deficiency, which could potentially be reduced by the maternal use of iodine-containing supplements and/or iodised salt. Further studies of women representing the social and regional diversity of the population will be needed to confirm our findings.

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Conflicts of Interest: The authors declare no conflict of interest.

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Appendix K Iodine-containing supplement use and adherence to the supplement recommendation in studies of Australian pregnant and lactating women since 2010

Author, Publication date (Date of study)	State	Sample size (n)	Iodine-containing supplement use (%)	Adherence to supplement recommendation[1] (%)
Pregnancy				
Rahman et al., 2011 (2009-2010)	Vic	62	50	
Clifton et al., 2013 (2009-2010)	SA	196	47	
Charlton et al., 2013 (2011-2012)	NSW	2011: 147 2012: 114	60 66	
El-Mani et al., 2014 (2012-2013)	NSW	152	68	
Lucas et al., 2014 (2012-2013)	NSW	142	70	
Martin et al., 2014 (2011-2012)	Vic	200		54
Condo et al., 2016 (2011)	SA	783	81	62
Malek et al., 2016 (2013)	National SA	455 402		23
Hine et al., 2018 (2012-2013)	WA	425	66	
Lactation				
Axford et al., 2011 (unknown)	NSW	60	45	
Duynh et al., 2016 (2012-2013)	SA	653	90	

[1]Daily iodine supplementation of ≥150 µg/d.