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**Iodine Status of Queensland Children and the Associations with Diet and Thyroid
Function**

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ABSTRACT

Iodine deficiency is often referred to the leading cause of preventable mental impairment in the world (1), although excessive consumption of iodine can have detrimental effects also (38). This essential micronutrient is fundamental in the intricate production of thyroid hormones which regulate metabolic processes, protein synthesis and somatic growth (1). Across the lifespan the body requires a constant small amount of dietary iodine, with the most critical period of iodine nutrition occurring from conception to the third year of life (1).

The iodine status of Australian children is variable across the country (11, 86, 111), with some regions deficient whilst others border on excessive (11). In order to counteract iodine deficiency amongst school aged children in some regions of Australian (11), Food Standards Australia and New Zealand (FSANZ) introduced the mandatory use of iodised salt in bread manufacturing from 2009 (12). With undesired consequences observed amongst other iodine sufficient populations exposed to iodine prophylaxis programs (5, 35), it remains unknown whether the approach to reduce iodine deficiency in Australia will be detrimental to children residing in the iodine replete regions (2, 8).

This thesis explores the influences of iodine nutrition (including iodine fortification) on children residing in a preciously iodine replete region of south east Queensland. Also, one of few Australian study's to evaluate the relationship between iodine status, dietary intake and thyroid analytes in paediatric cohorts.

The overall aim of this thesis is to report the iodine status of Queensland children after the introduction of iodine fortification to Australian bread products. It focuses on school aged children aged 8-10 years so that the results can be compared with surveys conducted prior to iodine fortification, and also includes young children age 2-3 years, as this age group were identified as being the most vulnerable to changes in dietary iodine exposure (10).

Chapters 1, 2 and 3 of this thesis discuss the background and history of iodine status globally and within Australia, followed by the objectives and methods implemented by this study. Chapter 4 examines the challenges in assessing iodine status. With the populations' median urinary iodine concentration (UIC) being the recommended determinant of iodine status (1), this paper discusses the challenges of categorising iodine status and the distribution of iodine status within a population using one spot urine sample. A total of 4 spot UIC samples from 51 young children and 30 school aged children were analysed and adjusted for inter and intra

variability typically observed when evaluating urinary iodine. The results show that the coefficient of variance (CV) of UIC for children aged 2-3 years were reduced by 35.6%, 36.5% and 39.7% when two, three and four samples were included in the adjustment respectively. Similarly, the CV of UIC for children aged 8-10 years was reduced by 24.7%, 30.7% and 34.7%. The paper concludes that using an adjusted UIC from multiple UIC samples reduces the large coefficient of variation typically observed between individuals and within individuals to provide a more accurate reflection on iodine status within the distribution of the cohort. It also confirms that the timing of urine collection is an important consideration when assessing iodine status.

Dietary sources of iodine and their impact on UIC amongst school aged children are explored in chapter 5. The adjusted median UIC for this age groups was determined to be 143.8ug/L (IQR 119.5-209.6ug/L). This paper reports that bread ($r=.37$, $p=0.02$) is the only statistically significant contributor of dietary iodine impacting UIC with 14% of variation in UIC explained by bread consumption. This is an important finding as whilst other surveys (119) suggest that the UIC of Queensland children has increased since the implementation of iodine fortification this paper provides the only data to report a direct association between bread intake and iodine status.

Chapter 6 reports the impact of iodine fortification on children aged 2-3 years, a cohort identified as being at risk of attaining excessive iodine status post iodine fortification (10). This paper reports that the percentage of UIC samples above the upper level for dietary iodine was almost double than expected. The adjusted median UIC was reported to be 223.3 μ g/L. With no validated criterion to assess UIC available for this age group, a crude dietary intake was calculated from the UIC based on an estimated urine output (80). The estimated median dietary iodine intake was 124.8ug/day (SD 47.0) with 9.8% of samples above the upper level of 200ug for dietary iodine for children within this age group. This status was not associated with iodine fortification in bread or any other foods. The chapter highlights the limitations of interpreting iodine status using the current WHO criterion for this particular age group. The paper concludes with emphasises that a universal criterion for children aged 2-3years using UIC as a marker of iodine nutrition is warranted.

The influence of discretionary use of iodised salt, supplement use and drinking water iodine concentration on UIC has not been previously explored in Australian children. The paper presented in chapter 7 shows a significant positive association between the use of iodised salt

on any occasion and the iodine status of children exposed to it. Iodised salt was consumed by 76.4% of 2-3 year olds and 66.6% of 8-10 year olds on any occasion. Independent t-tests revealed significant differences in UIC between those children receiving iodised salt and those who did not (2-3 year olds $p=0.029$; 8-10 year olds $p=0.041$). Approximately 14% of children consumed iodine supplements. Independent t-tests showed a significant mean difference in UIC ($p=0.013$) between children who consumed iodine supplements and those who did not in only 8-10 year olds. No significant correlations between DWIC and UIC were detected. These data have never been described elsewhere in Australia and provides a valuable contribution to reports compiled by the World Health Organisation. The paper concludes that other environmental factors, in addition to iodine concentration of drinking water, may have an impact on the inconsistent iodine status' observed across the country.

The important role of iodine in thyroid hormone production is discussed in chapter 8. As this part of the study was an additional option for participants, only Twenty-seven children (eleven 2-3 year old and sixteen 8-10 year olds) provide blood samples. The paper reports no associations between thyroid analytes and iodine status in either age group, although it does reveal that 1 in 5 children had some form of abnormality in their thyroid analytes. No children had fT4, fT3 or Tg levels outside the reference ranges. Seven children had one or more thyroid analytes outside the references ranges of TSH or TPOAb. Five children had a TSH (mean 2.7mU/L) above the recommended reference, all of whom had normal fT4 (mean 15.0pmol/L) and fT3s (mean 5.9pmol/L) and only one child with corresponding TPOAbs of 75U/mL. A difference of 23.5ug/L ($p= 0.021$) in Tg levels was observed between the two age groups. Familial history was only a predictor for TSH for both age groups combined ($\beta 0.19$, $p=0.016$, 95%CI 0.03-0.34). The paper concludes that the use of adult reference ranges and varying interpretations of laboratory results may cause some children with subclinical thyroid anomalies to remain undiagnosed. Finally, chapter 9 amalgamates the thesis by discussing the findings and limitations of the overall research project. The thesis concludes that the discretionary use of iodised salt has greatest influence on the iodine status of Queensland children. Dietary contributors of iodine vary according to age and thus the impact of the mandatory introduction of iodine in bread varies also.

DECLARATION BY AUTHOR

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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PUBLICATIONS DURING CANDIDATURE

Peer-reviewed publications

Samidurai AJ, Ware RS, Davies PSW. Advantages of collecting multiple urinary iodine concentrations when assessing iodine status of a population. *Acta Paediatrica*, 2015; 104: 524-529 doi: 0.1111/apa.13168

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Heffernan AL, Aylward LL, **Samidurai AJ**, Davies PSW, Toms LM, Sly PD, Mueller JF. Short term variability in urinary bisphenol A in Australian children. *Environment International*, 2014; 68:139-143 doi: 10.1016/j.envint.2014.03.0276

Non peer-reviewed publications

Walsh AJ. Adding iodine to your menu. *Belonging Early Years Journal*. 2012; 1:68-69

Walsh AJ. Food for thought. *Totline National Playgroup Magazine*. 2012; 3:16

Poster Presentations

Walsh AJ, Ware RS, Davies PSW. Collecting multiple urinary iodine concentration samples from each participant in population surveys – Is it worth it? Poster presentation at European Thyroid Association 37th Annual Meeting, 7-11th September 2013, Leiden, The Netherlands.

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Contributor	Statement of contribution
Samidurai AJ (Candidate)	<p>Conception and design (60%)</p> <p>Data collection and entry (100%)</p> <p>Reviewing literature (80%)</p> <p>Statistical analyses and interpretation (20%)</p> <p>Writing manuscript (100%)</p>
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CONTRIBUTIONS BY OTHERS TO THE THESIS

My supervisor Professor Peter Davies supervised the entire project. Consequently, Professor Davies was involved in the original concept and design, analysis, interpretation and revision of the ideas presented in this thesis. Dr Robert Ware provided statistical support to some parts of the project as described elsewhere.

**STATEMENT OF PARTS OF THE THESIS SUBMITTED TO QUALIFY
FOR THE AWARD OF ANOTHER DEGREE**

None.

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KEYWORDS

Iodine, children, urinary iodine concentration, iodine fortification, thyroid, dietary iodine, iodine status

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LIST OF ABBREVIATIONS

DIT	Diiodotyrosine
DWIC	Drinking Water Iodine Concentration
FFQ	Food Frequency Questionnaire
FSANZ	Food Standards Australia and New Zealand
fT3	Free Triiodothyronine
fT4	Free thyroxine
ICCIDD	International Council for the Control of Iodine Deficiency Disorders
I/Cr	Iodine/Creatinine ratio
MIT	Monoiodotyrosine
NHS	National Health Survey (Australia)
NINS	National Iodine Nutrition Survey (Australia)
NIS	Sodium Iodine Symporter
NNS	National Nutrition Survey
QLD	Queensland
WHO	World Health Organisation
TgAb	Thyroglobulin Antibody
Tg	Thyroglobulin
TPOAb	Thyroid peroxidase Antibodies
TSH	Thyrotropin (Thyroid Stimulating Hormone)
UL	Upper Level
UIC	Urinary Iodine Concentration

UIE Urinary Iodine Excretion

UNICEF United Nations Children's Emergency Fund

USI Universal Salt Ionisation

CHAPTER 1: INTRODUCTION

BACKGROUND

Iodine is an essential micronutrient required for normal growth and development (1). As an integral component in thyroid hormone production, the primary role of iodine is to regulate a range of physiological processes including; energy production, metabolic rate, protein synthesis and the development of tissues in the central nervous system (2-4). Iodine nutrition, or iodine status, can influence an individual's cognitive development, motor function, somatic growth and thyroid function (1,4). Appropriate iodine nutrition is particularly critical for young children, as brain development and physical growth can be impaired without adequate iodine intake (1).

As the leading cause of preventable brain damage in children globally, iodine deficiency has instigated considerable concern world-wide (5). It is for this reason, the World Health Organisation, UNICEF and the Iodine Global Network (IGN) (formally known as; International Council for the Control of Iodine Deficiency Disorders (ICCIDD)), promote global interventions to improve iodine status in an attempt to reduce the detrimental effects of iodine deficiency (1, 5-7). In countries where iodine prophylaxis interventions have been implemented, typically the entire national population is exposed to an acute increase of iodine (5-7). Although these interventions are intended to improve population health, there are some challenges in controlling adverse consequences that may be experienced by susceptible sub-population groups (7-9). For example, Australian children aged 2-3 years have been identified as being potentially susceptible to consuming excessive amounts of iodine as a result of the national iodine fortification program (10).

Differences in iodine status is observed between countries and within countries worldwide (5,7). Australia is not exempt from such variation and therefore children in some areas are iodine replete while others are iodine deficient (11). Overall, the 2003 National Iodine Nutrition Survey (NINS) reported that Australian school aged children were mildly iodine deficient (11), and consequently the mandatory use of iodised salt during bread manufacturing (except organic) was initiated in late 2009 (12). Contrary to the national average, however, school aged children residing in Queensland (QLD) were classified as being iodine adequate (11). Other than data reported by Li et al (11), at the commencement of this study, there were no other records of the iodine status of QLD children using UIC as a marker, thus the underlying reasons

as to why their iodine status exceeded the national average remained unknown. Furthermore, the impact of increasing dietary iodine through the iodine fortification of bread on QLD iodine replete children had not been assessed.

This research project reports the current iodine status of QLD children and establishes the key contributors to iodine status. Furthermore, this research project is the first Australian study to evaluate corresponding thyroid analytes with iodine status amongst a paediatric community cohort post iodine fortification. In addition to physiological markers of iodine status, the dietary patterns of children were investigated to ascertain the influence of food choices on iodine status and environmental iodine exposure is explored through evaluating the iodine concentration of drinking water.

AIMS AND OBJECTIVES

It is well accepted that monitoring iodine status within a population is an important part of preventative medicine (13, 14, 15). Taking into account that thyroid disorders can occur in areas with low and high levels of dietary iodine, in order to prevent disease, it is important that the iodine intake of a population is maintained within the recommended levels (9, 16-18). For this reason, the overall aim of this thesis is to ascertain the primary dietary iodine sources for QLD children and determine what impact iodine fortification of bread has on corresponding UIC. Secondly this thesis will establish whether a relationship between UIC and thyroid function exists and report the presence of any detected abnormalities.

Objective 1: Evaluate the effect of collecting multiple urine samples on the variance observed in urinary iodine concentration (UIC) in an attempt to reflect iodine status more accurately.

The iodine status of a population is traditionally assessed via morning spot urine samples for a number of reasons, notably convenience, cost and simplicity (1, 18). The accuracy of the approach, however, is largely affected by the significant within-person variation in UIC (1, 19, 20). Adjustment for within-person variation will have little effect, if any, on the mean value within a population but it will increase the precision of the variance within the population, reducing the proportion of individuals with very low or very high UIC (21). Considering the criterion for iodine status includes not only the assessment of median UIC but the distribution spread of UIC (1), it is important to adjust for these variances. Urinary iodine concentration data for QLD school children ascertained by the NINS prior to iodine fortification, represented

a positively skewed distribution (Eastman C. personal communication). From this cohort, 14.7% of UIC samples were categorised as having ‘more than adequate’ iodine nutrition and 3.4% were within the range of ‘excessive’ iodine nutrition prior to the introduction of iodine fortification (Eastman. C personal communication). Typically observed when assessing UIC (1, 19, 20), the large standard deviation 80.1 in conjunction with a mean of 135ug/L lead to a large CV of over 60%, indicating a large spread across the distribution of UIC and potentially misrepresenting the actual spread. Other Australian surveys have used repeated measurements of UIC in iodine surveys in older populations where at least one repeated measure was used (22, 23), however, this thesis aims to explore the advantages of up to four UIC samples from younger individuals. The outcome of this objective could improve the interpretation and classification of iodine status in population groups with potentially large iodine intake diversity.

Objective 2: Determine current iodine status of Queensland children aged 8-10 years and establish the level of significance that food consumption, especially bread, has on the individuals’ urinary iodine concentration.

According to the 2003-2004 Australian National Iodine Nutrition Study (NINS), south east Queensland school children between the ages of 8-10 years, had a median urinary iodine concentration of 146.8ug/L prior to mandatory fortification (Eastman C. personal communication). It was predicted, using data from the 1995 National Nutrition Survey (NNS), that with the implementation of iodine fortification in bread, children over the age of 2 years would increase their dietary iodine intake by approximately 54ug/day (10). This thesis attempts to establish the differences in UIC of QLD children pre and post iodine fortification and identify which foods have the most significant influence on UIC.

Objective 3: Document what proportion of 2-3 year old children consume above the upper level for dietary iodine post iodine fortification of bread, and report dietary determinants most influential on urinary iodine concentration.

The U.S. Agency for Toxic Substance and Disease Registry (ATSDR) argue that children are not small adults due to their continuing development and therefore may be more susceptible to varying levels of iodine (24). Vulnerability to adverse reactions in response to excessive amounts of iodine is proportional to developmental stage (24). When proposing mandatory iodine fortification in Australia, FSANZ acknowledged concern that children aged 2-3 years would be at greatest risk of exceeding the upper level (UL) for dietary iodine (10). Prior to the implementation of iodine fortification of bread, dietary intake surveys reported varying iodine

intakes between 95ug/day and 125.6ug/day for children within this age group (25, 26). It was predicted the mean dietary iodine intake for 2-3 year olds would increase by 37 ug/day, which meant a possible 6% of this populations' distribution would exceed the recommended UL of 200ug per day (10). This thesis is the first Australian study to explore what proportion of children aged 2-3 years consume above the upper level for dietary iodine since the implementation of iodine fortification of bread. Furthermore, the current study anticipated to establish a relationship between foods consumed and UIC for this age group.

Objective 4: Explore the influence of drinking water iodine concentration, discretionary use of iodised salt and supplement use on UIC in Queensland children.

Inconsistent iodine concentrations found in drinking water are considered to be one of the most significant contributing factors to differences observed in dietary iodine intake (11, 27, 28). It was suggested by the Australian NINS that disparity in environmental iodine levels may be a plausible explanation as to why the iodine status of school aged children varies from state to state (11). As there are no Australian records reporting the impact of drinking water iodine concentrations (DWIC) on the iodine status of populations, the need to determine the extent of this influence was of great importance. This thesis attempted to establish an association between DWIC and UIC.

Prior to the implementation of iodine fortification, the level of discretionary use of iodised salt and iodine supplement use by Australians had not been reported. Although governing bodies report on the global use of iodised salt (6, 7, 29, 30) until recently such data have been absent when referring to Australia. The 2012 National Health Survey (NHS) has since reported data on the use of iodised salt during cooking or meal times but the correlation between its use and UIC is yet to be established. One of the important features of this thesis was to compile such data in an attempt to contribute to the global monitoring of iodine status.

Objective 5: Evaluate the thyroid function of Queensland children and identify associations between thyroid hormone analytes and UIC.

Autoimmune thyroid disease, hypothyroidism, Graves disease, hyperthyroidism, sporadic goitre and some thyroid cancers are more common in iodine sufficient populations (31). Little is known about thyroid status of iodine replete QLD children. The 2003-2004 Australian National Iodine Nutrition Study reported that QLD children age 8-10 years had relatively larger thyroid glands when compared to the international standard for iodine sufficient school aged children (11). Nevertheless, no definitive associations between thyroid volume and UIC were

established (11). This thesis reviews literature reporting an altered incidence of thyroid disease resulting from increasing dietary iodine in sufficient populations and then evaluates the thyroid analytes and corresponding UIC of children aged 2-3 years and 8-10 years residing in the iodine replete region of QLD.

THESIS STRUCTURE

Following the introduction, literature review and methodology chapters, this thesis addresses its aims and objectives through a series of manuscripts. In total, 5 papers will be presented in this thesis, of which two are published, one is accepted for publication and one is under review. The remaining are submitted to peer reviewed journals.

The literature review revises the physiological metabolism of dietary iodine and various methods of assessing iodine status. It also discusses the challenges of interpreting iodine status using these conventional methods and presents the strengths and limitations of indicators of iodine nutrition. The literature review summarises the global relevance of iodine nutrition and then focuses specifically on the history and current iodine status of Australia. This chapter reveals gaps in Australian literature regarding the iodine status of Australian children and thus justifies the aims and objectives of the current thesis.

Following the review begins a sequence of papers, each addressing an independent aim previously stated in the introduction chapter. This thesis shows that the methodology of using UIC as an indicator of iodine status can be improved by collecting multiple samples from each participant so that intra and inter variability can be adjusted for (Chapter 4). Although such variability is expected to even out within large populations (1), this paper shows that for smaller cohorts there is an advantage in collecting multiple UIC samples. Normally considered a cumbersome approach (1, 29), it demonstrates that collecting multiple UIC samples from children is achievable and improves the accuracy of the distributions' iodine status within the group.

Subsequent papers (Chapters 5, 6 & 7) evaluate the iodine status of children aged 2-3 years and 8-10 years and dietary patterns. Very little Australian literature describe the relationship between dietary intake and UIC, and importantly in the context of this thesis, the relationship has never been discussed for iodine replete QLD children. These chapters explore reasons as to why the iodine status of QLD children differs from the national average and discusses concepts why iodine variability exists within Australia.

The prevalence of thyroid dysfunction in a healthy community paediatric cohort has not been investigated in Australia. Chapter 8 consists of a manuscript presenting an explorative convenience sample of thyroid analytes from a paediatric cohort and the significance to iodine status. This paper is an important contribution to Australian literature in the area of both endocrine medicine and micronutrient nutrition, as it is the first to report thyroid dysfunction within a seemingly healthy population.

Finally, the outcomes of each paper are collated and examined in Chapter 9. This chapter completes the thesis with reflections of the overall study, limitations, main findings and recommendations for further research.

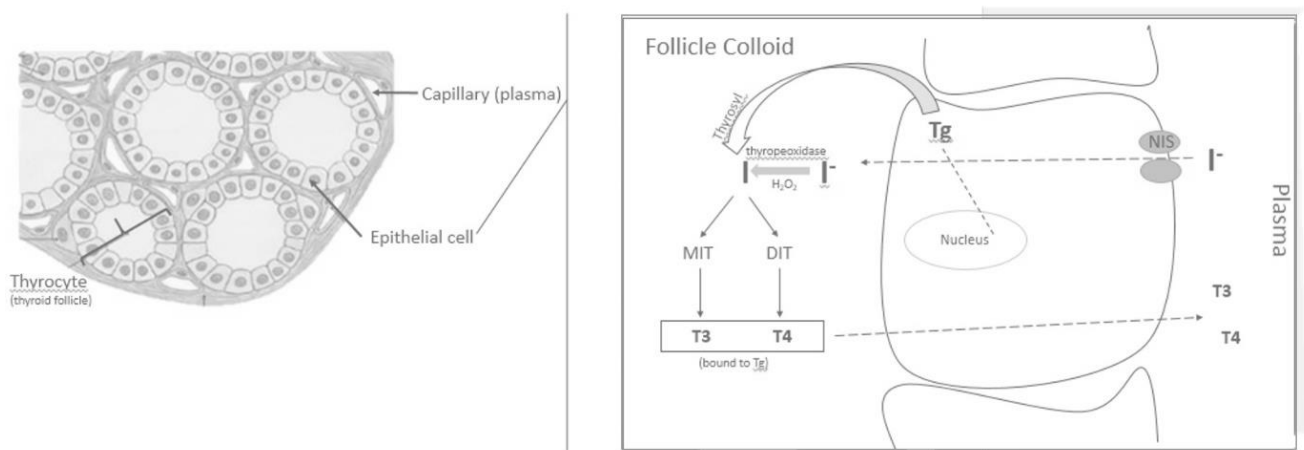
Appendices include copies of ethical approval documents approved by the University of Queensland Medical Research Ethics Committee, parent information statements and consent forms, child information statements, food frequency questionnaires, Queensland Medical Laboratory reference ranges and anthropometric record sheets.

CHAPTER 2: LITERATURE REVIEW

IODINE METABOLISM

Consumed in the form of inorganic iodide (I^-) or iodate (IO_3^-), the micronutrient iodine, is almost completely absorbed via the stomach and small intestine, after which it is then transported throughout the body bound to protein in the plasma (2, 3, 32). Whilst under normal conditions, small amounts (<3%) of iodine is cleared from the plasma by salivary glands, breast tissue, gastric mucosa and choroid plexus, with the remainder removed from the plasma via the kidneys (~85-90%) and the thyroid gland (~10%) (33, 34). Iodine accumulated by the kidneys is eventually excreted in the urine at a relatively constant rate, whilst iodine absorbed by the thyroid, initiates the production of both active and inactive thyroid hormones (33, 34). Located on the apical surface of thyrocytes found within the thyroid gland, a sodium/iodine symporter (NIS) actively transports iodide from the plasma through the epithelial cell at a concentration gradient 20-50 times greater than that in circulation (3, 33). In conjunction with hydrogen peroxide, the iodide molecule is then oxidised by the TSH regulated hemoprotein enzyme, thyroperoxidase, in order to yield iodine (3, 32-34). Iodine is subsequently bound to tyrosyl residues on the thyroglobulin protein stored within the epithelial cell, initiating a cascade of complex reactions to produce monoiodotyrosine (MIT) and diiodotyrosine (DIT) (33). These hormone precursors eventuate to thyroid hormones, active T3 and, to a greater extent, inactive T4 (Figure 2.1) (33). Iodine contributes to 65% of T4's molecular weight and 59% of T3 (34).

Figure 2.1 Iodine Metabolism and Thyroid Hormone Production



Once released into circulation by endocytosis, the half-life of T3 is 1.5-3 days and the half-life of T4 is 5-8 days (33, 34). Most of the T3 hormone within the plasma is a consequence of deiodination of T4 within the liver and other tissues (3). The process of deiodination, where an iodine atom is removed from T4 to create T3, is dependant of the presence of the selenium dominant enzyme, 5-deiodinase (2, 4, 35). The released iodine atom reverts back into circulation where it can be utilised by the thyroid again or excreted by the kidneys (2, 34). Within the plasma, iodine only has half-life of about 10 hours (34).

The process of thyroid hormone production, and thus the uptake of iodide by the thyroid gland, is regulated by a negative feedback mechanism controlled by the pituitary glands response to circulating T4 (3). The thyroid gland adapts to varying degrees of iodide exposure by regulating the uptake of iodide at the thyroid gland site, and consequently adjusts thyroid hormone synthesis to meet biological requirements (2, 3, 33, 34).

IODINE EXPOSURE

Environmental Iodine

Iodine is one of the most abundant micronutrients found in seawater (36). With oceans and seas occupying over 70% of the earth's surface, these water reservoirs are the primary source of iodine exposure to the animal and human food chain (36). Originating from molten at the earth's core, the concentration and molecular composition of iodine varies within the sea levels according to temperature, microorganisms, plant species and salinity (36, 37). Relevant to the Queensland population, within the Pacific Ocean, iodide is more prominent in surface water above the depth of 200 meters and iodate dominates the deeper water below (36). The transfer of iodine from ocean waters to the food chain occurs via three major processes; 1) iodised seawater is evaporated in a gaseous phase and subsequently precipitated over land, contaminating soils, leaching into crops/vegetation and fresh water sources 2) algae, seaweeds and salt water fish are incorporated into the animal/ human diet via food or supplements or 3) extraction of iodine from water with little sediments e.g. salt (36, 37). Existing in the ecosystem as either solid, liquid or gaseous form, human exposure to iodine can occur via drinking water, inhalation (i.e. Sea air), and consumption of foods sourced from the ocean/sea or grown in iodine enriched soil.

Iodine in Foods

The bioavailability of dietary iodine from most foods is reported to be up to 90% and is generally considered a nutrient of high yield (3, 32, 34). Considering the environmental origins of iodine, as expected, the major contributors of dietary iodine are found from marine sources (38). Seaweed or Nori, salt water fish, crustaceans and other seawater plants or animals notably contain high concentrations of iodine (38). Not surprisingly, countries where traditional cuisines are dominated by such foods, generally have high dietary iodine intakes (7, 38, 39). Within a balanced diet, the iodine contribution from foods can differ between countries, especially amongst milk/dairy, bread and egg samples (38, 40). The use or non-use of iodophors during the sanitation procedures conducted by dairy farmers has been shown to vary the iodine content of dairy samples (40). Likewise, whether chickens are fed an iodine enriched grain influences the iodine content of eggs or whether a food fortification has been implemented, the iodine content of food samples accordingly influence the amount of iodine consumed by the population (2, 15, 18, 39, 40). It is generally accepted that, milk/dairy products, eggs, fish/seafood and some cereals are the most dominate sources of iodine in the human diet (39, 40). The 22nd Total Diet Study analysed sample of Australian food considered to be commonly consumed by the general population (27). Out of the 97 foods analysed by the study, the top 20 foods with the highest amount of iodine (ug) per kilogram are shown in table 2.1 (27).

Table 2.1 The middle bound mean of the top 20 foods identified to have highest amount of iodine (ug) per kilogram according to 22nd Total Diet Study

Food Item	Iodine content (ug/kg)
Iodised salt	80 500.0
Nori sheets	17 333.3
Eggs boiled	366.0
Fish fillets	356.4
Prawns (cooked)	250.6
Salmon (canned)	233.3
Cheddar cheese (full fat)	229.0
Ice-cream (full fat)	213.3
Salt non – iodised	201.5
Chocolate milk	196.7
Fruit yoghurt	166.7
Cheese processed	174.0
Milk (modified low fat)	159.0
Cottage cheese	152.9
Milk (full fat)	133.0
Tuna Canned	108.7

Tomato sauce	107.5
Fish crumbed (oven bake)	106.8
Fish battered	103.2

IODINE BIOAVAILABILITY AND BIOACCESSIBILITY

Commonly described as U shaped, the association between iodine intake and thyroid disease has been well established, given that thyroid dysfunction is observed in both iodine deficient and iodine excessive populations (figure 2.2) (4). As a consequence of poor dietary iodine intake, iodine deficient populations are likely to show symptoms of functional and developmental disorders including; hypothyroidism, endemic goitre, intellectual impairment and cretinism (4, 5). It is estimated that nearly 2 billion people are affected by some degree of iodine deficiency (1), of which 29.8% or 246.2 million are school children (7). Conversely, populations consuming excessive amounts of dietary iodine are more likely to experience autoimmune hypothyroidism (40, 41), sporadic goitre, Graves disease, iodine-induced hyperthyroidism and some cancers (16, 31). Lower Intelligence Quotients (IQ) have been observed in school aged children residing in areas of both iodine deficiency and iodine excess (28, 43).

Figure 2.2 The u-shaped curve relationship with iodine intake and thyroid function.

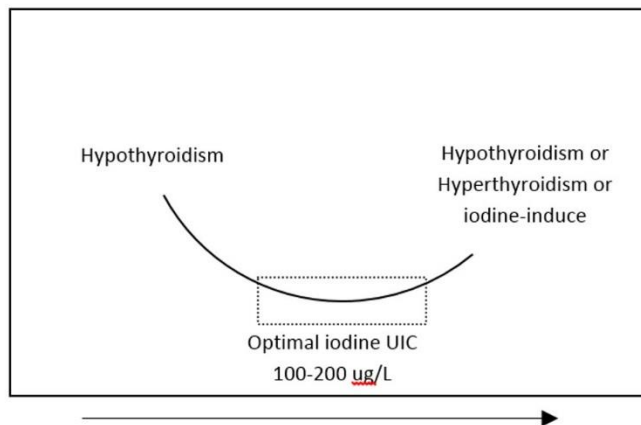


Figure 2.1. A theoretical model of the U- shape relationship between iodine status and thyroid function. If UIC is within the optimal levels of 100-200ug/L, the risk of thyroid related disease is at its lowest point.

Table adapted from source: *The U-shaped curve of iodine intake and thyroid disorders* (Laurberg, Pedersen et al. 2009)

Iodine Deficiency

Where iodine deficiency occurs, circulating T3 and T4 are low, which causes a response by the pituitary gland to secrete more TSH (3, 34). Consequently, the epithelial cell increases its sensitivity of NISs and its production of Tg, to accommodate a greater uptake of iodide (3,

34). As a result, hyperplasia and hypertrophy of the thyroid cells and gland occurs and a goitre may develop (3). Thyroid enlargement is particularly expected when there is a chronic iodine deficient intake below 50ug/day (34). Hypofunction of the thyroid gland is observed when iodine deficiency remains uncorrected. In neonates and children, the decreased production of T3 hormone is of concern, as this an essential growth hormone required for neurodevelopment and somatic growth (2, 3, 4, 9).

Iodine Excess

As discussed elsewhere, the thyroid gland can tolerate up to 1000 fold above recommended dietary iodine intake in healthy individuals (16). As expected, an increase in circulating hormones T3 and T4 are initially observed where there is an abundance of iodine, which can lead to hyperthyroidism (16). In some circumstances, an excess presence has been documented to stimulate T cells for an immune response to produce TPOAbs and TgAbs that interfere with the oxidation of iodide to iodine and the coupling of iodine with Tg to produce thyroid hormones (16). In such cases, autoimmune thyroiditis is observed which may result in the hypofunction of the thyroid gland (16).

Other Influences on Iodine Metabolism and Thyroid Hormone Synthesis

Nutrient Interactions

As an essential component in the deiodinase reaction, a deficiency in selenium may inhibit the utilization of iodine for thyroid hormone production and hinder the conversion of T4 to T3 (2, 16, 35). Its antioxidant properties play an important role in protecting the thyrocyte against oxidative damage caused by hydrogen peroxide (3, 16, 32). Likewise, Iron deficiency may reduce the activity of the heme dependant enzyme thyroperoxidase, thus reducing the availability of oxidised iodine for thyroid hormone synthesis to begin (34, 35). Vitamin A deficiency may increase the development of a goitre as the regulation of TSH stimulation is not adequately maintained by the vitamin A mediated TSH suppression gene (34). High levels of potassium, calcium, copper and fluorine have been noted to compete for iodine uptake by the NIS and thus compromise the bioavailability and bioaccessibility of iodine to the thyrocyte (32).

Goitrogens

Goitrogens are substances that either interfere with thyroid hormone production or inhibit the uptake of iodine by the NIS (2, 3). Such goitrogens may be present when such foods including; cabbage, cauliflower, broccoli and turnip, are consumed in high amounts (3, 32). Excessive consumption of foods including nuts, sweet potato and corn have been known to produce thiocyanates, which hinder the concentration gradient of iodine entering the epithelial cell (2, 32).

Chemicals

Certain chemicals within the environment can imitate goitrogens also. Perchlorate actively inhibits the function of sodium-iodine symporter (8). Thiocyanates, a by-product of cigarette smoke, inhibits the deiodination process, limiting the conversion of T4 to T3 (8, 34).

METHODS OF MONITORING IODINE STATUS

Urine

The process of analysing urinary iodine concentration (UIC) as a marker for a populations' iodine status is strongly supported by World Health Organisation (WHO) and has been used as an indicator in numerous studies around the world (1). The criterion for a population's iodine status according to UIC are described in table 2.2. Compared to thyroid size (which indicates chronic iodine exposure), UIC is regarded as the most practical marker for a populations iodine status because of its ease and effectiveness to assess acute iodine exposure within a large sample of individuals (1). The representation of immediate iodine exposure by method of urinary analysis, is a viable indicator on the basis that, most iodine not absorbed by the body eventually appears in the urine (1, 3).

Table 2.2 Criteria for assessing iodine nutrition based on median urinary iodine concentration of school aged children (> 6 years)

Median UIC (ug/L)	Iodine Intake	Iodine Nutrition
< 20	Insufficient	Severe iodine deficiency
20-49	Insufficient	Moderate iodine deficiency
50-99	Insufficient	Mild iodine deficiency
100-199	Adequate	Optimal iodine nutrition
200-299	More than adequate	Likely to provide adequate intake for pregnant/lactating women, but may pose a slight risk of more than adequate intake in the overall population.
≥ 300	Excessive	Risk of adverse health consequences (iodine induced hyperthyroidism, auto-immune thyroid diseases)

Table adapted from source: WHO et al. 2007 (1)

Evaluating and Expressing Urinary Iodine

Urinary iodine is usually expressed as; 1) median urinary iodine concentration or UIC (ug/L) (usually from first morning void urine samples), 2) urinary iodine excretion or UIE from 24 hour urine samples (ug/24-h or ug/day) or 24hr excretion (UIE), or 3) an iodine creatinine ratio (I/Cr x predicted 24hr urine output) (1, 44, 45). Throughout iodine literature UIC and UIE are used interchangeable, despite the expressions representing different estimates of iodine (44, 45). The WHO states that provided a sufficient number of spot urine samples are collected from the population, the added burden of collecting 24hr urine samples, especially from young children, is not necessary (1). Furthermore, there is no validated criterion for appropriate cut-offs describing the varying degrees of iodine status using UIE or I/Cr as an expression, as the estimate can vary between ages, genders and ethnicity (44, 45). As UIC is largely influenced by urine volume, which is known to vary day to day, but adjusting for the protein creatinine

(Cr) (product of muscle metabolism) can reduce the impact of this variation on the interpretation of iodine nutrition (20, 44, 45). The challenge in correcting for creatinine is that it is influenced by BMI, age, gender and ethnicity and has no validated cut-off criteria for iodine nutrition (20, 46). Recently, however, Montenegro-Bethancourt et.al. (2015), reported convincing data to suggest that UIC reasonably reflects actual 24-hr iodine excretion (UIE) but only when corrected for creatinine (45). Whilst the approach of correcting UIC for Cr may yield a more accurate representation of actual 24-hr urinary iodine excretion, for the purpose of a population survey, there remains no validated criteria to appropriately categorise the findings. Furthermore, WHO considers this process expensive and not necessary, as a sufficient number of samples collected from a population can also counterbalance the influence of such biological variances (1). Furthermore, in populations with adequate general nutrition, urinary iodine concentration has been shown to correlate well with the urine iodine/creatinine ratio and 24hr urine samples (33).

Consistent with the methodology used by the 2003-04 Australian National Iodine Nutrition Survey (NINS) and WHO recommendations, iodine measurements in this research project were expressed as UIC from multiple spot urine samples and ascertained by the ammonium persulfate digestion prior to a Sandell-Kolthoff reaction methodology (47). This procedure only requires 0.5-1.0ml of urine sample and is endorsed by World Health Organisation (1, 47).

When collecting urine samples, meticulous methodology is required to establish nearest representation of iodine status. Urinary iodine concentrations are expected to vary from day to day as well as within the day, particularly in populations with adequate iodine intake (19, 20). Provided there are a sufficient number of samples collected, it is accepted that a median UIC will provide an adequate representation of a population's iodine nutritional status (1). It is important, however, to also consider the distribution of UIC within the population as WHO describes an iodine adequate population as having a median UIC between 100-199ug/L with no more than 20% of UIC sample below 50ug/L (1).

Thyroid Function

Thyroid function is indicative of the thyroid glands response to iodine intake (1). Urinary iodine concentration is an indicator of iodine status but not of thyroid function (14), hence the reason why this current study analysed thyroid physiological markers to determine the association between thyroid function and UIC. Although sustaining optimal levels of dietary iodine is

expected to prevent thyroid dysfunction, populations with UICs which fall within normal limits may still be vulnerable to thyroid dysfunction as consequence to recent changes in dietary iodine (18).

Thyroid volume is a reflection of iodine nutrition history (14). It can be used as a long-term indicator of iodine exposure but does not represent acute iodine status (14). It has been noted in many studies where universal salt iodization (USI) has been introduced to iodine deficient areas, that it can take up to 10 years for thyroid volume to modify in response to change in iodine intake (14). For the purpose of this study, thyroid volume was the less preferred method of iodine status assessment because of its limited response to varying dietary iodine and methods used to determine its size can be inconsistent (15). As the objective of the current study is to measure the impact of iodine within a two to three-year time frame after introduction of iodine fortification in bread, the insensitivity of thyroid volume measurements made it an inappropriate method for this research project. Instead this is the first paediatric Australian study to collect thyroid physiological markers, Thyroid stimulating hormone (TSH), free thyroxine (fT4) and free triiodothyronine (fT3) and Thyroglobulin (Tg) to also detect acute iodine status. Thyroid antibodies, thyroid peroxidase antibody (TPOAb) and thyroglobulin antibody (TgAb) were used to detect any presence of autoimmune disease associated with iodine status.

Assessment of Dietary Iodine Intake

Nutritionists have long known that the assessment of habitual dietary intake is difficult due to a number of factors including daily, weekly and seasonal variation as well as under and over reporting (46). The assessment of iodine intake is further challenged by the influence of a number of factors including fertilisers, irrigations, sanitisers (dairy industry), grain feeds for livestock, rainfall and location (48). Assessing dietary iodine intake is predominately evaluated by using one or more of the following nutrition assessment tools; 24 hour recall, food frequency questionnaire and dietary records (49).

All methods of dietary assessment are subject to limitations and bias coinciding with application burden and cost (46). Due to their relative ease to administer, ability to capture long term variation in diet and consumption patterns, FFQs are the most common dietary assessment tool when evaluating dietary mineral intake in epidemiological studies (46, 49). The benefit of using a FFQ in dietary assessment is that it can reflect the habitual dietary patterns an individual

may adopt over a week, month or year (46, 49). At the time of commencement of this study, the number of validated iodine FFQs was limited but of those that did exist, compared to dietary records, the level of iodine intake established by FFQs was generally overestimated (50). Food frequency questionnaires are unreliable when determining absolute specific RDI cut-offs and therefore may not reflect precise dietary amounts (50, 51). They are useful, however, in classifying groups into high or low intakes as well as identifying the main dietary sources of iodine (50). It is for this reason, FFQs were used to estimate the ranking dietary intake of individuals but are not used to determine absolute measures of dietary intake (51). The method, however, is heavily reliant on memory recall, and may be limited to subjective interpretation of portion sizes leading to over or under estimates of micronutrient intake (46, 52). 24-hour recall dietary records measure short term intakes and may better quantify iodine foods consumed (49). Whilst 24-hour recalls and repeated 24-hour recalls dietary records have been shown to be better correlated to reference markers of nutrition (biological or additional dietary assessment), 24 hour recalls are subject to the same recall bias (especially regarding salt use) as FFQs (53). Furthermore, evidence suggests that 24-hour recalls are required to be repeated up to 10 assessment day or collected from a large sample size to have a good correlation with 24-hour urinary iodine excretion (53).

Weighed dietary records are expensive and cumbersome to implement (46, 49). Work from almost 30 years ago showed that to determine accurate measures of intake for a single nutrient of micronutrient required repeated data collection for many days (54). For example, Basiotis and colleagues (54) considered the number of days of intake data required to accurately estimate the value of an intake of specific nutrients within individuals. Although iodine was not considered specifically, it was noted that higher precision may be desired in the intake estimate of nutrient that is not stored in the human body for long periods (54). In this respect, they cite vitamin C as one such nutrient, but iodine would also fall into this category. Basiotis and colleagues (54), calculated that in order to obtain a precise estimate of vitamin C (precision was defined as an X-day average intake being within 10% of the 'true' average intake 95% of the time, the 'true' intake was based on analysis of food records collected for 365 consecutive days), data would need to be collected for about 230 days.

Bingham (55) used a slightly different approach and calculated that in order to achieve a measurement that was $\pm 10\%$ of the standard error of the average intake of vitamin C would require 36 days of daily records. Likewise, Bingham did not specifically study iodine, however,

the principle of collecting dietary records for such a high turnover nutrient, like iodine, could be relevant.

Such repeated measures were considered too cumbersome for the targeted population and was thought to potentially exacerbate existing recruitment challenges explained in chapter 3.

INTERPRETATION OF IODINE INDICATORS

Urinary Iodine Concentration

Numerous studies report high variability in UIC. Soldin described a circadian rhythm of urinary iodine concentration over a 24 hour period (20). Of the 3,023 urine spot samples collected over time, UIC in adults and children was found to be independent of the subjects' age and gender (20). The lowest concentration of urinary iodine was reported in the morning spot samples with a gradual increase in concentration over the rest of the day (20). In children, it was also observed that urinary iodine concentration peaked 4-5hours after a meal and then returned to base line between 21:00 to 22:00 hours (20). This observation is consistent with the knowledge that ingested iodine appears in the urine after an approximate 4-5 hour delay (56). Another cross-sectional study involving 60 volunteers, concluded that UIC from spot samples collected in the afternoon, between lunch and dinner time, have a stronger correlation to urinary iodine concentrations determined by 24hr collection compared to first morning spot samples (57). A 24 hour sample collection of urinary iodine in Denmark also supported the theory of variable iodine excretion throughout the day (58). The study concluded that due to lower levels of iodine observed in the morning urine collections, morning samples used to determine the iodine status of a population, could potentially underestimate the overall iodine status (19, 58). Furthermore, when evaluating the different expressions of UIC (24hr, I/L, I/Creatinine) a study analysing two population groups in Switzerland and Belgium confirmed that a single spot urine sample expressed as I/L underestimated the iodine status by 30-35% when compared with 24hr UIC (59).

Taking into account the obvious variations in urinary iodine excretion throughout the day, a single spot morning sample may not adequately represent the mean value of UIC over a 24 hour period (20, 56, 59). It is the opinion of some organisations that obtaining 24hour urinary samples is cumbersome and unnecessary in large populations (1). No matter what period of day spot samples are collected for urinary iodine analysis, it is important to consider the influence time has on sample collection when comparing data for iodine status (56, 58).

There is strong evidence to suggest that collecting afternoon samples to determine UIC will provide a better representation of iodine status (56, 57).

The Australian National Iodine Nutrition Study (NINS) conducted in 2003-2004 prior to the introduction of mandatory iodine fortification of bread, collected first morning urine samples from 294 QLD school age children which revealed a median UIC of 136.5ug/L (10). As previously discussed, the distribution of iodine status within the population meant that 14.7% of UIC samples were categorised as having 'more than adequate' iodine nutrition and 3.4% were within the range of 'excessive' iodine nutrition (Eastman. C personal communication). As UIC is lowest in the first morning void the distribution of samples within each WHO category may have been underestimated. Furthermore, the advantage of collecting afternoon UIC samples in an attempt to better reflect iodine status has not been explored in children. Considering the variations observed in UIC throughout the day, this thesis provides an important assessment on the value of collecting both a morning and afternoon UIC sample from each participant on two consecutive days. As later discussed, the approach implemented by the current study reveals a better representation of the distribution of iodine status for a cohort.

Justification for repeating data collection on two consecutive days is described in the Healthy Kids Queensland Nutrition Survey (60). According to this survey, information from successive data sets can be used to compare differences between subjects and within subjects (60). Furthermore, successive data sets also allow adjustments to be made to the data collected, which consequently reduces variance and strengthens the precision of extreme values found within the distribution (60). When observing the distribution of values, adjusted measurements reduce the variability or error which can occur day to day and express a better estimate of nutrient inadequacy or excess (25).

Considering one of the objectives of this proposed research project was to monitor the extreme values of iodine status in the population (particularly iodine excess), the value of collecting multiple UICs are further explained in Chapter 4.

Thyroid Analytes

Thyroid dysfunction can manifest as a variety of diseases. Hypothyroidism (underactivity of thyroid hormone production) can often present as autoimmune thyroiditis and/or Hashimoto's disease (61) and Hyperthyroidism (overactivity of thyroid hormone production) can present as

Graves disease (16). In the context of iodine supplementation the onset of thyroid disease in response to an increase in dietary iodine can be referred to as iodine-induced hyperthyroidism (16) or iodine-induced thyrotoxicosis (62).

The thyroid gland can tolerate relatively high amounts of iodine intake without adverse effect as demonstrated by Korean and Japanese populations (16, 63, 64). It is arguable that cultural cuisines such as seaweed products and fish dishes are popular foods in these countries and allow the thyroid to adapt to high levels of iodine over a lifetime. The relationship between thyroid dysfunction and higher iodine intakes is more often observed amongst populations exposed to a sudden increase of iodine rather than a prolonged exposure to high amounts (65). Theories as to why the thyroid responds acutely to a sudden increase in dietary iodine include; a predisposition to thyroid disease as indicated by the presence of thyroid nodules (16), injury to the thyroid follicular cells (66), and immune responses incorporating thyroid antibodies (67). A well-known mechanism used by the thyroid gland to manage an acute influx of iodine, is the Wolff-Chaikoff effect (68). This process involves blocking thyroid production by inhibiting thyroid peroxidase from converting inorganic iodide to organic iodine (68). The Wolff-Chaikoff effect is considered to be transient, however, in some individuals this response continues and thyroid dysfunction develops (68).

Thyroid volume is a reflection of iodine nutrition history (34). It can be used as a long term indicator of iodine exposure but does not necessarily represent present iodine status (14). It has been noted in many studies where universal salt iodisation (USI) has been introduced to iodine deficient areas, that it can take up to 10 years for thyroid volume to modify in response to change in iodine intake (14). It is for this reason, thyroid volume is the less preferred method of iodine status assessment because of its limited response to varying dietary iodine and methods used to determine its size can be inconsistent (15). Considering that the objective of this current study was measure the impact of iodine fortification within a timeframe of 2-3 years after implementation, the insensitivity of thyroid volume measurements rendered it an inappropriate method for this research.

Thyrotropin (TSH), fT3 and fT4

Thyroid stimulation hormone (TSH), free thyroxine (fT4) and free triiodothyronine (fT3) concentrations are routinely evaluated when testing thyroid function (68). Total thyroxine and total triiodothyronine are also common measurements (70). Nonetheless, fT4 and fT3 are

preferable markers to screen for thyroid dysfunction, because they not bound to protein and are therefore a stable reflection of available hormones (70). In the presence of acute iodine excess the thyroid responds by increasing serum TSH whilst decreasing fT4 and fT3 (70). The effect of varying iodine intake on thyroid test is outlined in table 2.3.

Table 2.3 The effect of iodine intake on thyroid hormone concentrations

Iodine nutritional status	TSH	free T4	free T3	Other
Iodine deficiency	↑	↓	↑	↓rT3*,↑Tg^
Acute Iodine excess	↑	↓	↓	
Acute iodine excess on nodular thyroid gland	↓	↑	↑	Possible development of thyrotoxicosis
Chronic iodine excess	-	-	-	

Table adapted from *Interpretations of Thyroid Function Tests and Their Relationship to Iodine Nutrition* (70) pg. 53
 * reverse Triiodothyronine
 ^ Thyroglobulin

Depending on the actual amount of change in dietary iodine intake, hormone levels may remain within normal limits even though a change in overall concentration levels may be observed (70). It is the accuracy of interpreting the change in these hormone levels that is particularly important to establish a correct diagnosis (70). The general interpretation of thyroid function test, TSH, fT4 and fT3 are outlined in table 2.4.

Table 2.4 General Interpretation of thyroid function tests

	Raised fT4 or fT3	Normal fT4 and fT3	Low fT4 or fT3
Raised TSH	Pituitary hyperthyroidism Resistance to thyroid hormone Recent ingestion of T4	Mild (subclinical) hypothyroidism	Primary hypothyroidism
Normal TSH		Euthyroid	Pituitary hypothyroidism Nonthyroidal illness
Low TSH	Primary hyperthyroidism	Mild (subclinical) hyperthyroidism	

Table adapted from *Interpretations of Thyroid Function Tests and Their Relationship to Iodine Nutrition* (70) pg. 49

Where hypothyroidism is prevalent, TSH levels are elevated above normal range and fT4 levels fall below normal range (71). Hyperthyroidism, on the other hand, is determined by a decrease in TSH and an increase in fT4 in addition to elevated fT3 (71). Although in adults, TSH is used as maker for detecting hyper- or hypothyroidism, in children TSH alone is known to have inconsistent normal ranges depending on age (71). Despite these inconsistencies, abnormal TSH often precedes other abnormal hormone levels and it is for this reason that measuring both TSH and fT4 is the most preferable method when evaluating thyroid dysfunction in children (71). The combined analysis of TSH, fT4 and fT3 will provide substantial diagnostic information for both hypothyroidism and hyperthyroidism to be detected if present (70, 71). Hence, in this thesis, the combined analysis of TSH, fT4 and fT3 was performed.

Thyroglobulin (Tg)

As the most abundant thyroid protein in the body, thyroglobulin (Tg) is a key precursor in the production of thyroid hormones (72, 73). Thyroid hormone is dependent on iodine uptake and its integration with thyroglobulin to initiate the production of mono- and di-iodotyrosine, which cascade into thyroid hormones, T4 and T3 (32, 74). Originating in thyroid tissue alone, Tg levels are reflective of three factors (74, 75);

1. the presence of differentiated thyroid tissue
2. the presence of any physical damage to, or inflammation of the thyroid gland
3. the amount of TSH stimulation

Thyroglobulin can be elevated where there is large thyroid volume, thyroid inflammation or high levels of TSH present (71).

The co-dependent relationship between iodine and thyroglobulin, suggests Tg is a sensitive marker for recent changes in iodine nutrition (74). The relationship between iodine intake and Tg levels are outlined in table 2.5.

Table 2.5 Thyroid status and iodine intake influence of thyroglobulin serum levels

	Iodine Intake	Serum Tg concentration
Eufunction of thyroid gland	Deficiency	Increased
	Adequate	Normal
	Excess	Normal or Increased
Hypofunction of thyroid gland	Deficiency	Decreased
	Adequate	Decreased
	Excess	Decreased
Hyperfunction	Deficiency	Increased
	Adequate	Increased
	Excess	Increased

This table is extrapolated from *Thyroglobulin as an Indicator of iodine Intake* (74)

Closely associated with UIC, Tg is becoming a popular indicator to use in increasing dietary iodine environments (68, 71). The WHO reference range for Tg in iodine sufficient children aged 5-14 years is 4-40 ug/L (1). As Tg levels reflect iodine uptake into the thyroid, Tg as an indicator will provide information regarding of how thyroid responds to increasing dietary iodine in iodine deficient, iodine adequate and more than adequate populations.

Thyroid Peroxidase Antibody (TPOAb) and Thyroglobulin Antibody (TgAb)

Measuring thyroid peroxidase antibody (TPOAb) and thyroglobulin antibody (TgAb) is a standard diagnostic tool when screening for autoimmune thyroid diseases (76). The immune system produces thyroid autoantibody proteins which act against other proteins in the thyroid tissue when there is an increase in dietary iodine or genetic tendency for autoimmune disease (66, 67, 76).

Thyroid peroxidase is responsible for catalysing the oxidation process of plasma inorganic iodide to organic iodine and in turn enables the beginning of thyroid hormone production (76). TPOAb is the protein that inhibits this oxidation process in the presence of autoimmune thyroid disease (76). Under the influence of the Wolff- Chaikoff effect, a sudden increase in dietary iodine has been associated with an increase of TPOAb and TgAb activity (76).

Higher intake of iodine may adjust thyroid hormone production and as a result change the prevalence of thyroid disease within a population (77). Using the discussed indicators of thyroid function, this thesis explored the presence of each analyte in the setting of iodine sufficiency.

Food Frequency Questionnaire

The recommended dietary intakes for children ages 2-3 years and 8-10 years are 90ug/day and 90-120ug/day respectively (2). Extrapolated from adult toxicity studies (2, 33), the upper limit of iodine intake for these age groups are 200 ug/day and 300-600 ug/day respectively (2). Identifying contributing food groups of iodine within populations, not only verifies the effectiveness of iodine fortification programs, but also aids in the development of recommendations which can be used to further improve iodine nutrition where necessary (40).

In other population surveys there has been no significant relationship between fortified bread and urinary iodine concentration (78), while other researchers report that iodine fortified bread is a major contributor to iodine status (79-81). Although reports indicate an increase in UIC of Australian school aged children since the implementation of iodine fortification of bread (82), this project provides evidence regarding to what significance iodine fortification of bread has had on the iodine status of QLD children in particular.

Prior to this thesis, there were no Australian data on the inter-dependant relationship between UIC and dietary iodine intake. Comparing dietary iodine sources between populations is difficult, as variations in iodine composition of foods, supplements and water can influence overall iodine intake (40, 32). Nevertheless, there are consistent trends suggesting milk, seafood and non-alcoholic beverages (mostly water) are significant contributors to iodine status (32, 38). This research project is the first mainland Australian study to investigate the influence of drinking water, iodised salt use and supplements on the iodine status of Australian children.

Although monitoring the effectiveness of iodine fortification in iodine deficient populations has generated much attention, there is little research that focuses primarily on monitoring the iodine status of iodine replete populations, especially after the introduction of an iodine prophylaxis program.

Little is known about the primary sources of dietary iodine for QLD children. Furthermore, there is little literature regarding the iodine intake of younger children (e.g. <5 years). For Australian children aged 2-3 years and 8-10 years in general, foods considered to be key contributors of dietary iodine prior to iodine fortification of bread included; milk, milk products, non-alcoholic beverages, fish and seafood dishes (2, 83). The discretionary amount of iodised salt used is difficult to quantify, but as most Australian children consume more than the recommended level of salt through processed foods, it is assumed that iodised salt used in bread manufacturing is now an important source (82). In some studies it has been identified by semi-quantitative food frequency questionnaires that milk and discretionary iodised salt remain to be major contributors of dietary iodine more so than iodine fortified foods, such as bread (84, 86). Other iodine fortification programs have not increased iodine status to an expected level, largely due the fact individuals consumed less bread than predicted (85).

Using an un-validated FFQ, one Tasmanian study evaluated dietary influences on UIC of 170 children aged 4-12years (86). The questionnaire assessed the intake of foods such as; iodised salt, ice-cream, cream cheese, soy milk, salt water fish, shell fish, seaweed products and preserved cherries (86). The study confirmed that anticipated dietary sources of iodine (such as dairy products) were correlated with UIC, but yoghurt or 'fruche' was the most significant contributor of dietary iodine because of iodophers used in its' manufacturing process (86). Likewise in South Australia, to most significant source of dietary iodine for toddlers prior to iodine fortification was yoghurt (83).

As aforementioned, thyroid function is not completely dependent on the amount of iodine ingested, but can also be subjective to barriers of iodine absorption (32). Selenium (a co-factor in thyroid hormone production) and iron deficiency have been reported to exacerbate thyroid dysfunction in iodine deficient populations (32, 35, 87). In addition to foods that enhance the utilisation of iodine by the body, a category of foods referred to as goitrogens are known to hinder iodine status (32, 34). Brassica vegetables such as cabbage, cauliflower, broccoli as well as turnip inhibit the uptake of iodine by the thyroid (32, 34). Dietary assessments which take into account the influence of these vegetables on iodine status are limited. Therefore this thesis considers the influence of these foods when assessing the relationship between food intake and UIC.

As mentioned previously, measuring micronutrient intake using food frequency questionnaires has its challenges. Bias associated with FFQs such as memory recall, interpretation of food

items and differing portion size descriptions interfere with the accuracy of the nutrient intake (88). Furthermore, determining what foods are associated with UIC is dependent on several dynamics. Consideration for environmental influences, processed or unprocessed foods and variations in iodine compositions of similar food types (i.e. milk, cheese, bread, fish) need to be accounted for (40). It is therefore imperative that the FFQs are designed unambiguously and results should be compared with other references of iodine status, such as biochemical markers (88). Excluding questions regarding supplement use and discretionary salt intake can also misrepresent dietary iodine intake (66, 88).

With the number of iodine fortification programs on the rise, there is growing concern about the adverse consequences being observed in some susceptible populations (8, 89). The significance of this study is that it provides information regarding the effect of increasing dietary iodine in QLD children and determines their susceptibility of adverse consequences as a result of mandatory iodine fortification.

GLOBAL IODINE STATUS AND IODINE PROPHYLAXIS

Prophylaxis is an intervention implemented to maintain health and prevent the spread of disease. Using urinary iodine concentration data from 150 national or subnational studies, a 2012 review reported that 32 countries were iodine deficient; 71 countries were iodine ‘adequate’; and 47 countries were considered to be ‘more than adequate’ or ‘iodine excessive’ (1, 7). The irreversible consequences suffered by iodine deficient populations has generated much attention worldwide (5), and are key motivators in the development of iodine prophylaxis programs to improve iodine status (1). In collaboration with UNICEF and Iodine Global Network (IGN), the World Health Organisation (WHO) provides clear guidelines regarding appropriate implementation and monitoring of iodine prophylaxis programs in *Assessment of Iodine Deficiency Disorders and Monitoring their Elimination* (1). Prophylaxis programs are unique to each country and may involve; universal salt iodination (USI), iodised salt bread fortification, iodine fortification of animal feed or direct oral supplementation (90). The prophylaxis programs discussed further in this chapter are outlined in table 2.6.

Table 2.6 Global examples of prophylaxis programs and experiences with UIC and thyroid response

Country/Province	Mode of Prophylaxis (concentration of iodine ppm)	Population of Interest	Year Prophylaxis Commenced (year of iodine nutrition assessment)	Assessment of Iodine nutrition/status	Median UIC outcome	Thyroid outcome
China (95)	Iodised salt (discretionary)	School children >13 years in 3 regions with previous history of mildly iodine deficient, more than adequate and excessive iodine status	1996 (2004)	UIE, FFQ, TSH, fT4, fT3, Tg, TPOAb, TgAb, thyroid ultrasound	88ug/L, 214ug/L and 634ug/L respectively	Cumulative incidence of overt hypothyroidism was 0.2 percent, 0.5 percent, and 0.3 percent, respectively; that of subclinical hypothyroidism, 0.2 percent, 2.6 percent, and 2.9 percent, respectively; Autoimmune thyroiditis, 0.2 percent, 1.0 percent, and 1.3 percent, respectively.
Spain (96)	Iodised salt and bread (60 ppm)	Total population	1985 (1989)	Census data	na	The incident rate of thyrotoxicosis (defined by depleted TSH and elevated T4), in the previously iodine replete region of Spain increased by 2.5 fold after prophylaxis
Spain (97)	Iodised salt and bread (60 ppm)	School aged children 6-7 years	1985 (2011)	UIC, FFQ, TSH	173ug/L	In total, 0.5% of children had known hypothyroidism (derived from the questionnaire) and 7.6% had TSH levels above reference values. Median TSH was higher in schoolchildren with family history of hypothyroidism.
Poland (100)	Iodised salt (30ppm)	School aged children 6-15 years	1997 (1992-2001)	UIC, thyroid ultrasound	Increased from 56ug/L (prior to fortification) to 103ug/L (after fortification)	Prevalence of goitre reduced from 14.5% to 5.2%

North Western Greece (98)	Iodised salt (40-60ppm) - voluntary	School aged children 12-18 years	1963 (2003)	UIC, TSH, T4, T3, Tg, TPOAb, TgAb, thyroid ultrasound	202.1ug/L	Incidence of thyroid anomalies increased 3 fold: Subclinical hypothyroidism 2.5%. Positive Anti-thyroid antibodies including those with subclinical hypothyroidism 10.6%. Hyperthyroidism 9.6%.
Denmark (76, 84) [Dan Thyr Study]	Iodised salt and baking products (13ppm)	Child bearing aged women 18-22 years	1998 (1997/98 – 2004/05)	UIC, 24-hr UIE, I:Cr, FFQ,	61ug/L (prior to fortification) to 101ug/L (after fortification)	A 50% increase in incidence of hyperthyroidism in the area with the most severe iodine deficiency previously (33).
Sri Lanka (104)	Iodised salt	Female school aged children 11-16 years	1993 (1998)	UIC, TSH, ft4, ft3, Tg, TPOAb, TgAb, thyroid ultrasound	105(1995), 152ug/L (1998), 154ug/L (2001), (across the age groups)	The prevalence of TgAb was elevated in all age groups: 14.3% in 11-year-old, 19.5% in 12-year-old, 44.1% in 13-year-old, 53% in 14-year-old, 52% in 15-year-old and 69.7% in 16-year-old schoolchildren by 2001.
Switzerland (106-108)	Iodised salt (20ppm)	[pilot study] children 3-15 years	1998 (1998-2000)	UIC, TSH, ft4, ft3, Tg, TPOAb, TgAb,	141ug/L (prior to fortification) to 162ug/L (after fortification)	TSH, ft3 and ft4 analytes significantly decreased compared to baseline, however, remained within an acceptable range.

Due to differences in exposure to salt supply and consumption, each country based intervention has a distinct approach to developing and implementing iodine prophylaxis (91). The methodologies used for monitoring the impact of iodine prophylaxis vary from country to country, thus making it difficult to directly compare the impact of iodine prophylaxis between population groups (92). Nevertheless, globally there are shared experiences between countries and these observations support the need to monitor iodine prophylaxis programs post-implementation (14, 30).

The introduction of iodine prophylaxis programs is usually successful in reducing the severity of iodine deficiency (90). Between 2007 and 2012, the number of countries classified as iodine deficient has decreased from 47 to 32 (93, 94). As the number of prophylaxis programs increase, there is now emerging concern for those populations at risk of developing iodine-induced autoimmune thyroiditis as a result of reaching iodine upper limits (8). The number of countries classified as, more than adequate or excessive, has increased from 34 to 47 (93, 94).

Iodine-related thyroid anomalies have been reported in several countries including China (95), Spain (96, 97) and Greece (98), despite exposure to iodine prophylaxis. The extent of which thyroid disease develops within a population after iodine prophylaxis is not only dependant on present iodine status, but on the iodine status of the population prior to iodine prophylaxis (65). Although it is assumed that previously mild to moderate iodine deficient populations are more likely to be predisposed to the adverse side effects of increasing dietary iodine (17), some studies show that iodine sufficient populations may also experience side effects in changing iodine environments (77, 65, 99).

Both hyperthyroidism and autoimmune hypothyroidism have been observed in iodine sufficient populations resulting from an acute increase in dietary iodine from salt iodization (17, 61). Despite the median UIC of a population remaining within acceptable limits, the prevalence of thyroid disease may still be observed despite only minor changes to dietary iodine intake (13, 31, 96, 99). These observations have been recorded in countries such as Spain and China.

In Spain, census data of a previously iodine sufficient province, showed an increase prevalence of thyrotoxicosis or Grave's disease within six months of introducing iodinated salt in bread and salt products (96). The incident rate of thyrotoxicosis (defined by depleted TSH and

elevated T4), in the affected region of Spain increased 2.5 fold (96). More recently Spanish school aged children were reportedly iodine replete with a median UIC of 173ug/L, however, the incidence of TSH anomalies was 7.6% despite only 0.5% having previously identified thyroid disease (97). The presence of thyroid dysfunction whilst iodine replete was associated with familial history (97) which illustrates that thyroid dysfunction may still occur even when exposed to acceptable iodine intake.

Like Australia, China is also a country with regions of iodine sufficiency and iodine deficiency (95). Following USI, hypothyroidism and autoimmune thyroiditis was more prevalent amongst those Chinese population groups who were either iodine sufficient or iodine excessive prior to the introduction of USI (95).

A Polish study observed the changing iodine status of 1471 iodine deficient school children between the years 1992/93 to 1999/2001, during which time salt was mandatorily fortified with iodine in 1997 (100). The baseline median UIC of 56ug/L increased to 103ug/L with an improvement in thyroid volume observed in only half of the regions investigated (100). This would support the assumption that although exposed to the same iodine prophylaxis, the thyroids response to such a change in iodine exposure will differ between individuals over time.

Likewise, in North-western Greece the prevalence of autoimmune thyroiditis amongst school aged children increased from 3.35% in 1994 with median UIC of 84 ug/L, to 9.6% in 2001 with median UIC of 202ug/L, whilst under the influence of ongoing voluntary iodine fortification of salt (98, 101). This study confirmed the findings of another evaluation which found children, aged 8-15 years, who were positive for thyroid autoantibodies (indicating autoimmune thyroiditis) had higher median urinary iodine concentrations than those children with negative antibodies (102). Furthermore amongst those who were positively identified as having thyroid antibodies in the presence of higher UIC (median 206ug/L), an increase in thyroid volume were also observed (102). It was concluded that although the iodine prophylaxis program was successful in eliminating iodine deficiency in parts of Greece, it has come at the expense of emerging autoimmune thyroid disease amongst children with sufficient iodine status (98, 102).

Denmark have reported marked differences in the prevalence of thyroid disease between populations differing only slightly in their iodine dietary intake (103). In the year 2000,

Denmark introduced mandatory iodization of salt with 13 ppm of iodine, and 4 years after implementation, observed a rising prevalence in hypothyroidism associated with the increasing iodine dietary intake of the population (77). Although the implementation of an iodine fortification program resulted in increasing the median UIC from 61ug/L to 101ug/L to establish iodine sufficiency, the Danish experience demonstrates that thyroid dysfunction can still exist within iodine replete populations (84).

A 1998 Sri Lankan study reported elevated TgAb in female children aged 11-16 years (104). The observation is consistent with a biological response to an acute rise in iodine intake as a result of salt iodination in 1993 (104). The observation was not prolonged, however, with a follow up survey reporting a decrease in the prevalence of thyroid goitre and thyroid autoimmunity between two independent groups of school girls, aged 11-16 years in 1998 (n = 401) and 2001 (n = 282) (104, 105). The initial prevalence of thyroid autoantibodies amongst the school girls in 1998, had significantly decreased by 2001 (105). It was observed, however, that thyroid autoimmunity was most likely to persist in regions where thyroid autoantibodies previously existed and therefore the onset of thyroid disease could not be completely attributed to an increase of dietary iodine (105).

A sample of 42 previously thyroid autoantibody-positive girls, aged 10-17 years, were included in a smaller prospective study conducted simultaneously with the larger study. The smaller study revealed an altered ratio in the types of autoantibodies present in those individuals exposed to the ongoing iodine prophylaxis (105). It was reported that in the event of increasing dietary iodine, the presence of thyroglobulin antibodies (TgAb) decreased in 2001, whilst the presence of thyroid peroxidase antibodies (TPOAb) increased, when compared to 1998 (105). This indicated the reversal of thyroid autoimmunity in some individuals, but in others, the onset of subclinical hypothyroidism (105). Again the two studies, suggest that depending on the participants preceding thyroid function, a varied immune response can occur when there is an acute change in dietary iodine. An interesting observation in this study, however, was the median UIC of the total population did not change significantly between studies from 1998 to 2001 suggesting that the thyroid gland eventually adapted to the changes in iodine intake in the majority of individuals.

Several studies monitoring the impact of iodine prophylaxis in Switzerland have documented the success of increasing UIC in children aged 3- 15 years to iodine sufficiency status (106-108). In a pilot study it was observed that the UIC of children increased at a faster rate than

adults (108). Despite having the higher UIC increase rate when compared to adults, thyroid biochemical markers confirmed that children were not subject to any side effects of the increasing dietary iodine (108). Although the study was limited to a small sample size of 12 participants, the overall decrease in the average TSH observed amongst children remaining within acceptable limits. Converse to the other studies, the Switzerland experience suggests that changing dietary iodine intake can impact thyroid function without causing dysfunction.

A challenge in comparing the outcomes from these global experiences to the QLD population is that each study had its own strengths and limitations. The Polish study only investigated thyroid volume and no physiological markers whilst the Danish study only measured thyroid physiological markers but not UIC. Some studies only used census data (96), excluded children from participation (95), had only small sample sizes or omitted the inclusion of food analysis with iodine status indicators (105). These differences emphasise that the effect of iodine prophylaxis within a population cannot necessarily be predicted by other experiences.

It has been suggested that the detrimental side effects resulting from iodine prophylaxis have hindered the progress of much needed iodine supplementation in some deficient populations (91). Therefore the importance of accurately documenting observed changes that occur within a population undergoing probable shifting iodine status, will give perspective on the reality of these concerns and will potentially instigate better implementation procedures.

AUSTRALIAN IODINE STATUS

Between 1994 and 2006, ninety-four countries participated in urinary iodine national nutrition surveys, effectively collating data reporting iodine status for 91.1% of the world's population (1). The Australian National Iodine Nutrition Study (NINS), conducted between July 2003 and December 2004, incorporated 1709 year 4 school children (typically aged 8-10 years) from New South Wales, Victoria, South Australia, Western Australia and Queensland (11). Overall it was concluded that Australian school aged children had inadequate iodine intake based on a median urinary iodine concentration (UIC) of 96 ug/L (109).

Children in New South Wales, Victoria and South Australia were considered to be mildly deficient or borderline deficient with UICs of 89ug/L, 73.5ug/L and 101ug/L respectively (11). On the other hand, Western Australia and Queensland children showed evidence of iodine sufficiency, with UIC of 142.5ug/L and 136.5ug/L respectively (11). The results of this national survey were used by FSANZ when iodine fortification was being considered, which eventually

led to the mandatory use of iodised salt in bread manufacturing commenced in October 2009 (110). FSANZ mandated the use of salt containing 25-65mg/kg of iodine (potassium iodide/iodate or sodium iodide/iodate) during the manufacturing of all bread and bread products, only excluding organic bread and salt garnishes (12). It was forecast, that the amount of iodine fortification required to improve the national iodine status, would not cause iodine sufficient children in WA and QLD to exceed the optimal range for iodine (10). These estimates were based on expected dietary intakes (10) and although best efforts to reduce the likelihood of adverse effects in response to iodine prophylaxis, it has been argued that dietary intake alone is not an adequate indicator of the functional consequences which can arise when a sudden change in iodine consumption occurs (15). Therefore, monitoring tangible outcomes, such as biological markers, is essential.

Prior to the Australian National Iodine Nutrition Study, several sub-national studies also raised the concern of re-emerging iodine deficiency in certain regions of Australia (111, 112, 113). Independent of the Australian NINS, a sample of Melbourne school aged children were found to be mildly iodine deficient with a median UIC of 70ug/L (111). Additionally, evidence of emerging iodine deficiency was also observed amongst pregnant women and neonates throughout Sydney hospitals (114, 115).

When investigating the iodine status of Australian children, these studies were predominately conducted in the south-eastern states of Australia which excluded Queensland from their analysis. Furthermore, the methodologies used in these sub-national studies were limited to spot urine samples and thyroid volume assessments (111, 112, 113). Prior to the commencement of this study, there were no published mainland Australian data evaluating the combined association between three key indicators of iodine status; thyroid function, dietary iodine intake and urinary iodine concentration from a paediatric cohort.

With inconsistent urinary iodine concentrations observed between states within Australia, there has been a call for further investigation as to why such variations occur (11). Suggestions explaining why Queensland children have adequate iodine levels compared to the national level include; possible use of iodised salt, increased iodised milk contamination or higher levels of iodine in the drinking water (11). Nevertheless, these assumptions were unconfirmed and in order to identify any unique influences that contribute to the iodine status of QLD children, it was required to collectively measure the impact of dietary iodine on the thyroid function and iodine status.

The current iodine prophylaxis program is not a first in Australia. The issue of iodine deficiency was initially confronted in Tasmania during the mid 1960's (116). Up to 20 ppm of potassium iodate substituted potassium bromate in bread improver in April 1966, increasing the mean daily intake of iodine between 80-270ug depending on sex and age (116). In the latter months of 1966, a peak in the incidence of thyrotoxicosis was observed in thyroid clinics throughout the state (116, 117). Similar to the conclusions made by FSANZ prior to the current Australian fortification program, it was predicted that the amount of iodine required to eliminate iodine deficiency in Tasmania, would not contribute to excessive iodine consumption (10, 117). Although it was originally thought that iodine fortification would not trigger adverse side-effects in normal healthy individuals, the sudden rise in thyrotoxicosis after the commencement of iodine fortification instigated some concern (117). Even though the incidence of goitre seemed to decline amongst some schoolchildren, thyrotoxicosis persisted (117). Five years after iodine was fortified in bread, newly reported cases of thyrotoxicosis eventually began to lessen, nevertheless iodate bread improvers were removed from the manufacturing process in 1976 due to other contaminants such as iodine in water and iodophors in milk sanitation influencing iodine intake (117).

This Tasmanian experience reinforces the concern that thyroid dysfunction can potentially be a side-effect of an acute increase in dietary iodine. The observation also highlights other variable dietary or environmental factors which can contribute to the overall iodine intake of a population and in turn exacerbate the impact of a sudden rise in dietary iodine.

More recently, however, the problem of iodine deficiency has re-emerged in the state of Tasmania. In the year 2000 it was reported that median UIC of Tasmania children aged 4-17 years was 84ug/L (113). It is believed that inconsistent monitoring of various iodine fortification programs employed by Tasmania has resulted in irregular iodine status and thyroid dysfunction over the years (118). As observed in the state of Queensland, iodine status in Tasmania was not uniform across different regions (118). Eliminating social economics, age and gender influences, geography proved to be the only variable impacting UIC (118). Without dietary intake measures or recorded drinking water iodine concentrations, it is not possible to elaborate why geography has such a significant influence on UIC. Nevertheless, voluntary fortification of bread with iodine was reintroduced in 2001 and since then, the UIC of Tasmanian children aged 8-11 years has improved (81). Investigations measuring the impact on thyroid function or dietary intake of this most recent mainland Australian iodine fortification

program are yet to be conducted and therefore possible side-effects of its implementation cannot be scrutinized.

Since the commencement of this thesis, the 2012 Australian National Health Survey (NHS) reported the UIC of QLD school aged children to be 165.9ug/L since iodine fortification of bread was introduced (119). This increase is 29.4ug/L greater than the baseline data reported by Li and colleagues (11) but not to the extent which was predicted by FSANZ (10). Although it is assumed that the iodised salt consumed via bread is responsible for this increase in UIC (82), a direct correlation has not been determined. Furthermore, the biological response by the thyroid to this dietary increase of iodine has not been elsewhere assessed amongst school aged children.

Skeaff and colleagues (83) did, however, most recently report data on the UIC and Tg levels of preschool children prior to the introduction of iodine fortification in Australia. Investigators reported a mean daily iodine intake of 2-3 year olds as 71ug/day, with a corresponding median UIC of 122ug/L, in 68 South Australian pre-school children prior to the implementation of iodine fortification in Australia (83). It was predicted that the introduction of mandatory iodine fortification would increase the median UIC of this cohort to approximately 130-160ug/L. With this expected increase, it was estimated that approximately 10-14% of preschoolers within this group could be at risk of attaining an UIC >300ug/L, which is considered excessive in other populations (83). The challenge of categorising iodine status according to UIC is further explored in chapter 6, however, with FSANZ also expressing concern that children within this age group were at risk of consuming excessive amounts of iodine as result of iodine fortification through bread (10), at the commencement of this study, the outcome of that risk was unknown.

The importance of iodine prophylaxis in iodine deficient regions is undeniably essential to prevent irreversible disease. On the other hand, when blanketing iodine prophylaxis programs, it cannot be disregarded that there is a potential for iodine sufficient regions to experience undesirable side-effects. Without complete dietary iodine intake records or databases that document existing thyroid disease in Queensland, it is difficult to accurately predict how children in regions of varying iodine status will respond to a sudden change in iodine intake. The implication of possible side-effects could impede the optimal health outcomes intended by the implemented Australian iodine fortification program. The need to monitor the impact of Australian iodine fortification is critical.

CHAPTER 3: RESEARCH DESIGN AND METHODOLOGY

STUDY DESIGN

An observational study of children aged 2.00-3.99 years and 8.00-10.99 years, conducted in south east Queensland during November 2011 to March 2013. The study was separated into 2 parts, A and B. Part A, required a morning urine sample (first urine void after waking) and an afternoon urine sample (first urine void after midday meal) on two consecutive days from each participant. Simultaneously, carers collected water samples from the source the participant drank from on the days of urine collection. Carers completed a FFQ for each participating child. The chief investigator (A. Samidurai) reviewed the completion of FFQs at time of sample collection where basic anthropometrics were also recorded. Part B, was an optional addition to Part A where carers could consent to a venous blood sample collection from their child participating in Part A for the purpose of thyroid hormone analysis.

Setting

By definition the south east Queensland region incorporates an area north of the NSW border to Noosa and stretches to the west to include the Locker Valley and Somerset (approximately 120km radius).

Figure 3.1 Geographical area of south east Queensland.

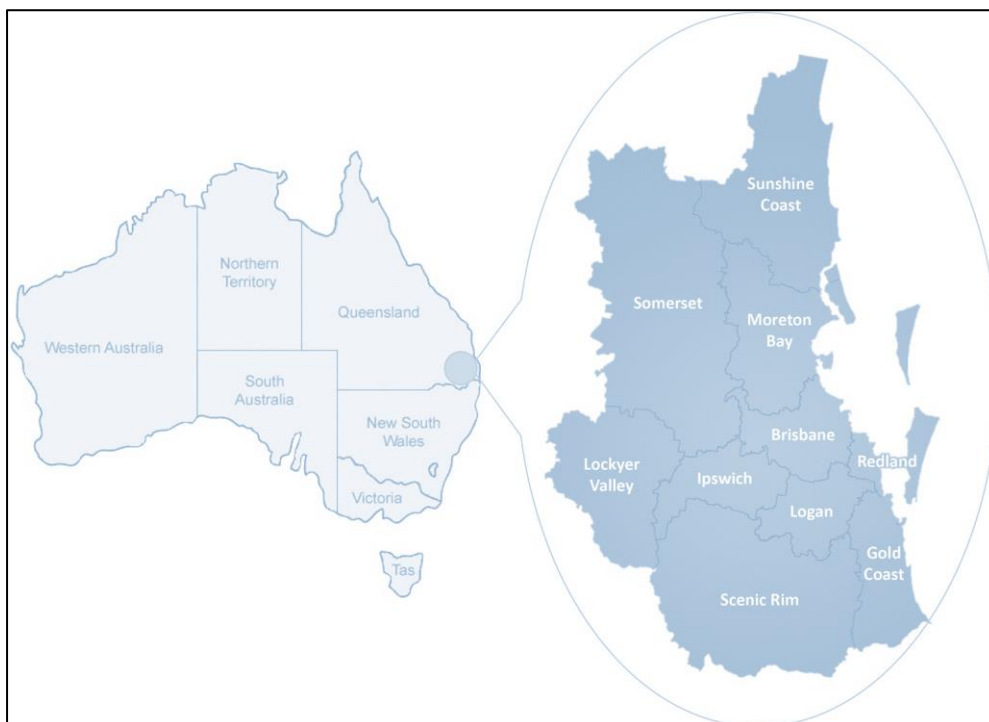


Figure 3.1 shows south east Queensland encompassing 12 government regions, it has estimated total population of 3.27 million people, with almost 1 million of those residents under the age of 14 years (120). The main catchment reservoirs sourcing water to this region include Little Nerang and Hinze Dam, Wivenhoe Dam and Brisbane River, including Somerset Dam (SEQ Water, personal communication, February 2010). Desalinated water is also integrated into the drinking water supply, using water from the Coral Sea (SEQ Water, personal communication, February 2010). Whilst the eastern region borders the Pacific Ocean the western regions are mountainous. There are 122 Primary schools in the region (121). Prior to the introduction of iodine fortification of bread, this region was considered to be iodine replete with a median UIC of 137.0ug/L (Eastman C personal communication).

SAMPLE SIZE

Differences estimated in dietary iodine as a result of iodine fortification (10) are used to calculate a possible 46ug/L increase to be detected in the UIC. Using this expected change as a reference, calculations to establish an appropriate sample size are explained in table 3.1.

Table 3.1 Calculations for sample size ages 8-10 years using baseline data from the Australian National Iodine Nutrition Survey

Australian National Iodine Nutrition Study : Queensland data source C. Eastman

Mean : 152.7 ug/L

Median : 136.5 ug/L

SD: 80.1

Kolmogrov-Smirnov test for normality revealed skewed data

Therefore, log₁₀ performed on data and revealed normal distribution

Mean : 2.130

SD: 0.216

Combined the original Mean and the difference to be detected (46ug/L) to establish a predicted Mean after iodine fortification

$152.7 + 46 = 198.7$ ug/L

Expressed as ratio of the original mean

$198.7/152.7 = 1.301$

log₁₀ of expressed ratio = 0.1143 therefore, this value can be used with new log₁₀ SD, 0.216 required to be detected $0.1143/0.216$ (SD) = 0.529SD

Sample Size (N) = $16/f^2$ where f^2 = difference to be detected in SD units

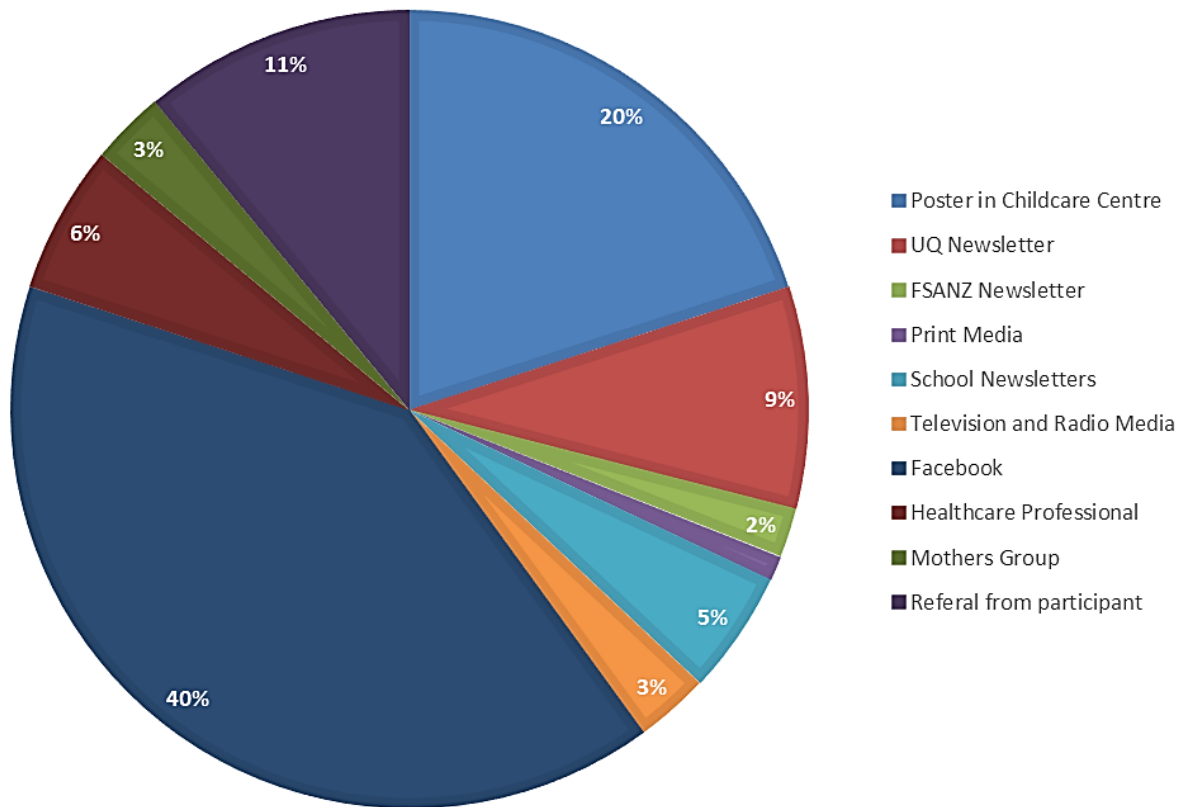
$N = 16/0.53^2 = 56.9$ therefore, **57** children are required in each sample size to detect a significant difference of 46ug/L in urinary iodine concentration within the population.

For children aged 2-3 years it was estimated that their dietary iodine intake would increase by 38ug/day (10), implying that UIC could potentially increase by approximately 32.3ug/L. At the time of commencement of this study there were no baseline data of UIC available for this population group, it was therefore not possible to calculate a difference between UIC before and after mandatory iodine fortification. Nevertheless, it had been established, using the calculations from the 8-10 year old group, that when monitoring changes in UIC it would be desirable to detect a change of at least half a standard deviation in the data. Therefore, as with the older age group, a total of 64 participants was the anticipated number to recruit.

METHOD OF RECRUITMENT

A cluster sample scheme, as suggested by WHO, was attempted through primary schools within Queensland. Applications and appeals to the Department of Training and Education in Queensland to permit this process were, however, denied. Consequently, recruitment strategies focused on assembling a convenience sample through various forms of advertisement; posters in childcares, newspaper articles, television news reports, letters to local general practitioners and child healthcare workers, mothers groups, school newsletters (private institutions) and paid advertisement through social media (figure 3.2). At the time of recruitment the use of social media in public health surveys was still emerging, and in this circumstance, the method was justified by other population health surveys using a similar approach to yield representative samples (122-124). Using Facebook allowed targeted advertisement to community members who profiled as being between the ages of 18-45 years residing in areas of Gold Coast, Brisbane, East Brisbane, West Brisbane, South Brisbane, Sunshine Coast and Wide Bay Burnnet. The Facebook page was shared on other community pages in order to improve its reach. Facebook generated the largest number of respondents (n=76).

Figure 3.2 The proportion of respondents to the various mediums of advertisement for the current study.



PARTICIPANTS

Of 191 respondents to advertisements, a total of 70 children aged between 2.00 years and 3.99 years (2-3years olds) and 37 children aged between 8.00 years and 10.99 years (8-10year olds), were recruited into the study. Fifty one children aged 2-3 years and thirty children aged 8-10 years completed the survey. The majority of participants resided within 100km of Brisbane city. Figure 3.3 shows the approximate spread of participants across the south east Queensland according to postcode. Reasons for attrition included; no longer wanting to participate, no longer responding to communication or failure to complete survey in study timeframe or failed to complete at eligible age (before/after birthday) (figure 3.4). The method of recruitment is detailed in each publication chapter.

Figure 3.3 Approximate distribution of participants throughout south east Queensland according to postcode

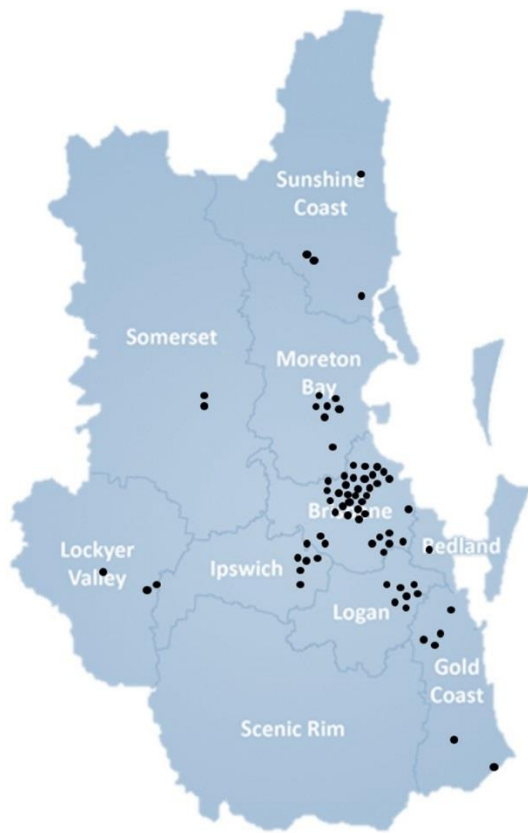
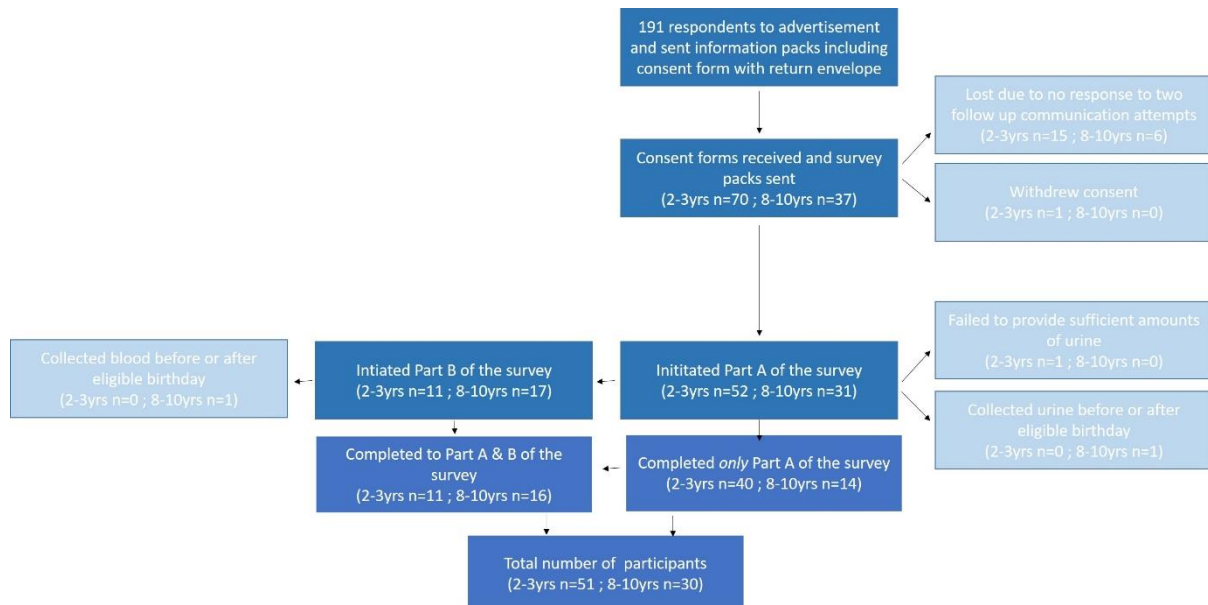


Figure 3.4 Retention of respondents and participants in the current study



SIEFA

Socio-economic status (SES) was measured at the postcode level using the Socio-Economic Indexes for Areas (SEIFA) Index of Relative Disadvantage, in an attempt to determine how well the recruited participants represented the broader population. This index was developed by the Australian Bureau of Statistics (125). Participants were categorised as lower SES (SIEFA decile 1-3) /middle SES (SIEFA decile 4-7)/ upper SES (SIEFA decile 8-10) (table 3.1).

Table 3.2 The proportion of postcodes within each rank of socio-economic status according to Socio-Economic Indexes for Areas (SEIFA) Index of Relative Disadvantage for south east Queensland total population, 2-3year old participants and 8-10 year old participants.

	All SEQ postcodes	2-3 year olds	8-10 years olds	2-3 year olds (thyroid analyte)	8-10 years (thyroid analyte)
Lower SES (decile 1-3)	12.8%	13.2%	0%	18%	0%
Middle SES (decile 4-7)	33.4%	11.7%	46%	27%	25%
Upper SES (decile 8-10)	53.2%	70.5%	54%	55%	75%

It is obvious that although the recruited 2-3 year olds showed a better representation of the greater south east Queensland population, children from the 8-10 year old cohort were over represented in the middle and upper SES deciles. The impact of SES on iodine status has reportedly had no effect in Australia previously, which is further explained in the subsequent chapters.

For those who participated in Part B (thyroid analytes) of the survey, the SES distribution of participants is also outlined in table 3.2. No children participating in either part of the survey had any known thyroid disease or had prior investigations for thyroid disease. The differences in known family history between those children who participated in part B and those who did not are described for both age groups in table 3.3. Table 3.3 also describes the percentage of those carers who had a prior knowledge about thyroid function prior to the survey.

Table 3.3 The percentage of participants with family history of thyroid disease and carers knowledge of thyroid function prior to survey

	2-3 year olds Part A	2-3 years olds Part A and B	8-10 year olds Part A	8-10 year olds Part A and Part B
Fathers family history	5.0%	27.5%	7.0%	0%
Mothers Family history	18.0%	27.5%	29.0%	31.0%
No family history	67.0%	45.0%	43.0%	63.0%
Unknown	10.0%	0.0%	21.0%	6.0%
Previous awareness of thyroid function	64.0%	85.0%	87.0%	100.0%

OUTCOME VARIABLES

Urinary Iodine Concentration

Each participant received a de-identified labelled urine sample specimen cup and was instructed to collect a first morning urine sample on day one. On the same day, a second urine sample was collected after the midday meal. Carers labelled each sample with the date and time of collection. This process was repeated on the following day. For participants aged 2-3 years, caretakers/guardians were asked to assist in the urine sampling collection procedures and to place cotton wool balls in nappies if the child was not toilet trained. Urine samples were stored in participants' home freezer until collection by investigator. Urine samples that were collected from home site were transported to the Children's Nutrition Research Centre, Royal Children's Hospital, Brisbane, in an insulated cooler with ice bricks. Samples were stored at -20°C until sent via courier on ice to Westmead laboratory, Sydney for analysis.

Iodine status was categorised according to UIC using the WHO criterion for iodine status as shown in table 2.2 (1). Urinary iodine concentration were ascertained by ammonium persulphate digestion before a Sandell-Kolthoff reaction method and reported in ug/L, as recommended by WHO (1, 47). For consistency, this is also the same method performed by the NINS and was conducted at same laboratory at Laboratory Endocrinology, Pathology West/ICPMR, Westmead Hospital, Sydney.

A single sample of water was repeatedly measured 30 times by the Sandell-Kolthoff method at the laboratory, prior to total sample analysis. Of the repeated measurements, the Standard Error

of Mean was 3.9ug/L and Coefficient of Variance was 2%, which were within the acceptable range outlined by the guidelines (47).

Food Frequency Questionnaire

Adapted from the Healthy Kids Queensland Survey (60), the complete semi quantitative FFQ is in Appendix 1. Written instructions, with examples of responses, were provided to carers who self-administered the FFQ on behalf of their participating child.

The FFQ was divided into four sections. Section 1 comprised of a total 110 food items to estimate the frequency of foods consumed, daily, weekly or monthly over the past 12 months. Within this section 10 food types i.e. bread and cereals, dairy, salt, etc., were further expanded into specific food items i.e. thin bread, bread rolls, flat bread, or organic. The reason food items were specified in this section was to primarily observe whether children who consumed bread, consumed bread that was fortified with iodised salt. As all Australian bread, except organic bread, is fortified with iodised salt, it would have been useful to compare the iodine status of children who consumed bread fortified with iodised salt and those who consumed bread that was not fortified with iodised salt (e.g. organic bread), and/or which items of bread were the greatest contributors to overall bread consumption. Other foods included in the FFQ were identified by the 22nd Total Diet Study as having various concentrations of iodine (ug) per kilogram or were considered to be foods commonly consumed by children i.e. tomato sauce (27). Foods known to have goitrogenic properties such as broccoli, cabbage and cauliflower were also included in the survey, as well as salt and iodised salt use. Serving size was based on standard serving sizes outlined by the Australian Dietary Guidelines – Healthy Eating for Children (126). The information collected from this section was used to report the data presented in chapter 7.

Section 2 focused on 16 food items, to capture the number of serves of specified food types consumed per day. The data from this section were used in two ways 1) to find associations between number of overall bread serves consumed and UIC, 2) Compare the UIC of children consuming high (>2 serves/day) vs low serves (\leq 1 serve/day) of bread. Serving size was based on standard serving sizes outlined by the Australian Dietary Guidelines – Healthy Eating for Children with measurements expressed as slice, cup, ½ cup or ml (126). Examples of what was considered a serve were included in the questions e.g. 1 serve of milk equates to 250ml. For the purpose of this thesis, foods and beverages of particular interest were bread, milk, yogurt,

sushi, nori, water, eggs, saltwater fish and brassica vegetables (broccoli, cauliflower, cabbage), as they are assumed to be important sources of dietary iodine for Australian children (2, 10). The information collected from this section (section 2) was used to report the data presented in chapters 5, 6 and 7.

Sections 3 and 4 capture general demographics of the participating children. Information obtained included postcode, country of birth, home smoker status, known thyroid conditions of the child and familial thyroid conditions. This information was used to determine social economic deciles within chapters 4,5,6,7 and 8. Data pertaining to participants' individual thyroid health history and family history were incorporated in chapter 8. None of the children resided in homes with a smoker, so this information became redundant in further analysis.

Completeness of the FFQ was reviewed by a nutritionist at the home visit when frozen urine samples were collected and anthropometric measurements taken.

Thyroid analytes

Venous blood samples were collected at various Queensland Medical Laboratories (QML) collection centres. Blood sample were drawn into a 'yellow ss tube' (8.5ml capacity) which was labelled, centrifuged and transported by QML staff to the QML Brisbane laboratory. A minimum of 5mls was required to provide at least 2ml of serum. After centrifugation, analysis for TSH, fT4, fT3, TPOAb and TgAb were preformed using the Siemens Centaur Method and Tg levels were obtained using the Siemens ImmaLite 2000.

Drinking water iodine concentration

Drinking water was collected from the source from which the child drank on the days of urine collection. In total, two water samples were collected for each child which were frozen and analysed using the same laboratory and method as describe for attaining UIC.

EXPLANATORY VARIABLES

Each participant was allocated a serial number corresponding as their identification for urine samples, blood samples and FFQ. Upon sample collection height was measured using a portable stadiometer (to the last completed mm) and weight was be recorded using digital scales (to 0.1kg). Children were asked to empty pockets, remove shoes and excess clothing before measurements were recorded. Anthropometric procedures replicated those described in the Healthy Kids Queensland Survey as provided in Appendix 2 (60).

Age, gender, post code of residence, place of birth, smoking or non-smoking home, language spoken, Indigenous identity and familial history of thyroid disease were captured in the FFQ. A socioeconomic rank was obtained for each individual by linking Socio Economic Indexes for Areas (SEIFA) data at the postcode level (125).

ETHICS

The study was reviewed and approved by the Medical Research Ethics Committee, The University of Queensland (#2001000125) (Appendix 3). Informed written consent was attained from carers of participating children (Appendix 4).

STATISTICAL ANALYSIS

Stata statistical software (StataCorp, College Station, TX, USA), SPSS (IBM, NY, USA) and Microsoft Excel (Microsoft Corporation, WA, USA) were used for statistical analysis through the thesis. Details of statistical analysis of arising data from this thesis are outlined in each publication chapter.

CHAPTER 4: IMPROVING THE MEASURE OF IODINE STATUS USING UIC BY REPEAT MEASURES TO REDUCE THE VARIATION.

INTRODUCTION

As discussed the variability of UIC between individuals and within individuals is well documented. As a result, the coefficient of the variance (CV) is large and may inaccurately represent the distribution of UIC samples in each category of iodine status, especially those in the upper and lower extremes. This paper demonstrates that by collecting multiple UIC samples, the CV is reduced and consequently changes the interpretation of iodine status within a cohort.

MANUSCRIPT DETAILS

The manuscript details are as follows:

Samidurai AJ, Ware RS, Davies PSW. Advantages of collecting multiple urinary iodine concentrations when assessing iodine status of a population. *Acta Paediatrica*, 2015; 104: 524-529 doi: 0.1111/apa.13168

The manuscript has been reformatted to fit the requirements of the thesis.

ADVANTAGES OF COLLECTING MULTIPLE URINARY IODINE CONCENTRATIONS WHEN ASSESSING IODINE STATUS OF A POPULATION.

Abstract

Aim: Evaluate the effect of collecting multiple (four) urine samples on the extensive variance often observed within a cohort when determining iodine status via urinary iodine concentration (UIC).

Methods: Fifty one children aged 2-3 years and thirty children aged 8-10 years participated in the study in south east Queensland, Australia. Each child's four urine samples were analysed using ammonium persulphate digestion before a Sandell-Kolthoff reaction method. Analysis of variance techniques were used to assess the effect of using multiple urine samples.

Results: The median UICs were 223.3ug/L and 141ug/L for 2-3 year olds and 8-10 year olds respectively. The coefficient of variance (CV) of UIC for children aged 2-3 years were reduced by 35.6%, 36.5% and 39.7% when two, three and four samples were included in the adjustment respectively. Similarly, the CV of UIC for children aged 8-10 years was reduced by 24.7%, 30.7% and 34.7%.

Conclusion: Although the practicality and cost of collecting multiple UICs needs to be considered, collecting multiple UIC samples from each participant provides a better reflection of a cohort iodine status.

Introduction

Iodine is an essential micronutrient required for thyroid hormone synthesis and metabolism (1). Appropriate dietary iodine intake is particularly crucial for young children as it influences both cognitive and physical growth (1). The iodine status of a population is traditionally assessed via morning spot urine samples for a number of reasons, notably convenience, cost and simplicity (19). The accuracy of the approach, however, is largely affected by the significant within-person variation in urinary iodine concentration (UIC) (1, 19). Adjustment for within-person variation will have little effect, if any, on the mean value within a population but will increase the precision of the variance within the population, reducing the proportion of individuals with very low or very high UIC (21).

As 85%-90% of dietary iodine appears in the urine within 4-5 hours after ingestion, UIC has been used as a viable indicator of population iodine status for a number of decades (1, 20, 32, 56). With its relatively acute renal clearance, UIC also reflects recent dietary intake. For this reason, UIC variation will replicate the variation in the diet throughout the day and between days. Studies report that in both adults and children UIC shows a significant circadian rhythm, being lowest in the morning and peaking in the early afternoon (19, 56, 57, 127).

Population iodine status is categorised according to the median UIC determined in that population (1). The World Health Organisation (WHO) and International Council for Control of Iodine Deficiency Disorders (ICCIDD) defines an iodine sufficient population as having a median UIC between 100-299ug/L, with no more than 20% of the population below 50ug/L (1). The cut offs used to determine iodine status are shown in table 4.1. WHO and ICCIDD acknowledge the impact of between-person variance when assessing iodine status, and thus recommend the recruitment of large sample sizes to reduce its influence on the overall iodine status of the population (1). Although this approach is one way to increase the precision of estimates, an alternative is to take repeated measures on the same individual. Although the effect of within person variation can be decreased by collecting twenty-four hour urine samples from individuals (20), this method is considered to be too cumbersome and impractical, especially in young children (1). Consequently, the iodine status of a population is typically determined by the analysis of a single spot UIC from a number of individuals (1). Without allowing for within-person variation, it has been argued that single spot morning urine samples may under estimate a population's median UIC and overestimate the frequency of samples in the tails of the distribution (19, 20, 56, 57, 127). This could misrepresent the distribution of

iodine status within the population, which may inadvertently impact the evaluation of any iodine nutrition intervention that may be in process.

Two Australian studies have demonstrated that collecting more than one urine sample from each individual in a population survey can significantly reduce the within person variation and strengthen the precision of extreme values within the distribution (22, 23). This approach of reducing within-subject variation in nutrition assessment has been used before on a number of occasions (60). Effectively therefore the approach reduces the standard deviation (SD) or interquartile range (IQR) and hence the variability of the data (60). Although proven to be effective in adolescents (22) and older cohorts (23), the practicability of this approach has not yet been determined in iodine nutrition surveys involving young children. Validation of this approach in young children will provide a more accurate representation of the proportion of children experiencing iodine deficiency or iodine excess.

The aim of this study was to assess the reduction of within person variability by collecting a number of samples and the relative merit of collecting multiple samples.

Subjects and Methods

Recruitment

Children aged 2.00-3.99 and 8.00-10.99 years residing in south east Queensland, Australia were eligible to participate. Convenience samples were recruited between November 2011 and March 2013 via various advertisement mediums including newspaper, television, radio, posters and social media. This method of recruitment has successfully yielded representative samples in other public health surveys (122-124). Informed written consent was attained from parents or guardians of participating children. The study was reviewed and approved by the Medical Research Ethics Committee, The University of Queensland (#2001000125).

Sample Collection

Carers collected from their child one spot morning and one spot afternoon sample (first morning void and first urine following midday meal) from their child. Collection was repeated on the next day. Cotton wool balls were placed in the diaper of those participants not toilet trained to collect urine. Thus a total of four urine samples were collected from each participant. Height and weight anthropometrics were measured to determine Body Mass Index (BMI) using standard formula. Socio-economic status (SES) was measured at the postcode level using the

Socio-Economic Indexes for Areas (SEIFA) Index of Relative Disadvantage. This index was developed by the Australian Bureau of Statistics and ranges from 1 to 10, with a low score indicating greater disadvantage (125).

Sample Analysis

All urine samples were stored at -18°C until assayed. UIC was determined using ammonium persulfate digestion before a Sandell-Kolthoff (47) reaction. Iodine status was categorised using the WHO criterion (1) for assessing iodine nutrition based on median UIC. For this method, the CV of repeated measurements of samples, in the range of iodine concentration found in urine, has been reported as being less than 2% (47). Iodine status categories determined by WHO and ICCIDD cut-offs as described in table 1.1.

Statistical Analysis

To calculate an adjusted UIC value for each child using the following formula: Adjusted UIC = [(person's UIC – group mean) x ($s_b \div s_{obs}$)] + group mean (22). To calculate the between-person (s_b) and total (s_{obs}) standard deviations the data was Log-transformed before we undertook a repeated measures analysis of variance. To investigate the effect of additional urine samples, UICs for each child were initially calculated using only the first sample collected, then the first two samples only, then the first three samples, and finally all four samples were included. For each number of samples analysed, the frequency of samples within WHO cut-offs and the corresponding exact binomial 95% confidence intervals were calculated. An adjusted UIC value calculated using all four samples was used in a multivariate regression model to assess the influence of SES and BMI on UIC. Mean morning and afternoon UIC means were calculated. T-tests were performed to investigate differences between mean morning UIC and mean afternoon UIC. The distribution of UIC for a single sample, and after correction for repeat samples, is displayed using kernel density estimates with an Epanechnikov kernel function and bandwidth of 50. Statistical Significance was set at 0.05 and all tests were two-tailed. Stata, SPSS and Microsoft Excel were used to perform statistical analysis.

Results

Participation

Fifty one children (30 males and 21 females) aged 2.00-3.99 years and 30 children (18 males and 12 females) aged 8.00-10.99 years completed the survey. Table 4.1 displays demographic, social and clinical characteristics of the sample.

Table 4.1 Descriptive demographics of 2-3 and 8-10 year olds in south east Queensland included in this study.

	2-3 year olds Mean ± SD	8-10 year olds Mean ± SD
Age	2.91 ± 0.5	9.4 ± 0.8
BMI	16.2 ± 2.7	17.0 ± 2.8
Sex Ratio (M:F)	1.4:1	1:1.5
SES (decile)	(1-3) 13.2% (3-7) 11.7% (7-10) 70.5%	(1-3) 0% (3-7) 46.0% (7-10) 54.0%

Urine Iodine Concentration

The UIC mean, median, standard deviation (SD) and interquartile interval (IQI) for raw data and each adjusted sequence are described in table 4.2 for both age groups. The adjustments had greatest effect on the extreme frequencies within the distribution, but little effect on the mean or the median, as would be expected. Essentially, repeating the UIC measurements provided a more accurate reflection of the proportion of samples were above or below the optimal iodine category cut off.

Table 4.2 Descriptive statistics of single and multiple urinary iodine concentrations for children aged 2-3 years and 8-10 years in south east Queensland cohort

Age (years)	Number of samples used in analysis	Mean (ug/L)	Coefficient of Variance (CV)	Standard deviation (SD) (ug/L)	Median (ug/L)	IQ Interval (ug/L)	Proportion of samples below 100ug/L	Proportion of samples above 299ug/L
2-3	1	246.07	56.5	139.1	237.0	142.0-308.0	13.7	25.5
	2	221.4	38.2	84.6	224.6	161.5-265.9	5.8	20.0
	3	220.9	37.8	83.4	224.3	162.0-264.9	5.8	20.0
	4	219.2	36.1	79.1	223.3	163.9-261.5	5.8	20.0
8-10	1	166.9	48.0	80.1	141.0	106.5-249.0	23.3	10.0
	2	159.1	36.6	58.2	143.07	115.8-219.6	7.0	0.0
	3	157.7	34.1	53.7	143.5	117.9-213.6	7.0	0.0
	4	156.8	32.3	50.7	143.8	119.5-209.6	10.0	0.0

The reduction in the standard deviation of the distribution when two or more samples are used in the analysis can be calculated using the ratio between S_b and S_{obs} , where S_b is the between persons standard deviation and S_{obs} is the total standard deviation (22). Using this approach, the SD of the log-transformed values for children aged 2.00-3.99 years were reduced by 35.6%, 36.5% and 39.7% when two, three and four samples were included in the adjustment respectively. For those aged 8.00-10.99 years SD values were reduced by 24.6%, 30.1% and 33.8% when two, three and four samples were included in the adjustment respectively.

The median UIC for children aged 2.00-3.99 years was 223.3 ug/L (IQI 163.9-261.5ug/L), indicating a more than adequate iodine status. Adjusted data indicated that 57% (95%CI 43%-69%) of children were above the cut off for iodine adequacy. A total of 37% (95%CI 24%-52%) of children were classified as more than adequate and 20% (95%CI 10%-32%) were classified as excessive for iodine status.

Likewise, the overall estimated median UIC for children aged 8.00-10.99 years was 143.8ug/L (IQR 119.5-209.6ug/L), indicating an adequate iodine status. Adjusted data indicated that 27% (95%CI 12%-46%) of children were above the cut off for iodine adequacy. No children in this cohort were classified as excessive for iodine status.

The effect of the adjustment has greatest effect on the spread of samples, contracting the IQI from 166.0ug/L to 97.6ug/L for 2-3 year olds and 142.5ug/L to 90.1ug/L for 8-10 year olds. Furthermore, the SD reduced from 139.1ug/L to 79.1ug/L for 2-3 year olds and 80.1ug/L to 50.7ug/L for 8-10 year olds.

No association was found between UIC and either BMI or SES in either age group. Mean afternoon samples (228.5 ug/L (SD 127.8) 2-3 years and 203.5 ug/L (SD 81.3) 8-10 years) in both age groups were higher than morning samples (190.5 ug/L (SD 127.4) 2-3 years and 145 ug/L (SD 81.5) 8-10 years), although these were non-significant.

Discussion

The distribution of UIC within a population is highly variable and not normally distributed (1, 20, 56). This study shows that the overall variance within a UIC distribution lessens when extra samples collected from each participant are used. It has been demonstrated, that compared to a single sample in our younger group of children, two UIC samples obtained from each participant within a population can reduce the variance within the distribution by up to 35.6%, three UIC samples up to 36.5%, and 4 UIC samples up to 39.7%. Although increased samples have little effect on the median UIC, they do substantially reduce the variation – with the consequence that the number of individuals recorded in both extreme tails is reduced.

In this study, collecting 4 urine samples instead of one spot sample from each participant resulted in reducing the proportion of samples categorised as iodine excessive from 25.5% to 20% in 2-3 year olds and 10.0% to 0.0% in 8-10 years old. Similarly, the proportion of samples classified as having insufficient iodine status reduced from 13.7% to 5.8% in 2-3 year olds and 23.3% to 10% in 8-10 years old. Although the variance continued to reduce as the number of samples included in the adjustment increased, the greatest absolute reduction in distribution spread was observed when the second sample was included in the adjustment. The frequency of samples in the upper and lower extremes of the distribution was relatively unchanged when the third and fourth sample sets were included.

Comparable with the findings of the current study, other analyses achieved similar results when the similar adjustment was applied to other multiple urine sets (22, 23). A study involving 84 adults (aged 60-95 years), collected a total of 3 urine samples, one week apart, from each participant (23). Charlton and colleagues reported 21% reduction in the SD when adjustment included two urine samples and 17% reduction when 3 urine samples were used (23). As found in our current study they found that using more than two samples did not have significant extra advantage (23).

Mackerras and colleagues collected second spot urine samples from a subgroup participating in an iodine nutrition survey conducted in the Northern Territory, Australia. Of the 376 Aboriginal teenage participants (mean age 17.8 years), 82 gave one repeat urine sample, approximately one year after the original sample was (22). The authors reported a 31% reduction in the distribution spread after the adjustment was performed using two samples (22). Despite differences in measurement intervals, the results between the studies are consistent, reporting an overall reduction in the variability by 25-30%. These studies also reinforce the benefit in collecting at least one extra sample from each participant to provide a more accurate reflection of the actual iodine status distribution within a population.

A review of current global iodine status included data from 48 populations, representing 17 countries, (128) and investigated the frequency distribution of iodine status amongst iodine sufficient populations. The aim of this study was to determine whether the upper limit of no more than 20% of samples below 50ug/L was acceptable. Some critics previously expressed the view that the 50ug/L cut-off allowed for a too great of a proportion of individuals experiencing iodine deficiency within a population (128). From populations varying in size from 50 to 1666, school aged children (n= 26, 270) between the ages of 6-14 years had median values between the iodine sufficient range (100-200ug/L) and 13, 636 children had median values >200ug/L. The proportion of samples <50ug/L were 7.8% and 2.6% respectively (128). When groups with more than one UIC sample collected from each participant were analysed independently, values below <50ug/L were not found (128). Whilst the authors concluded that a 20% of samples below 50ug/L remained suitable to categorise long standing iodine deficiency, an accepted frequency of samples above 300ug/L to categorise iodine excess was not discussed. The authors did, however, acknowledge that levels above 300ug/L may coincide with hyperthyroidism in previously iodine deficient populations (128). Although, this review emphasises the need for larger sample sizes to account for between-person variability, it also

features the advantage in collecting successive samples from individuals to reduce the overall variability and convey a potentially more accurate classification of iodine status frequency. Reducing the within-person variability by including multiple samples in the analysis has little effect on the population's iodine classification according to the median UIC, however, the inclusion of multiple samples may affect the percentage of samples above or below cut offs for iodine status classification. This could be advantageous in populations with median UIC values bordering either end of the iodine adequate range (ie 100-199ug/L).

The current study is limited by the small sample size recruited in each age group. This reduces the power and increases the standard error of the population mean. However, as shown in figures 4.1 and 4.2, by collecting multiple consecutive UIC samples the findings of the current study are strengthened by reducing the effect of within-person variability to an extent similarly observed in larger studies. Furthermore, consistent with circadian rhythm studies (19, 57, 127), the current study observed higher UICs in the afternoon urine samples than first morning void. Although these differences were determined to be statistically insignificant, the pattern of including both lows and peaks in daily UIC increases the possibility of representing actual iodine status (56, 57). This is not to suggest, however, that collecting four UIC samples is representative of individual iodine status, as it is advised that at least 10 repeated urine samples are required to ascertain individual iodine status (129).

Figure 4.1 Corrected multiple sample distribution vs single sample distribution for 2-3 year olds in a south east Queensland cohort.

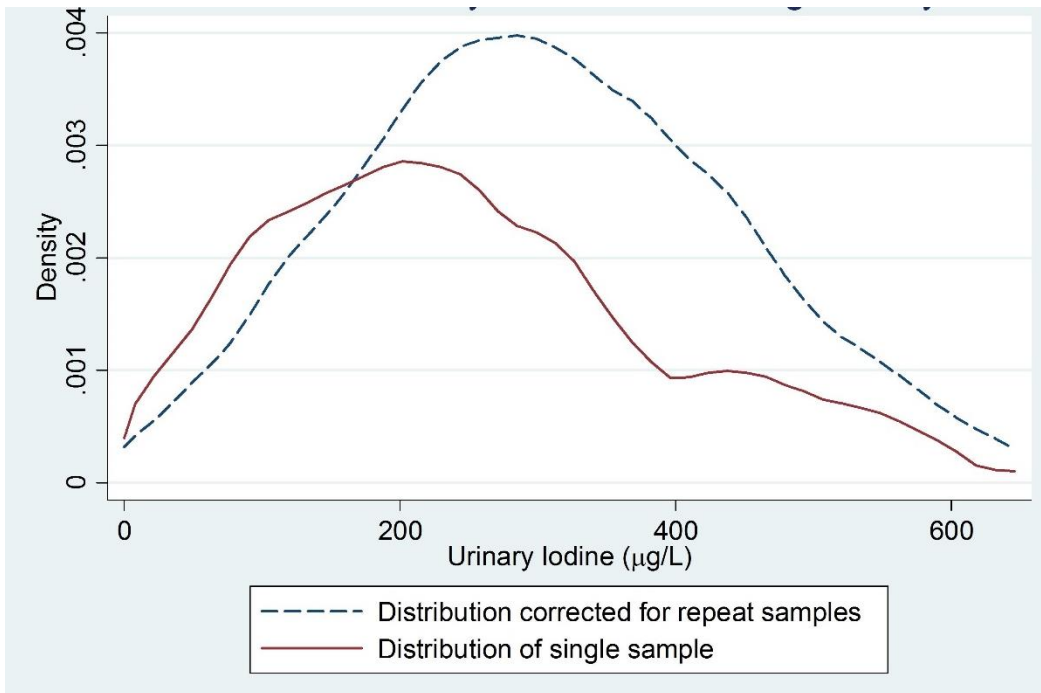
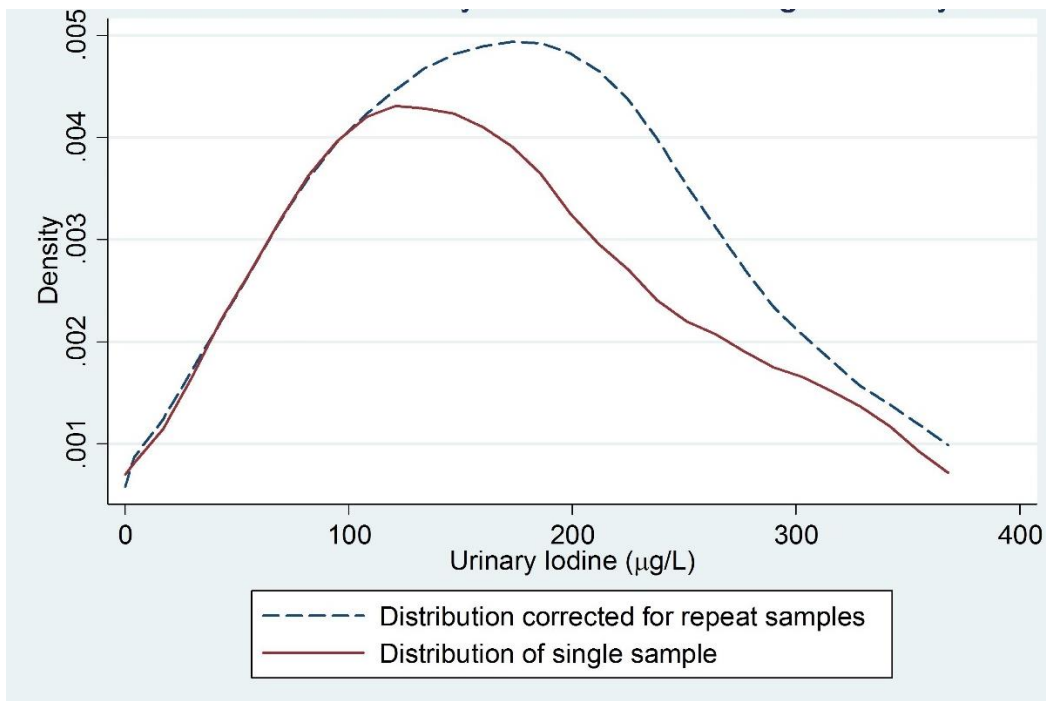


Figure 4.2 Corrected multiple sample distribution vs single sample distribution for 8-10 year olds in a south east Queensland cohort.



When collecting urine samples, meticulous methodology is required to establish accurate representation of iodine status. It is currently accepted, provided there are a sufficient number of samples collected, median UIC will provide an adequate representation of a population's iodine nutritional status (1, 30). The current study together with other studies, suggests that the traditional methodology of collecting one spot morning sample from each individual may not accurately reflect the actual distribution of iodine status within a population. The practicality and cost of collecting multiple UICs from each participant needs to be considered, however, the benefit of reducing the variance by up to 35% simply by collecting at least two consecutive UICs instead of one, may provide a more accurate reflection of the populations iodine status and may influence what intervention, if any, is required to maintain optimal iodine nutrition. Further research investigating the influence of timing when collecting repeat samples, i.e. morning and afternoon, may also further improve a populations estimated iodine status.

CHAPTER 5: THE CURRENT IODINE STATUS OF SCHOOL AGED CHILDREN IN QUEENSLAND

INTRODUCTION

This chapter presents the iodine status of school aged children aged 8-10 years residing in south east QLD as determined by this thesis study. It explores the dietary influences on iodine status, including habitual intake of iodine fortified bread, and discusses possible reasons as to why the iodine status of QLD is above the national average.

MANUSCRIPT DETAILS

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THE INFLUENCE OF MANDATORY IODINE FORTIFICATION OF ON THE IODINE STATUS OF AUSTRALIAN SCHOOL CHILDREN RESIDING IN AN IODINE SUFFICIENT REGION

Abstract

Aim: To counter emerging iodine deficiency mandatory iodine fortification of bread was introduced throughout Australia in 2009. This study investigated the impact of iodine fortification on the iodine status of school aged children living in the iodine replete state of Queensland, and investigates which foods have greatest influence on overall iodine status.

Methods: A convenience sample of 30 children aged 8.00-10.99 years living in south east Queensland, Australia, provided spot morning and afternoon urine samples on two consecutive days. Iodine status was categorised by the World Health Organisation criterion using urinary iodine concentration (UIC). Semi-quantitative food frequency questionnaires (FFQ) completed by carers were used to investigate which foods were having the greatest influence on UIC. Analysis of variance was used to reduce the within person variation observed in UIC and the data were log transformed before statistical analysis.

Results: Adjusted median UIC was 143.8ug/L (IQR 119.5-209.6ug/L) indicating iodine sufficient status. No samples were above the cut off for excessive UIC. Bread was the only statistical significant contributor to UIC ($r=.37$, $p=0.02$) with 14% of variation in UIC explained by bread consumption. UIC increased by 8.7% for each additional serve of bread.

Conclusions: Iodine fortification of bread has increased the iodine status of school aged children in this Queensland cohort. Despite the small sample size in this study, improvements in methodology allowed its findings to be comparable to other, larger surveys.

Introduction

Iodine is an essential micronutrient required for normal growth and development (1). Iodine status can influence an individual's mental development, motor function, somatic growth and thyroid function (34). Monitoring iodine status of populations is considered an important part of preventative medicine (13). Children are particularly vulnerable to inappropriate dietary iodine intake, with impaired cognitive function and thyroid dysfunction being documented amongst both iodine deficient and iodine excessive populations (43, 130, 131).

The irreversible consequences experienced by iodine deficient populations have generated global attention (5) and are key motivators in the development of iodine prophylaxis programs with the aim being to improve the iodine status of at risk populations (1, 13). The World Health Organisation (WHO) and International Council for Control of Iodine Deficiency Disorders (ICCIDD) established criteria (table 1.1) to assist with the assessment and definition of iodine status using median urinary iodine concentration (UIC) of a population as a determinant (1). As 90% of consumed iodine appears in the urine mostly after 4-5 hours of ingestion, UIC is considered the most immediate reflection of iodine nutrition intake (1, 3). The criteria identifies iodine sufficient populations as not only having a median UIC between 100ug/L-199ug/L but also no more than 20% of its samples should be below 50ug/L (1). Thus not only is the central tendency of a populations' UIC important, but also its spread.

Globally, 130 countries have implemented iodine nutrition monitoring (1). In 2003-2004 Australia conducted its first National Iodine Nutrition Survey (NINS) (3). Mild to moderate iodine deficiency was observed in almost 50% of school-aged children (11). Investigators reported a national median UIC of 96ug/L, below the lower limit of adequate iodine intake of 100ug/L (11). However, iodine levels varied across Australia, with some states such as Queensland and Western Australia having adequate iodine status with median UICs of 136.5ug/L and 142.5ug/L respectively (11). Subsequent Australian surveys (5, 103) reinforced the inadequate iodine status of some Australian school-aged children and consequently prompted Food Standards Australia and New Zealand (FSANZ) to introduce the mandatory use of iodised salt in all bread manufacturing (except organic) from late 2009 (12). Bread was chosen by FSANZ as the most suitable vehicle to increase the dietary iodine intake because the 1995 National Nutrition Survey (NNS) reported that Australians consume approximately 75-85% of their salt via processed foods, and of that percentage, 50% of salt is consumed via bread and cereal products (10).

Whilst deliberating on the mandatory use of iodised salt in bread manufacturing, concerns were raised that a blanket fortification program could cause iodine sufficient regions to attain more than adequate or excessive iodine intakes (132). Previous attempts of iodine fortification in Tasmania, Australia in the mid 1960's resulted in a rise of thyrotoxicosis (116). FSANZ, however, predicted that the amount of iodine fortification required to improve the Australian iodine status would not cause iodine sufficient regions, Western Australia and Queensland, to exceed the optimal range for iodine (10). Thus the risk of adverse effect as a result of mandatory iodine fortification was considered low (132).

It was estimated that iodine fortification in bread would increase the dietary iodine intake of children over the age of 2 years by approximately 54ug/day (10). Taking into account the bioavailability of dietary iodine (3) it could be assumed that as a result of iodine fortification, the median UIC of Queensland school aged children would increase by approximately 48ug/L, suggesting that the population median UIC would rise to be within the range of 180-185ug/L. Although this median level is not considered excessive, due to large variations in UIC (20), the proportion of samples within the distribution that could reach beyond the excessive bounds needs to be considered also.

Speculation that excessive iodine intakes may occur due to iodine fortification programs can hinder the success of much needed iodine fortification programs amongst iodine deficient regions (30). Documenting changes in iodine status within a population exposed to an acute rise in dietary iodine provides an important insight into the merit of objecting concerns and improves the implementation of existing fortification programs to benefit the majority (30).

This study investigated the iodine status of school aged children residing in the iodine sufficient region of Queensland, three years after iodine fortification was introduced to Australian bread manufacturing. The study aimed to identify the proportion of children's UIC samples at the lower and upper extremes of the populations' UIC distribution, and investigate associations between dietary sources of iodine, in particular bread consumption and iodine status.

Methods

Recruitment

Children aged 8.00-10.99 years residing in south east Queensland, Australia, were eligible to participate. A convenience sample of participants were recruited via various advertisement

media including newspaper, television, radio, posters and social media between November 2011 and March 2013. This approach has been shown to attain representative samples in other public health surveys (122, 124). Informed written consent was attained from carers of the participating child. The study was approved by the Medical Research Ethics Committee, The University of Queensland (#2001000125).

Sample Collection

Carers supervised the collection of four urine samples from each child over two day consecutive days (first morning urine void, and first urine after midday meal).

Semi-quantitative food frequency questionnaires (FFQ) adapted from the Healthy Kids Queensland survey (60) were completed by carers. Foods included in the FFQ were those identified by the 22nd Total Diet Study (27) and FSANZ (2) as notable sources of dietary iodine. Each food was described as a serve with a specified quantity ie. 1 serve of bread = 2 slices or 1 medium roll or 1 flat bread. There were 5 daily frequency options for milk, bread, egg, yogurt, sushi, nori, brassica vegetables and salt water fish ie. never eats, 1 serve, 2 serves, 3 serves or 4 or more served per day. Brassica vegetables were also included because of their known goitrogenic affect to impair iodine uptake. For sub-categories of food, there were 10 frequency options; never, less than 1 serve per day, one per day, 2 per day, 3 or more per day, once weekly, 2-4 weekly, 5-6 serves weekly, less than 1 serve per month, 1-3 serves per month. For example, bread was split in the sub-categories of 'roll, flat, slice, bagel, turkish, crumbs, fruit bread' 'organic bread' or 'homemade bread', each with corresponding serving sizes. The questionnaire recorded demographic and clinical characteristics, including postcode of residence. Height and weight of the children were measured using standard anthropometric techniques. Body Mass Index (BMI) was calculated using standard formula. Socio-economic status (SES) was measured at the postcode level using the Socio-Economic Indexes for Areas (SEIFA) Index of Relative Disadvantage. This index was developed by the Australian Bureau of Statistics (125). Participants were categorised as lower SES (SIEFA decile 1-3) /middle SES (SIEFA decile 4-7)/ upper SES (SIEFA decile 8-10).

Sample Analysis

All urine samples were stored at -18°C until transported on ice to Westmead Hospital, Sydney for analysis by the same laboratory used by the NINS. UIC was established using ammonium

persulfate digestion before a Sandell-Kolthoff reaction (47). Iodine status was categorised using the WHO criterion¹ for assessing iodine nutrition based on median UIC.

Statistical Analysis

Using the mean 152.9ug/L, and standard deviation 80.1, of UIC in 8.00-10.99 year olds from Queensland school aged children included in the NINS (Eastman C personal communication), and after adjusting for non-normality of the data, it was calculated that a sample size of 57 was required to detect the 48ug/L increase in UIC predicted by FSANZ. Stata statistical software (StataCorp, College Station, TX, USA), SPSS (IBM, Armonk, NY, USA) and Microsoft Excel were used for statistical analysis. Analyses of variance was used to reduce the within person variation observed in UIC before statistical analysis using the following formula: Adjusted UIC = [(person's UIC – group mean) x ($s_b \div s_{obs}$)] + group mean (22). All four UIC samples were used to obtain an overall adjusted UIC, which was used in subsequent calculations. Descriptive statistics were calculated for UIC.

Paired t-tests were used to determine the difference between morning and afternoon UIC, taking into account repeated measures. The percentage of individuals in each WHO iodine category was calculated, along with exact binomial 95% confidence intervals. Univariate regressions were performed for each identified food to determine the dietary iodine contribution to iodine status. Foods that had a correlation of greater than 0.32 (ie. $R^2 > 10\%$) were considered to be important.

Differences in UIC between high (≥ 2 serves/day) and low (≤ 1 serve/day) bread intake groups were assessed using student t-tests. T-tests were also performed to detect a difference between the UIC of school children pre-fortification and post-fortification.

Results

Participants

Thirty children aged 8.00-10.99 years (18 male, 12 females) completed the survey. Basic demographics are shown in table 5.1.

Table 5.1 Characteristics of school aged (8 -10 years) participants in iodine nutrition surveys conducted in Queensland.

	Current Study	2011-12 National Health Measures Survey(12)	2003-04 Australian National Iodine Nutrition Study(3)
Median UIC ug/L (IQR)	143.8 (119.5-209.6)	165.9 (not reported)	136.5ug/L (104.3-183.8)
Mean Age ± SD	9.4 ± 0.8	Not reported	9.1 ± 0.4
Sex Ratio (F:M)	1:1.5	Not reported	1.3:1
Mean BMI	17.0	Not reported	17.0
Sample size	30	Not reported	294
Urine collection	Total of 4 samples (2 morning and 2 afternoon)	One spot morning sample	One spot morning sample
Dietary assessment	Food Frequency Questionnaire	24 Food Recall	Not preformed
Methodology to determine UIC	Sandell-Kolthoff spectrophotometric	plasma-mass spectrometry	Sandell-Kolthoff spectrophotometric
Social economic status (SEIFA deciles)	Lower 0%	Not reported	Not reported
	Middle 46%		
	Upper 54%		

Urine Iodine Concentration

The adjusted median UIC for children aged 8.00-10.99 years was 143.8ug/L (CV32.3%, IQR 119.5-209.6), with no samples above the cut off for excessive UIC (>299ug/L), indicating adequate iodine status according to WHO criterion (table 5.2). The mean difference between morning and afternoon UIC samples was 44.8ug/L (95%CI 25.7-64.1), with UIC being highest in the afternoon ($p=0.001$). No significant difference was detected between the UIC of 8.00-10.99 year olds prior to iodine fortification and the UIC of children included in the current study ($p= 0.38$).

Table 5.2 WHO criterion of iodine status and corresponding percentage of UIC samples in each category.

Median Urinary Iodine Concentration range (ug/L)	Iodine status according to WHO and ICCIDD criteria	Proportion of children aged 8-10 years with iodine status	n
<20	Severe iodine deficiency	0% (95% CI 0%-11%)	0
20-49	Moderate iodine deficiency	0% (95% CI 0%-11%)	0
50-99	Mild iodine deficiency	7% (95% CI 1%-22%)	3
100-199	Adequate iodine intake	67% (95% CI 47%-83%)	19
200-299	More than adequate iodine intake	27% (95% CI 12%-46%)	8
≥300	Excessive iodine intake	0% (95% CI 0%-11%)	0

Food Frequency Questionnaire

No participant consumed ‘organic’ or ‘home made’ varieties of bread. Due to limited dietary variation because of a small sample size, and taking into account of those children who consumed bread, consumed bread fortified that was fortified with iodine, correlations between dietary intake of food types and UIC were based on the reported number of serves consumed per day captured in section 2 of the FFQ. Individual food items were not further explored. Bread consumption was significantly associated with UIC ($r=0.37$, $p=0.02$) with 14% of variation in UIC explained by bread consumption. UIC increased by 8.7% for each additional serve of bread. The median number of serves of bread consumed was 2 per day, which was calculated to equate to 136.2 grams as shown in table 5.3 (based on average weight (g) of 2 slices of bread, 1 median roll or 1 flat bread as defined by AUS NUT 2011-2013 (27)). There was a significant difference in UIC between groups consuming high (≥ 2 serves/day) vs low amounts of bread (≤ 1 serve/day) ($p=0.02$). No other examined food type was significantly associated with UIC as shown in table 5.4.

Table 5.3 Median intake of foods considered to be main source of dietary iodine.

Survey	Current Survey	National Health Survey 2011-2012(31)	National Nutrition Survey 1995(39)
Age of children (years)	8.00-10.99	9-12	8-11
Bread (g) ± SE	65.0 ± 6.7	76.0 ± 6.0	91.0 ± NA

Table 5.4 Predictors of urinary iodine concentration in unilinear regression analyses.

Predictor	Standardised Coefficient (β)	t	Significance
Bread	0.37	2.10	0.04
Milk	0.10	0.54	0.58
Egg	0.30	1.70	0.09
Yoghurt	0.01	0.02	0.98
Sushi	0.09	0.50	0.62
Nori	0.11	0.61	0.54
Saltwater Fish	0.09	0.52	0.60

Discussion

This is the first mainland Australian study to report iodine fortified bread as statistical significant predictor of UIC since the iodine prophylaxis program began. The samples from school aged children within this cohort were classified as iodine sufficient, with a median UIC of 143.8ug/L with no samples falling above the cut off for excessive UIC. Surprisingly, the median UIC of this cohort was only 6.5ug/L greater than the median UIC of QLD school aged children prior to iodine fortification, and was substantially lower than the expected increase of 45.9ug/L. Nevertheless, bread consumption was the only food identified to have a significant, positive, influence on UIC and thus the iodine fortification of bread has had a considerable impact on the iodine status of these children.

This study is greatly limited by its small sample size, however, by collecting multiple spot samples from each participant, the large variance typically affecting the distribution of UIC

samples within a group was improved. As described elsewhere (133), the adjustment for inter and intra variability in UIC reduced the coefficient of variance by 34.7%. This reduction is comparable to larger iodine surveys where the same adjustment was performed using larger sample sizes (22, 23). Furthermore, the total number of spot UIC samples used in this adjustment (n=120) exceeds the recommendation of 86 spot samples required for $\pm 10\%$ precision range with 90% CI (133).

In this circumstance, the sample size was adequate to detect the significance of bread consumption but not the difference in UIC between pre and post iodine fortification cohorts. Although the method of recruitment may have impacted on the length of the recruitment process, participant demographic profiles enrolled in the current survey are comparable to larger nutrition surveys (table 5.1). As previously mentioned, the statistical adjustment performed by the current study to reduce the impact of variability, in the addition to including an afternoon sample in the analysis improves the accuracy of the sample interpretation (20, 22, 23).

Recently, the Australian Government released data from the 2011-2012 National Health Survey (NHS). It reported a median UIC of 165.9ug/L for QLD school aged children (aged 8.00-10.99years) (119) also indicating an iodine sufficient status after iodine fortification. The median UIC reported by the NHS is 22.1ug/L greater than the current studies median UIC and 29.4ug/L greater than the UIC of children prior to iodine fortification. Neither of the studies conducted post iodine fortification, reached the estimated UIC of 180-185ug/L. Children in both the current study and the NHS survey (82) consumed less bread than that reported by the 1995 National Nutrition Survey (25) (table 5.3) used by FSANZ to estimate expected changes in dietary iodine (10), and could explain why the expected median UIC was not reached. Bread products were reported by the NHS to be a significant contributor to UIC for the general population, however, the level of this significance was not identified for children 8.00-10.99 years (135). Although there are differences in sample size, methodology and geography, the results of both of these surveys are comparable reaffirming that the iodine fortification of bread has significantly impacted the iodine status of school aged children.

Following mandatory iodine fortification in New Zealand, the median UIC of New Zealand school aged children 8-10 years increased from 68ug/L to 113ug/L after iodine fortification was introduced to bread (79). As observed in the current study the consumption of bread was significantly ($p= 0.017$) associated with increasing UIC (79). Likewise bread was the main

contributor to iodine status within the New Zealand cohort. Although prior to fortification, dietary modelling to predict the outcome of iodine fortification in bread was not available for this age group, in other groups the observed increase in UIC also did not reach expected levels (136). As with the Australian experience, it could be hypothesised that the assessments made by FSANZ to predict the outcome of iodine fortification were also over estimated.

Typically dairy products, particularly milk, have been described as important contributor to iodine status in other populations (11, 27). The current study found no significant impact of milk consumption on iodine status, which also consistent with New Zealand's findings (79). The National Total Diet Study report the mean iodine concentration of Australian milk to be 133-159ug/L (27), and New Zealand milk to be 94-102ug/L (137). These concentrations are remarkably lower than countries where milk is a significant contributor, such as the United Kingdom (UK), where the average milk concentration is 300ug/L (138). Furthermore, the practice of using iodophors in at least one milk sanitation procedure is adopted by only half of Australian dairy farmers (139) whilst the use of iodophors is routinely recommended in the UK (140). As the use of iodophors has been shown to directly impact the iodine concentration of corresponding milk (141), its limited use may explain why milk may not be a major contributor of dietary iodine in this Australian cohort.

International bodies advise that iodine fortification should be monitored following implementation to evaluate the effectiveness of iodine fortification, and thus maintain its purpose (1, 5). Importantly this study confirms that the objective to positively influence the iodine status of school aged children through the means of iodine fortification using bread was successful although not to the expected level. The iodine status of school aged children in this cohort is greater than that of Queensland school aged children prior to iodine fortification, however, there was no indication of excessive iodine status. Therefore concerns of iodine excess as result of iodine fortification are not applicable to this group of children. Further investigations are required to determine the level of significance iodine fortification of bread has had in other regions of Australia and whether its impact is successful in alleviating iodine deficiency in vulnerable groups.

CHAPTER 6: THE IODINE STATUS OF QUEENSLAND PRESCHOOL CHILDREN

INTRODUCTION

There are very little data on the iodine status of Australian young children aged 2-3 years. This particular age group was identified by FSANZ as being at risk of consuming above the upper level for dietary iodine as result of introducing iodine fortification to bread manufacturing in Australia. This paper shows that the proportion of children aged 2-3 years potentially consuming above the upper level for dietary iodine is almost double than first expected. The contribution from iodine fortified bread, however, doesn't appear to be influential in this result, instead this paper presents other plausible reasons why the iodine status of these children is higher than expected.

MANUSCRIPT DETAILS

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THE IODINE STATUS OF QUEENSLAND PRESCHOOL CHILDREN AFTER THE INTRODUCTION OF MANDATORY IODINE FORTIFICATION IN BREAD: AN EXPLORATORY STUDY USING A CONVENIENCE SAMPLE.

Abstract

Aim: Appropriate dietary iodine is essential for thyroid hormone synthesis, especially in young children. Following an iodine fortification in bread initiative, approximately 6% of Australian preschool children were expected to have an excessive iodine status. The aim of this study was to document the current iodine status of preschool children using urinary iodine concentration (UIC) as a biomarker of iodine intake.

Methods: A convenience sample of fifty-one preschool children, aged 2-3 years, were recruited from south east Queensland. UIC was ascertained from spot morning and afternoon urine samples collected on two consecutive days and food frequency questionnaires were completed for each participant. Dietary iodine intake was extrapolated from UIC assuming 90% of dietary iodine is excreted in urine and a urine volume of 0.5L/day.

Results: A median UIC of 223.3µg/L was found. The calculated median dietary iodine intake was 124.8ug/day (SD 47.0) with 9.8% of samples above the upper level of 200ug for dietary iodine for children within this age group. No foods were associated with UIC.

Conclusion: Limited by sample size and recruitment strategies, no association was found between usual food intake and UIC. Extrapolated dietary iodine intake indicated that children within this cohort consumed adequate amounts of dietary iodine, although the number of children consuming above the upper limit of 300ug/day was almost double of expected. The development of a UIC criteria to assess appropriate parameters for varying degrees of iodine status is required for the monitoring of iodine nutrition in this vulnerable age group.

Introduction

Insufficient dietary iodine remains to be the leading global cause of preventable brain damage amongst children (1). The essential role of dietary iodine in thyroid hormone synthesis is well documented. Inadequate or excessive dietary iodine intake can negatively impact thyroid function as well as cognitive development, motor function and somatic growth (34). According to the World Health Organisation (WHO) and International Council for Control of Iodine Deficiency Disorders (ICCIDD), the most crucial period to obtain adequate dietary iodine is from the second trimester of pregnancy through to the third year of life (1).

Despite the importance of iodine status for preschool children, historically little is known about this specific age group. As recommended by WHO, many iodine surveys focus on neonatal or school-aged populations (1) due to the relative ease of accessibility. Typically the iodine status of a population is described as urinary iodine concentration (UIC) as it reflects almost 90% of dietary iodine intake (1, 3). This method of reporting has only been validated for school aged children, however, and thus the classification of iodine status in younger children can be difficult. Nevertheless, more data are emerging regarding the iodine status of preschool children during this critical period of development.

During deliberations around the introduction of iodine fortification in Australia, children aged 2-3 years were identified as potentially susceptible to attaining excessive iodine status resulting from the national public health intervention (10). Queensland children were particularly at risk of exceeding the upper limit for iodine, as the region was previously identified as being already iodine replete prior to iodine fortification for school aged children (11). The National Iodine Nutrition Survey (NINS) reported a median UIC for Queensland school aged children of 136.5ug/L, 42% greater than the national median (11). With no available UIC data concerning Australian children aged 2-3 years at the time of the fortification proposal, Food Standards Australia and New Zealand (FSANZ) used 1995 National Health Survey data to estimate dietary iodine intakes for this age group (10). The mean dietary iodine intake was estimated to be 95ug/day (5ug/day above the recommended daily intake (RDI) of 90ug/day), indicating adequate iodine intake in 97-98% of the 2-3 year old population prior to implementation. It was predicted by FSANZ that the dietary iodine intake of 2-3 year olds would increase by 38ug/day (10) resulting in 6% of children within this age group potentially exceeding the upper level (200ug/day) for dietary iodine intake (10).

More recently, Skeaff and colleagues published the mean daily iodine intake of 2-3 year olds as 71ug/day, with a corresponding median UIC of 122ug/L, in 68 South Australian pre-school children prior to the implementation of iodine fortification in Australia (83). Investigators (83) predicted that the introduction of mandatory iodine fortification would increase the median UIC of this cohort to approximately 130-160ug/L. With this expected increase, it was estimated that approximately 10-14% of preschoolers within this group could be at risk of attaining an UIC >300ug/L, which is considered excessive in other populations (83).

Since the implementation of iodine fortification in Australia, predictions estimating the iodine status of children aged 2-3 years using UIC as a biomarker remains unreported. The aim of this study is to investigate the current iodine status of preschool children in Queensland aged 2-3 years after the implementation of iodine fortification and determine what proportion of children exceed the recommendations for adequate iodine status.

Methods

Participants

Children aged 2.00-3.99 years residing in south east Queensland were eligible to participate. A convenience sample of participants were recruited via various advertisement mediums including newspaper, television, radio, posters and social media. This approach has proven to yield representative samples in other public health surveys (122, 124). Recruitment was from November 2011 to March 2013. Informed written consent was attained from carers of the participating child. The study was reviewed and approved by the Medical Research Ethics Committee, The University of Queensland (#2001000125).

Sample Collection

Carers collected one spot morning urine sample and one spot afternoon urine sample from their child, on two successive days. Urine was collected from cotton wool balls placed in the diaper of those participants not toilet trained.

Cares completed semi-quantitative food frequency questionnaires (FFQ) adapted from the Healthy Kids Queensland survey (60) were completed by carers. Foods included in the FFQ were those identified by the 22nd Total Diet Study (27) as notable sources of dietary iodine. Each food was described as a serve with a specified quantity ie. 1 serve of bread = 2 slices or 1 medium roll or 1 flat bread. There were 5 daily frequency options for milk, bread, egg, yogurt,

sushi, nori, brassica vegetables and salt water fish ie. never eats, 1 serve, 2 serves, 3 serves or 4 or more served per day. Brassica vegetables were also included because of their known goitrogenic affect to impair iodine uptake. For sub-categories of food, there were 10 frequency options; never, less than 1 serve per day, one per day, 2 per day, 3 or more per day, once weekly, 2-4 weekly, 5-6 serves weekly, less than 1 serve per month, 1-3 serves per month. For example, bread was split in the sub-categories of 'roll, flat, slice, bagel, turkish, crumbs, fruit bread' 'organic bread' or 'homemade bread', each with corresponding serving sizes. The questionnaire recorded demographic and clinical characteristics, including postcode of residence.

The questionnaire recorded postcode of residence. Child height and weight was measured by investigators.

Sample Analysis

All urine samples were stored at -18°C until assayed. Urinary iodine concentration was determined using ammonium persulfate digestion before a Sandell-Kolthoff reaction (47). Body Mass Index (BMI) was calculated using standard formula. Socio-economic status (SES) was measured at the postcode level using the Socio-Economic Indexes for Areas (SEIFA) Index of Relative Disadvantage. This index was developed by the Australian Bureau of Statistics (125). Participants were categorised as lower SES/Middle SES/ upper SES.

Statistical Analysis

Analyses of variance methods were used to combine the four UIC sample measurements used into one overall adjusted UIC estimate for each participant (133). Samples were combined according to the formula: Adjusted UIC = [(person's UIC – group mean) x (s_b ÷ s_{obs})] + group mean (22). To calculate the between-person (s_b) and total (s_{obs}) standard deviations the data was Log-transformed before we undertook a repeated measures analysis of variance. The advantage of combining all four samples is that it improves each individual estimate by reducing the coefficient of variance (CV) of the data and therefore decreases the probability of overestimating the proportion of low or high UIC samples. The WHO categorisation for iodine status according to UIC is only validated for older children with a urine output of 1L, therefore, assuming young children have urine output of approximately 0.5L per day (142) iodine intake was extrapolated from the median UIC using the following formula: Dietary iodine intake per day (ug/day) = median UIC (ug/L) x 100/9 x 0.5L (33). The approximate normality of the data

was assessed using the Kolmogorov–Smirnov test and data was log-transformed to improve normality. The percentage of UIC samples <100ug/L and >199ug/L were computed, along with exact binomial 95% confidence intervals. The association between number of dietary serves/day and the natural logarithm of UIC was investigated using spearman correlations and mixed-effects linear regression. All four readings from the child were analysed, with type of food included as a fixed effect and child included as a random effect. Mixed-effects models account for the correlation of UIC values recorded from the same child. Effect estimates are presented as mean differences and 95% confidence intervals. Statistical Significance was set at $p < 0.05$. Stata, SPSS and Microsoft Excel were used to perform statistical analysis.

Results

Participation

Fifty one children (30 (59%) males) returned urine samples and dietary records. Table 6.1 describes demographic and clinical information including, age, sex, weight, height, BMI and SES. The weight and height of children in this study was comparable to those recruited in the 2012 National Health Survey (NHS) considering the additional 12 month age range of those recruited by the 2012 NHS (BMI 16.5 (0.2) and 16.8 (0.7) respectively).

Table 6.1 Descriptive demographics of Queensland children aged 2-3 years old participating in the current survey compared to children recruited in the 2012 Australian National Health Survey

	Current survey (n=51)	2012 Australian National Health Survey
Age range (years)	2.00-3.99	2.00-4.99
Mean Weight (kg) Female (SE), Male (SE)]	14.3 (0.6), 15.7 (0.3)	16.4 (1.7), 16.9 (1.1)
Mean Height (cm) Female (SE), Male (SE)	93.1 (1.4), 97.3 (1.0)	98.7 (0.5), 100.1 (0.5)
Mean BMI (SE)	16.5 (0.2)	16.8 (0.7)
Social economic status	Lower 13.2% Middle 11.7% Upper 70.5%	NA

Urine Iodine Concentration

The median UIC for children was 223.3 ug/L (25th-75th percentile = 163.9-261.5ug/L). Adjusting for all four urine samples reduced the CV from 56.5% to 36.1%, as described elsewhere (132). The distribution of UIC samples showed 6% (95%CI 1%-16%) <100ug/L and

57% (95%CI 42%-71%) >199ug/L. The UIC adjusted for urine volume (UIC x 0.5L/day) was 112.3ug/L with 43% (95%CI 29%-58%) <100ug/L and 2% (95%CI 0%-10%) >199ug/L.

The median dietary iodine intake extrapolated from UIC was 124.8ug/day (SD 47.0) with 9.8% of samples above the upper level of 200ug for dietary iodine for children within this age group.

Food Frequency Questionnaire

No participant consumed ‘organic’ or ‘home made’ varieties of bread. Due to limited dietary variation because of a small sample size, and taking into account of those children who consumed bread, consumed bread fortified that was fortified with iodine, correlations between dietary intake of food types and UIC were based on the reported number of serves consumed per day captured in section 2 of the FFQ. Individual food items were not further explored. Table 6.2 describes the average intake of each food consumed by children and the correlation with log UIC as reported by spearman correlation. No foods were found to be significantly associated with log UIC. Table 6.3 reports mean differences in log UIC with every 1 serve increase of foods. No further significant associations were detected by the mixed-effect linear regression, with the exception of egg and salt water fish. Whilst difficult to interpret and explain, the significant negative association with egg (-0.07, p= 0.02) and fish (-0.3, p=0.03) consumption and log UIC is minor.

Table 6.2 Number of serves of food consumed by Queensland 2-3 year olds reported by FFQ and correlation with log UIC.

Foods (1 serve)	Median serve per day (IQR)	r	p-value
Bread (2 slices of bread or 1 medium roll or 1 medium flat bread)	1.0 (0.5, 1.0)	-0.02	0.45
Milk (250mls)	2.0 (0.5, 1.0)	0.04	0.39
Whole egg (1 whole egg)	0.5 (0.5, 0.5)	-0.29	0.01
Yogurt (200g yogurt)	0.5 (0.5, 1.0)	-0.03	0.41
Sushi with nori (1 roll)	0.5 (0.0, 0.5)	-0.07	0.31
Nori foods (1 nori snack sheet)	0.0 (0.0, 0.5)	0.09	0.26
Brassica vegetable (½ cup cooked)	0.5 (0.5, 1.0)	0.09	0.25
Salt water fish	0.5 (0.0,1.00)	-0.41	0.001

Table 6.3 The mean difference in log UIC observed with each increase of one serve of food consumed by 2-3 year old children.

Foods (1 serve)	Mean Difference of UIC with each additional serve of food	95%CI	p-value
Bread (2 slices of bread or 1 medium roll or 1 medium flat bread)	-0.1	"-0.3, 0.1"	0.25
Milk (250mls)	0.0	"-0.1, 0.2"	0.92
Whole egg (1 whole egg)	-0.7	"-1.2,-0.1"	0.02
Yogurt (200g yogurt)	-0.1	"-0.5, 0.2"	0.47
Sushi with nori (1 roll)	-0.1	"-0.6, 0.3"	0.53
Nori foods (1 nori snack sheet)	0.1	"-0.4, 0.5"	0.82
Brassica vegetable (½ cup cooked)	0.0	"-0.3, 0.3"	0.89
Salt water fish	-0.3	"-0.6, -0.1"	0.03

Discussion

WHO and ICCIDD both recommend using the median urinary iodine concentration (UIC) of a population to determine the iodine status of that population (1), as it is generally accepted that approximately 90% of ingested iodine is excreted in the urine (1, 3). They further recommend that no more than 20% of samples in any population lie below 50ug/L, the cut-off for moderate iodine deficiency (1). This recommendation emphasises the importance of considering the population-level distribution of iodine status in addition to the median UIC. The challenge is that WHO cut offs are only validated for children ≥ 6 years of age with an approximate urine volume of 1L/day, and therefore the criterion can be misinterpreted when applied to other populations. Furthermore, the requirements of young children are different than their older counterparts and appropriate cut-off ranges for the varying degrees of iodine status according to UIC are unknown. Nevertheless, of the few international studies that have assessed UIC in children 2-3 years, urine volume is measured (i.e. 24hr excretion) by some (143-146), and not by others (147-149) when categorising iodine status. This inconsistency in categorising UIC

for this age group consequently makes comparisons of iodine status difficult between preschool cohorts.

The current study is one of few measuring the UIC of Australian preschool children aged 2-3 years. The median UIC of children within this cohort was 223.3ug/L with the majority (57%) of UIC samples above 199ug/L, however, when adjusted for an approximate urine volume (0.5L/day for younger children), the median UIC was 112.3ug/L with the majority (43%) of UIC samples below 100ug/L. The crude median UIC indicates 'more than adequate' iodine status according to the WHO criterion and the adjusted for urine volume UIC suggests 'adequate' iodine status in this cohort. Furthermore, the spread of samples within the two median UICs, indicate conflicting interpretations of UIC and thus the classification of iodine status for preschool children using the current WHO criteria poses confusion when observing the global iodine nutritional status of this age group.

Korean preschool children (aged 2-3 years) were categorised as 'iodine excessive', with a median UIC of 561.8ug/L derived from spot urine samples not adjusted for urine volume (range 34.8-32240ug/L) (148). Additional studies categorising iodine status according to the WHO criterion without further adjustment for urine volume include, data from France, reporting the median UIC of pre-schoolers to be 134ug/L, suggesting 'iodine adequacy' (149) and data from New Zealand in 6-24 month old children reporting 'mild iodine deficiency' with a median UIC of 67ug/L (147). Conversely, data from Belgium (143), Germany (144) and India (145) reported median UICs of 101ug/L, 65-71ug/L, 220ug/L respectively leading to classifications of 'iodine deficiency', 'iodine deficiency' and 'more than adequate' iodine status respectively, from 24 hour urine samples and extrapolated dietary intake.

The current study reports an extrapolated dietary iodine intake of 124.8ug/day which is almost twice the 65ug/day EAR (2) for children within this age group. This intake is markedly above the 95ug/day reported by the 1995 National Nutrition Survey (25) and the 71ug/day consumed by South Australian preschool children (83) prior to iodine fortification, yet comparable to the 2007 Australian Children's Nutrition Survey reporting a national average intake of 126ug/day (26) and the most current 2012 National Health Survey reporting 149ug/day iodine intake for 2-3years olds (82). Each assessment of dietary iodine reported by these studies are obtained by dietary records, each with its reporting limitations. The current study is the first to report an extrapolated dietary iodine representation based on biomarkers that measure absolute intake and are calculated by a well excepted biological clearance (33).

The proportion of samples in this study likely to be associated with a dietary intake above the upper level was approximately 10%. This finding is consistent with that reported by the NHS which indicates approximately 13% of 2-3 year old Australian children consume dietary iodine above the upper level. Both of these estimates are two times greater than originally forecast by FSANZ. It should be noted, however, that other populations tolerate long-standing excessive iodine status without ill effect, generating discussions that the upper level for iodine sufficiency is too low (64). Furthermore, the upper limit established for dietary iodine were based on iodine balance studies performed in the early 1970's involving 12 malnourished Senegalese children after one month of rehabilitation (150, 151). Thus, the extrapolation of these results from such an unusual cohort may have implications on its relevance to present-day children with current environmental and dietary exposures. Henceforth, it is important to examine any biological effect that may exist under the conditions of 'excessive iodine' status before assuming a detrimental outcome.

The current study failed to find any associations between dietary serves of food usually consumed and UIC. Understanding that dietary iodine is excreted in the urine approximately 4-5 hours after ingestion (3, 20), a FFQ may not have accurately reflected its influence on immediate UIC. Food frequency questionnaires have typically over reported food intake in other surveys (88, 50), likewise in this survey one carer reported their child ate 4 serves of bread (total eight slices) per day and another reported an intake of 3 serves of brassica vegetables (total 1 ½ cups) per day. Both scenarios are unlikely to accurately reflect the typical intake of a pre-school child. Not attaining the required sample size may have had implications on achieving a broader representation of dietary intake. Although limited by recruitment response, the methodical strengths of the current study is the collection of multiple urine samples from each participant. As reported elsewhere (133), by obtaining more than one urine sample from each participant, the within-person variation typically observed when assessing UIC was reduced to a level similarly achieved by larger studies (22, 60). This method of adjustment does not counteract the advantage of recruiting larger representative samples, however, which allow for varying degrees of subject hydration and other biological variations (1). Recruitment via convenience sampling has its limitations, not excluding the higher SES areas overrepresented in the current study. No association between UIC and SES has been reported elsewhere in Australia, however (86, 146) and thus the external validity of this representative sample is unlikely to be impacted. Despite these limitations, the current study has effectively reported the UIC of a small cohort of 2-3 year old children and provided an

example of challenges and considerations that need to be addressed before assessing iodine status at the population level.

Normally a cohort considered too challenging to include in such a survey, this study demonstrates that the evaluation of iodine status using UIC in 2-3 years olds is not only viable, but important. The number of preschool children in this cohort exceeding the upper limit for dietary iodine is estimated to be almost double than previously expected. Limited by sample size and recruitment methods, the influence of iodine fortification of bread or other dietary contributors were not detected in this cohort. The effect of habitual fluid intake and smaller urinary volume needs to be explored when classifying iodine status according to UIC. To improve consistency in the monitoring of this important public health issue during this critical development period for children aged 2-3 years, a criterion outlining appropriate UIC cut-offs to describe varying degrees of iodine status is required.

CHAPTER 7: ENVIRONMENTAL IODINE AND DISCRETIONARY IODISED SALT EXPOSURE

INTRODUCTION

This manuscript explores the influence of drinking water iodine concentration, discretionary use of iodised salt and supplement use has the iodine status of Queensland children. This is the first Australian study to present data of the relationship between drinking water iodine concentration and UIC as well the use of iodised salt by households and the corresponding UIC of residing children.

MANUSCRIPT DETAILS

The manuscript details are as follows:

Samidurai AJ, Davies PSW. Discretionary use of iodised salt and iodine supplements, but not drinking water, impacts the iodine status of an iodine replete Australian paediatric cohort. Submitted for publication to Australian and New Zealand Journal of Public Health.

The manuscript has been reformatted to fit the requirements of the thesis.

DISCRETIONARY USE OF IODISED SALT AND IODINE SUPPLEMENTS, BUT NOT DRINKING WATER, IMPACTS THE IODINE STATUS OF AN IODINE REPLETE AUSTRALIAN PAEDIATRIC COHORT.

Abstract:

Aim: Investigate the impact of drinking water iodine concentration (DWIC), use of iodised salt and iodine supplements on the urinary iodine concentration (UIC) of children.

Methods: Children aged 2-3 years and 8-10 years were recruited from Queensland, Australia. Multiple UICs and DWIC's were collected over two days. Food frequency questionnaires recorded salt and supplement use.

Results: The median UIC for children aged 2-3 years (n=51) and 8-10 years (n=30) was 223.3 ug/L and 143.8ug/L respectively. No significant correlations between DWIC and UIC were detected. Iodised salt was consumed by 76.4% of 2-3 year olds and 66.6% of 8-10 year olds on any occasion. Independent t-tests revealed significant differences in UIC between those children receiving iodised salt and those who did not (2-3 year olds $p=0.029$; 8-10 year olds $p=0.041$). Approximately 14% of children consumed iodine supplements. Independent t-tests showed a significant mean difference in UIC ($p=0.013$) between children who consumed iodine supplements and those who did not in only 8-10 year olds.

Conclusions: Discretionary iodised salt importantly contributes to the iodine status of children. Public health initiatives to reduce overall salt intake may minimize this impact. The use of iodised salt by households on any occasion has an important influence on children's iodine status.

Introduction

The amount of iodine consumed by children can influence normal growth, especially cognitive development and maturation (1). Ensuring that populations consume the correct amounts of iodine has become an important public health initiative (1, 152). Several national iodine surveys report variations in iodine status within and between cohorts of populations residing in the same country (3, 130, 153). Reasons as to why such variations occur have been associated with geographical location, variation in drinking water iodine concentration (DWIC), salt and supplement use (153, 154). With variation in iodine status observed throughout Australia (11), factors explaining these variances are yet to be explored.

Iodine enters the food chain in the form of iodide through an ecosystem that cycles evaporated iodide or iodate from the ocean to land soils and water reservoirs (155). When this system is disturbed by floods, landslides or drought, the amount of iodine that enters the food chain is consequently affected (155). Differences in environmental iodine exposure is often recognised as a key contributor to the spatial distribution of iodine status within populations (11, 130, 153-155). For example, variations in DWICs have been associated with iodine deficiency and excess in Denmark (154). Likewise in China, regional differences observed in drinking water were related to iodine status and goitre prevalence in sub-national populations (153, 156). Reports from East Africa (157) and South Africa (158) reinforce a relationship between DWIC and the iodine status of exposed populations.

Within Australia, the incidence of iodine related thyroid dysfunction has been associated with geographic location since the 1930's (159), and early investigations during the 1960's describe seasonal related differences in iodine status of populations residing in southern mountainous areas (160).

Using the standard World Health Organisation (WHO) criterion for adequate iodine status, that being a median urinary iodine concentration (UIC) between 100-199ug/L (1) the 2003 Australian National Iodine Nutrition Survey (NINS) reported a national median UIC of 101ug/L, concluding Australian children were borderline deficient (11). New South Wales, Victoria and South Australia, with median UICs of 89ug/L, 73.5ug/L and 101ug/L respectively, were below or bordering the limit for iodine adequacy (11). Conversely, Queensland and Western Australia reported iodine adequacy with median UICs of 142.5ug/L and 136.5ug/L respectively (11). Other subnational studies continue to report geographic differences in iodine

status between cohorts within Australia (22, 113). In response to growing concern about emerging iodine deficiency in Australia, Food Standards Australia and New Zealand (FSANZ) introduced the mandatory use of iodised salt in the production of all breads, except organic (12). Although drinking water, salt use, and changing milk sanitisation techniques have been proposed as reasons to explain variations observed in iodine status (11), these relationships remain unsubstantiated.

South east Queensland encompasses 12 government regions with an estimated total population of 3.27 million people, of whom almost 1 million of those residents under the age of 14 years (120). The main catchment reservoirs sourcing water to this region include Little Nerang and Hinze Dam, Wivenhoe Dam and Brisbane River, including Somerset Dam (SEQ Water, personal communication, February 2010). Desalinated water is also integrated into the drinking water supply, using water from the Coral Sea (SEQ Water, personal communication, February 2010). Whilst the eastern region borders the Pacific Ocean the western regions are mountainous. Prior to the introduction of iodine fortification of bread, this region was considered to be iodine replete with a median UIC of 137.0ug/L and it remains unclear whether drinking water has an impact on corresponding UIC of children within this region.

According to WHO, the definition of iodised household salt, is salt iodised to a level of 15-40mg/kg (1). FSANZ mandates that iodised salt sold in Australia must have a range of 25-65mg/kg of iodine (161). The 2012 National Health Survey (NHS) reported that approximately 25% of Australian households use iodised salt in either cooking or at the table (135). The impact of this usage on iodine status has not been determined however. Furthermore, it is not known what proportion of Queensland children take supplements and whether these supplements contain iodine, thus also possibly influencing their iodine status.

This study aims to investigate the impact of DWIC, salt use and supplement use on the UIC of two age groups of Queensland children. It compares its findings with the national average to determine whether any differences found can explain why the iodine status of Queensland children is greater than the national average.

Methods

Recruitment

Children aged 2.00-3.99 and 8.00-10.99 residing in south east Queensland, Australia, were eligible to participate. A convenience sample of participants were recruited via various advertisement media including newspaper, television, radio, posters and social media between November 2011 and March 2013. This approach has been shown to attain representative samples in other public health surveys (122-124). Informed written consent was attained from carers of the participating child. The study was approved by the Medical Research Ethics Committee, The University of Queensland (#2001000125).

Sample Collection

Carers supervised the collection of four urine samples from each child over two consecutive days (first morning urine void, and first urine void after midday meal). On the same two days, carers collected one water sample per day from the tap from which the child predominantly drank from on those days. A semi-quantitative food frequency questionnaire (FFQ) completed by carers recorded usual water consumption by number of glasses (250ml) drunk per day. If the participant consumed a supplement on the day of UIC collection, the amount of iodine in that supplement was recorded in mcg/L via the FFQ. The questionnaire recorded demographic and clinical characteristics, including postcode of residence.

Discretionary use of iodised or non-iodised salt or sea salt or iodised sea salt was identified by the FFQ. The frequency of salt use was recorded in the form of i.e. often (1-4 times daily), occasionally (1-6 times a week) or rarely (<1-3 times a month) and whether it was added to cooking or at the table was documented.

Height and weight of the children were measured using standard anthropometric techniques. Body Mass Index (BMI) was calculated using the standard formula. Socio-economic status (SES) was measured at the postcode level using the Socio-Economic Indexes for Areas (SEIFA) Index of Relative Disadvantage (125). This index was developed by the Australian Bureau of Statistics. Participants were categorised as lower SES (SIEFA decile 1-3) /middle SES (SIEFA decile 4-7)/ upper SES (SIEFA decile 8-10).

Sample Analysis

All urine and water samples were stored at -18°C until assayed. UIC and DWIC was established using ammonium persulfate digestion before a Sandell-Kolthoff reaction at Westmead Hospital, Sydney (47). Iodine status was categorised by the WHO criterion based on median

UIC for children aged 8-10 years. As there is no UIC criterion for children aged 2-3 years, dietary iodine intake was extrapolated from UIC assuming 90% of dietary iodine is excreted in urine and a urine volume of 0.5L/day. As described elsewhere (133), an adjusted UIC value was calculated using all four samples to reduce the impact of inter and intra subject variability typically observed in UIC samples.

Statistical Analysis

Stata statistical software (StataCorp, College Station, TX, USA), SPSS (IBM, Armonk, NY, USA) and Microsoft Excel were used for statistical analysis. The adjusted UIC values were log transformed to achieve normality. An average DWIC was calculated from the water samples collected. Drinking water consumption was converted to litres and then multiplied by the iodine concentration ug/L determined by laboratory analysis to provide the amount of iodine ingested via water. Where the laboratory DWIC was <10ug/L, a value of 5ug/L was allocated to that sample as this was the lowest detection limit. An independent t-test was performed to compare the UICs of 2-3 year olds and 8-10 year olds. For each age group, correlation was used to determine the relationship between UIC and DWIC. Unpaired T-test were used to compare the mean UIC of children receiving or not receiving iodine supplements and children receiving or not receiving iodised salt. The percentage of households using iodised salt in various circumstances was calculated in Microsoft Excel. Statistical Significance was set at 0.05.

Results

Participation

Fifty one children (30 males and 21 females) aged 2-3years and 30 children (18 males and 12 females) aged 8-10years completed the survey, predominately residing within 100km of Brisbane CBD. Demographics of height, weight, BMI and SES are shown in table 7.1. No association was found between UIC and BMI or SES in either age group.

Table 7.1 Descriptive demographics of Queensland children aged 2-3 years and 8-10 years.

	Mean Age ± SD	Mean Weight (kg) ± SD	Mean Height (cm) ± SD	Mean BMI ± SD	SES (decile)	
2-3 year olds	2.9 ± 0.5	16.5 ± 1.1	95.6 ± 6.4	16.5 ± 1.4	Lower	13.2%
					Middle	11.7%
					Upper	70.5%
8-10 year olds	9.4 ± 0.8	32.1 ± 7.4	136 ± 0.1	17.0 ± 2.8	Lower	0%
					Middle	46%
					Upper	54%

Urine Iodine Concentration

The median UIC for children aged 2-3 years was 223.3 ug/L (SD 84.6, IQR 163.9-261.5ug/L). The calculated median dietary iodine intake was 124.8ug/day (SD 47.0) which almost twice that of the 65ug/day EAR for dietary iodine.

The median UIC for children aged 8-10years was 143.8ug/L (SD 58.2, IQR 119.5-209.6ug/L), indicating iodine sufficient status. There was a significant difference in the log transformed UIC ($p=0.002$) between the two age groups (mean difference 0.135 log UIC) (95% CI 0.050-0.221).

Drinking Water Iodine Concentration

The average daily water intake of children aged 2-3 years was 0.94L (SD 0.33) with a mean DWIC of 16.4ug/L (SD 8.2, IQR 7.7–23.5ug/L), converting to the mean amount of iodine consumed via drinking water being 15.4ug/L (SD 9.9, IQR 6.2–22.6ug/L). The average daily water intake of children aged 8-10 years was 1.2L (SD 0.59) with a mean DWIC of 14.7ug/L (SD 9.2, IQR 5.0–23.0ug/L), converting to the mean amount of iodine consumed via drinking water being 15.9ug/L (SD 12.1, IQR 6.7-21.6ug/L). In both age groups there was no correlation between amount of iodine consumed via DWIC and UIC ($r=0.13$, $p=0.17$ for 2-3year olds; $r=-0.29$, $p=0.06$ for 8-10 year olds).

Iodised Salt Use

The frequency and type of salt use for both age groups are shown in table 7.2. On any occasion (salt added to cooking or at the table), 76.4% ($n=39$) of 2-3 year old received iodised salt, 15.7% ($n=8$) used non-iodised salt and 7.9% ($n=4$) used no salt. Table salt was added by 50.9% ($n=26$) to meals, and of which 92.3% ($n=24$) was iodised salt. There was a significant

difference ($p=0.029$) in the log transformed UIC values between children receiving iodised salt and not receiving iodised salt (mean difference 0.132 log UIC) 95% CI 0.013 – 0.251).

On any occasion (salt added to cooking or at the table), 66.6% ($n=20$) of 8-10 years old households used iodised salt, 26.6% ($n=8$) used non-iodised salt and 6.6% ($n=2$) used no salt. Table salt was added by 53.3% ($n=16$) of 8-10 year old children to any meal, of which table salt 87.5 % ($n=14$) was iodised. There was a significant difference ($p=0.041$) in the log transformed UIC values between children receiving iodised salt and not receiving iodised salt (mean difference 0.128 log UIC) 95% CI 0.251– 0.005).

Table 7.2 The proportion of children aged 2-3 years and 8-10 years exposed to iodised or non-iodised salt according to frequency and method.

Age group (years)	Frequency of salt use	Iodised salt in cooking	Non-iodised salt in cooking	Iodised table salt	Non-iodised table salt	Iodised sea salt	Sea Salt
2-3	Often (%)	9.8	3.9	5.9	5.9	27.5	9.8
	Occasionally (%)	33.3	13.7	13.7	11.8	11.8	15.7
	Rarely (%)	19.6	11.8	7.8	17.6	5.9	15.7
	Never (%)	37.3	70.6	72.5	64.7	45.1	58.8
8-10	Often (%)	13.3	3.3	3.3	0.0	3.3	13.3
	Occasionally (%)	16.7	6.7	13.3	3.3	3.3	13.3
	Rarely (%)	53.3	73.3	73.3	83.3	86.7	70.0
	Never (%)	23.3	16.7	10.0	13.3	6.7	16.7

Comparisons in UIC mean, median and interquartile range (IQR) of children who received iodised salt on any occasion verses those who did not, are shown for both age groups, in table 7.3.

Table 7.3 Differences in UIC of those children consuming iodised salt vs no iodised salt and those children taking iodine supplements vs no iodine supplements.

Age group (years)	Dietary source	Mean (ug/L)	Median (ug/L)	IQR (ug/L)
2-3	Iodised salt [†]	259.81	254.70	223.37-306.03
	No iodised salt	206.85	189.50	136.19-247.82
	Iodine supplement	205.28	174.40	152.80-265.51
	No iodine supplement	223.95	227.68	171.93-259.22
8-10	Iodised salt [†]	174.31	161.81	133.80-219.96
	No iodised salt	132.82	131.45	108.34-155.32
	Iodine supplement [‡]	229.81	219.96	219.63-230.15
	No iodine supplement	148.21	134.58	111.66-168.37

[†] Significantly different to the no iodised salt group $p < 0.05$
[‡] Significantly different to the no iodine supplement group $p < 0.05$

Supplement Use

Only 14 children aged 2-3 years took supplements, with only 7 of those supplements contained iodine, with a mean of 51.8ug (min 9.3ug, max 75ug) of iodine. There was no significant difference between the mean UICs of 2-3 year old children who received an iodine supplement and those who did not.

In the 8-10 year old age group, 5 children took supplements, with 4 of those supplements contained iodine, with a mean of 93.7ug (min 75ug, max 149.3ug) of iodine. There was a significant difference ($p=0.013$) in the log transformed UIC values between 8-10 year old children receiving iodine supplements and not receiving iodine supplements (mean difference 0.217 log UIC) 95% CI 0.385 – 0.048).

Comparisons in UIC mean, median and interquartile range (IQR) of children who received iodine supplements versus those who did not, are shown for both age groups, in table 7.3.

Discussion

This is the first iodine nutrition survey to investigate the predictors of iodine status within a known Australian iodine replete cohort. The UIC of 2-3 year olds was significantly greater than the UIC of 8-10 year olds. This is consistent with larger surveys reporting an inverse relationship between UIC and age amongst children (119, 162). It should be noted, however,

the criterion for categorising iodine status using UIC is only validated for school aged children and thus comparisons of UIC between age groups may be affected by interpretation of UIC and other biological factors.

Both age groups in this study were found to be consuming adequate amounts of iodine. This is supported by the results of the National Iodine Nutrition Survey (11) with the recent 2012 Australian Health Survey (119, 81), which both reported Queensland to be an iodine replete region. The average DWICs were 16.4ug/L and 14.7ug/L for 2-3 year olds and 8-10 years respectively which were only slightly higher than the national average of 11.3ug/L (163). Furthermore, the average iodine consumed from water was not significantly associated with UIC, and therefore, we cannot explain the higher UIC of Queensland children, compared to the national average, by DWIC exposure. This observation maybe limited by water samples being only collected from one region of Queensland with mostly shared water sources (Personal communication, SEQ Water). Further cross-sectional DWIC comparisons from known regions with various grades of iodine status needs to be conducted to adequately determine the impact of DWIC on iodine status.

Overall, in both age groups, only approximately 14% of children consumed iodine supplements on a regular basis, which is markedly above the national average of up 3.7% of children consuming supplements in similar age groups (164). Nevertheless, supplementation made little contribution to the UIC of children aged 2-3 years, but did significantly increase the UIC of children aged 8-10years. The average iodine content of the supplements ingested by the older age group was almost double of that consumed by their younger counter parts (93.7ug vs 51.8ug) and thus this observation was expected. Furthermore with only a small proportion of children taking supplements overall, it is unlikely that this determinant provides much explanation to the national UIC variation.

Importantly, this study is the first to evaluate the impact of discretionary iodised salt use on the UIC of Queensland children. WHO recommends a national coverage of >90% of households using iodised salt (1). This study has shown that 76.4% and 66.6% of households with a child aged 2-3years or 8-10 years, use iodised salt on any occasion (either in cooking or at the table). Although this coverage is lower than the standard set by WHO, it is above the estimated national coverage of 26.6% of households with a 2-3 year old member and 25% of households with a 9-13 year old member using iodised salt in cooking alone (82). The use of iodised salt reported by the current study also indicates, the percentage of households purchasing iodised

salt is higher than previously reported in 2003 indicating only 11% of households purchase iodised salt (165). According to the 2012 NHS the average national use of iodised salt at the table was less than 15% for both age groups (82). It is apparent the discretionary use of iodised salt is an important source of dietary iodine as its contribution was a significant contributor to UIC in both age groups. With the use of iodised salt in both cohorts greater than the national average, the higher UIC observed in Queensland when compared to the national UIC may be partly explained by this determinant. It could be argued, however, that the method of recruitment performed in this study may subject these observations to bias, as participants may have had a previous interest in iodine nutrition and thus more likely to be inclined to use iodised salt.

Although discretionary salt use is difficult to quantify, table 7.2 describes the proportion of children exposed to salt, whether it be iodised or non-iodised, according to frequency and method of use. The most frequent exposure to iodised salt for 2-3 year olds is the use of iodised salt added to cooking at least 1-6 times a week, with iodised salt mostly only added to meals <1-3 times a month. For 8-10 year olds, the exposure to iodised salt was less frequent with the use of iodised salt added to cooking or at the table mostly being <1-3 times a month for both occasions. Interestingly, in this age group, the exposure of iodised salt is most likely to occur when salt is added at the table. More autonomy at the meal times may explain why the elder age group receives more salt at meal times than their younger counterparts.

The observation of adding salt to meals, however, could directly conflict the Australian dietary guidelines recommendation to limit the intake of foods and drinks containing added salt, due to the association with hypertension (166). Excluding discretionary salt, the 2012 NHS reported that children aged 2-3 years and 8-10 years exceeded the upper level for salt intake through the consumption of processed foods alone (167), of which 25% of salt consumed through cereal and cereal products. This confirms the use of bread as a viable option to incorporate iodised salt fortification in an attempt to improve the national iodine status (168). Since the commitment at the 2013 United Nation World Assembly to reduce global salt intake by 30% by 2025, the challenge of maintaining adequate iodine intake whilst reducing overall salt consumption begins (169).

Considering the positive impact bread (Chapter 5) and discretionary iodised salt use has on the UIC of children within this cohort, the implications of reducing overall salt intake on the iodine status of these children needs to be considered. From a public health perspective, a balance

needs to be achieved where negative health consequences of excessive salt consumption are avoided without compromising the benefits of iodised salt on the iodine status of children.

This study has surveyed the impact of three variables on UIC and provides possible insight to the varying iodine status observed throughout Australia. The use of iodised salt on any occasion by the household was the greatest predictor of iodine status for all children. Although DWIC had no effect on UIC in both age groups, the limitation of only collecting samples from a confined area has restricted the ability to appropriately assess this variable. A cross sectional investigation across a number of regions is required to adequately determine the impact geography or DWIC has on UIC.

CHAPTER 8: THYROID ANALYTES

INTRODUCTION

This manuscript present novel data of serum thyroid analytes from a healthy, iodine replete paediatric cohort. It explores the influence of UIC on thyroid analytes and identifies any abnormalities. This paper discusses the importance of interpreting thyroid function using thyroid analytes and it relevance to community settings.

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The manuscript has been reformatted to fit the requirements of the thesis.

THYROID ANALYTES OF A SMALL AUSTRALIAN, IODINE REPLETE, PAEDIATRIC COHORT.

Abstract

Aims: Healthy thyroid function is essential throughout the early years of life to establish normal neurodevelopment and physical growth. Thyroid function is not well defined for mainland Australian children.

Methods: Twenty-seven children (eleven 2-3 year old and sixteen 8-10 year olds) from south east Queensland provided blood samples, four urine samples and carers completed questionnaires pertaining to individual and familial thyroid history.

Results: All children were iodine replete with UICs of 174.4ug/L and 165.4ug/L for 2-3 year olds and 8-10 year olds respectively. No children had fT4, fT3 or Tg levels outside the reference ranges. Seven children had one or more thyroid analytes outside the reference ranges of TSH or TPOAb. Five children had a TSH (mean 2.7mU/L) above the recommended reference, all of whom had normal fT4 (mean 15.0pmol/L) and fT3s (mean 5.9pmol/L) and only one child with corresponding TPOAbs of 75U/mL. A difference of 23.5ug/L ($p=0.021$) in Tg levels was observed between the two age groups. Familial history was only a predictor for TSH for both age groups combined ($\beta 0.19$, $p=0.016$, 95%CI 0.03-0.34).

Conclusions: Although iodine replete, thyroid abnormalities were observed in this small community cohort. Larger community based surveys are needed to explore these findings within the wider population.

Introduction

Healthy thyroid function is essential throughout the early years of life, as an adequate supply of thyroid hormones establish normal neurodevelopment and physical growth (1). The clinical implications of thyroid dysfunction in children may include poor linear growth, delayed bone maturation, pubertal disorders, neurological impairment (170, 171). Emerging data also suggest links with autism (172) and attention deficit disorders (173). In some regions of the world, the consequence of thyroid dysfunction within the community has an estimated economic cost of up to \$1 billion (US) per (174). Although thyroid dysfunction is well identified in older adult populations (175-177), the occurrence of thyroid dysfunction is not so well defined for mainland Australian children.

Thyroid function can be indicative of the thyroid glands response to iodine intake (1). As an integral component in thyroid hormone production, the micronutrient iodine is routinely surveyed within population surveys using the median urinary iodine concentration (UIC) as a marker (1). The World Health Organisation collaborated with UNICEF and the Iodine Global Network (IGN) to establish guidelines for assessing a population's iodine status and associated risk of thyroid dysfunction (1). The guidelines suggest several markers of assessing iodine status, including thyroglobulin, thyroid volume, and the most widely used, UIC (1). The presence of thyroid dysfunction is expected in iodine deficient populations where the median UIC is <100ug/L and iodine excessive populations where the median UIC is >299ug/L (1, 4). This non-invasive, low cost approach to assessing thyroid function provides an indirect indication of whether thyroid dysfunction may exist within a population. Without analysing thyroid hormones directly, however, the nature of thyroid function is only insinuated and therefore not accurately defined. Furthermore, iodine replete populations are not necessarily devoid of thyroid dysfunction, as it has been detected in populations with median UICs within the acceptable range of 100-199ug/L (17, 95).

Although thyroid analyte screening for neonates has been standard practice for some time in Australia (175), reports of varying degrees of iodine status amongst Australian preschool (83) and school aged children (11, 111) may also suggest possible discrepancies in thyroid function.

The Australian National Iodine Nutrition Survey (NINS) revealed the prevalence of iodine deficiency amongst school aged children in year 2003/2004 with a national median UIC of 96ug/L (109). Western Australian and Queensland were an exception, however, attaining

median UICs of 142 ug/L and 135.6ug/L respectively, indicating iodine adequacy (11). Although the survey found no association between thyroid volume and UIC (11), this may not necessarily indicate an absence dysfunction. Furthermore, thyroid hormone analytes represent recent dietary iodine intake, whilst thyroid volume is a reflection of iodine intake over time (14). As it can take up to 10 years for thyroid volume to modify in response to iodine intake, thyroid volume may not be a sensitive enough indicator to determine present thyroid function in such young children (14, 15).

In Queensland, an individual paediatric endocrinologist reported thyroid disorders ranked 4th out of 13 reasons for referral (178), suggesting that some classifications of thyroid dysfunction may exist in the iodine replete state. The prevalence of thyroid dysfunction within a healthy community paediatric cohort has not yet been explored in Queensland.

The current study aimed to examine the thyroid analytes from a small sample of preschool and school aged children residing in the iodine replete state of Queensland and attempts to describe any abnormalities that may exist.

Methods

Recruitment

Children aged 2.00-3.99 years and 8.00-10.99 years, residing in south east Queensland, were eligible to participate as per criterion requirements of a larger iodine survey (132). Convenience samples were recruited between November 2011 and March 2013 via various advertisement mediums including newspaper, television, radio, posters and social media. This method of recruitment has successfully yielded representative samples in other public health surveys (122-124). Informed written consent was attained from carers of participating children. The study was reviewed and approved by the Medical Research Ethics Committee, The University of Queensland (#2001000125).

Sample Collection

Carers collected a total of four urine samples (first spot morning void and first spot urine void after midday meal) from their child on two consecutive days. Cotton wool balls placed in the diapers collect urine from those participants not toilet trained. A non-fasting venous blood sample was drawn into a 'yellow ss tube' until at least 5ml of blood was collected. Carers completed a questionnaire capturing previous known thyroid disease, familial history of

thyroid disease (parent or grandparent with disease), and socio demographic details. Height and weight were measured to determine Body Mass Index (BMI). Socio-economic status (SES) was measured at the postcode level using the Socio-Economic Indexes for Areas (SEIFA) Index of Relative Disadvantage. This index was developed by the Australian Bureau of Statistics and ranges from 1 to 10, with a low score indicating greater disadvantage (125). Participants were categorised as lower SES (SIEFA decile 1-3) /middle SES (SIEFA decile 4-7)/ upper SES (SIEFA decile 8-10).

Sample Analysis

Urine samples were stored at -20°C until couriered on ice to Westmead Hospital, Sydney for analysis. UIC was determined using ammonium persulfate digestion before a Sandell-Kolthoff (47) reaction with a sensitivity limit of 5ug/L and a precision of <20ug/L. For this method, the CV of repeated measurements of samples, in the range of iodine concentration found in urine, has been repeated as being less than 2% (47).

Serum Thyrotropin (TSH) (intra and inter assay variability at 0.7U/mL, CV2.5% and CV5.3% respectively and at 18.9U/mL, CV%2.4 and CV% 2.0 respectively), free-thyroxine (fT4) (intra and inter assay variability at 6.1pmol/L, CV4.7% and CV4.6% respectively and at 39.9pmol/L, CV%2.2 and CV% 2.7 respectively), free triiodothyronine (fT3) (intra and inter assay variability at 2.9pmol/L, CV3.1% and CV4.0% respectively and at 14.2pmol/L, CV%2.5 and CV 2.8 respectively), thyroid peroxidase antibody (TPOAb) (intra and inter assay variability at 70.8U/mL, CV6.8% and CV3.4% respectively) and thyroglobulin antibody (TgAb) (intra and inter assay variability at 71.0U/mL, CV5.8% and CV6.2% respectively) were analysed using the Siemens ADVIA Centaur chemiluminescence assay and Thyroglobulin (Tg) levels were obtained using the Siemens ImmaLite 2000 immunoassay system. All thyroid pathologies were determined by QML Pathology, Brisbane, who provided the following laboratory references ranges; TSH was 0.40-4.00 mU/L, fT4 was 10-20 pmol/L, fT3 2.8-6.8 pmol/L, TPOAb <60 U/mL, TgAb <60 U/mL and Tg <44.0 ug/L.

Statistical Analysis

As described elsewhere (133), an adjusted median UIC value was calculated using all four urine samples to reduce the coefficient of variance (CV) by up to 40%. UIC, TSH and Tg data were log-transformed to improve normality before statistical analysis. Independent two-tail t-test was performed to determine differences in mean UIC, TSH, fT4, fT3 and Tg between the

two age groups. Separate linear regression analysis was used to determine the effect of UIC, age, gender, familial history and SES on TSH, fT4, and fT3. Stata statistical software (StataCorp, College Station, TX, USA), SPSS (IBM, Armonk, NY, USA) and Microsoft Excel were used for statistical analysis. Statistical significance was set at 0.05.

Results

Fifty one children (30 males and 21 females) aged 2-3 years and 30 children (18 males and 12 females) aged 8-10 years completed the survey, of which 11 (2 females) and 16 (5 females) respectively, agreed to blood analysis.

The median UIC for children who provided blood samples was 174.4ug/L (IQR 108.8 - 234.3ug/L) for 2-3year olds and 165.4ug/L (IQR 132.0 -220.1ug/L) with a non-significant UIC difference of 8.4ug/L between the two age groups. None of the children had known prior thyroid dysfunction, nor had been tested for thyroid conditions previously. Table 8.1 describe the percentiles of TSH, fT4, and fT3 for each age group and the age groups combined.

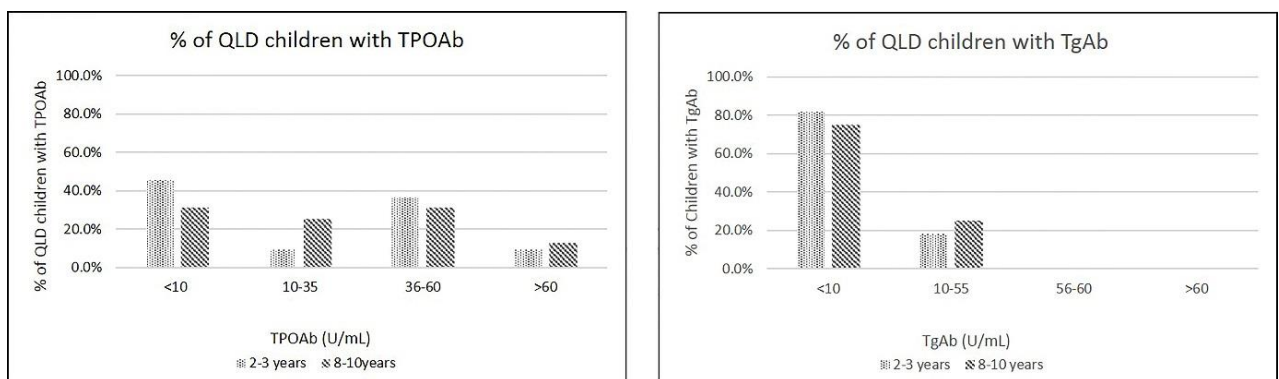
Table 8.1 Percentiles for thyroid analytes of Queensland children

	n	Percentiles							Median	Mean (SD)
		2.5	5	25	50	75	95	97.5		
TSH(mU/L)	27	0.5	0.9	1.9	2.7	3.4	4.5	4.8	2.4	2.7 (1.1)
All										
2-3 years	11	0.5	0.8	1.8	2.5	3.2	4.1	4.5	2.5	2.5 (1.0)
8-10 years	16	0.5	0.9	2.1	2.9	3.7	4.9	5.2	2.9	2.9 (1.2)
fT4(pmol/L)	27	12.5	12.9	14.1	15.0	15.9	17.1	17.5	15.0	15.0 (1.3)
All										
2-3 years	11	12.2	12.6	13.9	14.9	15.8	17.2	17.6	15.0	14.9 (1.4)
8-10 years	16	12.4	12.8	14.2	15.1	16.0	17.4	17.8	15.0	15.1 (1.4)
fT3(pmol/L)	27	4.9	5.1	5.6	5.9	6.2	6.7	6.8	6.6	5.9 (0.5)
All										
2-3 years	11	4.5	4.7	5.4	5.9	6.4	7.1	7.2	6.1	5.9 (0.7)
8-10 years	16	4.9	5.1	5.6	5.9	6.2	6.7	6.8	6.6	5.9 (0.5)

There were difference between the median Tg value found in the two age groups (20.8 ug/L for 2-3 year olds; 11.6ug/L for 8-10 year olds). In order to test if this difference was statistically significant, the data were firstly logged to correct for a non-normal distribution. The difference

between the logged values was significant ($p= 0.021$). A mean Tg of 27.7ug/L (95%CI 19.5-36.0ug/L) for 2-3 year olds and 16.0ug/L (95%CI 10.5-22.5ug/L) for 8-10 year olds was obtained. A combined mean of TSH 2.7mU/L (95%CI 2.2-3.1mU/L), fT4 15.0pmol/L (95%CI 14.4-15.4pmol/L) and fT3 5.9pmol/L (95%CI 5.6-6.1pmol/L) were attained. No children had fT4, fT3 or Tg levels outside the reference ranges. Five children had a TSH above the recommended reference of 4.0mU/L (range 4.2-4.8mU/L), all of whom had normal fT4 and fT3's and only one child with corresponding TPOAbs of 75U/mL. TPOAbs were detectable above the sensitivity limit of 15U/mL in 16 children, however, only 3 children had titres above the reference > 60U/mL. TgAb were detected above the sensitivity limit of 15U/mL in 6 children, however, none of those exceeded the accepted reference of <44ug/L. The distribution of TPOAbs and TgAbs are shown in figure 8.1.

Figure 8.1 The distribution of TPOAb and TgAb within a small Australian paediatric cohort.



Regression analysis of SES, gender, age category and UIC, when performed separately on the data combined from both age groups ($n= 27$), were not significant predictors for any thyroid analytes fT3, fT4 and TSH. The small sample size prevented multiple regression analysis to be performed.

Familial history was only a statistically significant predictor for TSH ($\beta 0.19$, $p=0.016$, 95%CI 0.03-0.34).

Discussion

This study describes the influence of iodine status, gender, SES, age and familial history on thyroid analytes in a small, iodine replete, paediatric cohort residing in Australia. The only

significant relationship found between the various predictors of thyroid functions and thyroid analytes was family history and TSH. Although no children had no known previous thyroid disease, 14% had at least one thyroid analyte outside the recommended reference range. The reference ranges used, however, were not paediatric specific and thus the interpretation of these results should be applied with caution.

Used as a primary indicator to detect hypothyroidism or hyperthyroidism within an individual, TSH is considered to be the most important analytes when screening thyroid status (179). The current study reports modest subclinical hypothyroidism (defined as elevated TSH with normal fT4 (179) in 5 children. The continuing physiological changes throughout childhood cause the application of adult reference ranges to paediatric cohorts to be widely debated and considered unreliable by some (180-183).

Establishing paediatric reference ranges, however, are limited by analytical methods, sample size, environmental exposure, genetic susceptibility, age, gender and hospital based cohorts used as standards as well as ethical considerations of bleeding healthy community children (180, 181). The International Federation of Clinical Chemistry and Laboratory Medicine recommend the 2.5th and 97.5th centiles to be used to define a reference range suitable for representing 95% of the central tendency of a population (184). A Western Australian study explored age specific TSH cut-offs from a laboratory database of community samples and reported a 2.5th and 97.5th percentile range to be 0.74-4.02mU/L for 5-10 year olds (males and females inclusive) (185). Further studies establishing paediatric reference ranges using the same analytical method as the current study are shown in table 8.2.

Table 8.2 Various paediatric reference ranges for TSH

Age (years)	Established TSH reference range (mU/L)	Reference cohort	Exclusion Criterion	Laboratory Equipment	Reference
1-5 (n=76)	0.42-4.79	Hospital based	Hyper-/hypothyroidism, diseased, drugs known to interact with thyroid, outliers	ADVIA Centaur (chemiluminometric assay)	Hubner, U., Englisch, C., et al (2002)
6-10 (n=138)	0.48-4.67				
1-5 (n=2782)	0.75-6.57	Health service database from non-hospitalised patients attending community clinics	Positive antibodies, thyroxine treatment, drugs known to interact with thyroid	ADVIA Centaur (chemiluminometric assay)	Strich, D., Edri, S., et al (2012)
6-10 (n=3531)	0.79-6.0				
1-5 (n=218)	0.80-6.26	Health service database from non-hospitalised patients attending community clinics Hospital based laboratory database	Positive antibodies, thyroxine treatment, drugs known to interact with thyroid drugs known to interact with thyroid, eating disorders, pituitary disease	ADVIA Centaur (chemiluminometric assay) ADVIA Centaur (chemiluminometric assay)	Strich, D., Edri, S., et al (2012) Kapelari, K., Kirchlechner, C., et al (2008)
6-10 (n=315)	0.80-5.40				

When applied to the current study TSH 2.5th and 97.5th percentile range (0.9 - 4.8mU/L), it appears that this group of children are within normal limits for their age. Furthermore, the European Thyroid Association Guidelines for Management of Subclinical Hypothyroidism in Pregnancy and in Children only considers a TSH value >5mU/L abnormal (171). Nevertheless, differences observed within these paediatric references ranges, such as, reference cohort (ethnicity, sick/healthy, iodine status), exclusion criteria, population size and statistical adjustments need to be considered when applying these references ranges to this Queensland cohort.

In some circumstances TPOAbs have peaked after the introduction of an iodine prophylaxis program, particularly in previously iodine replete communities (17, 61, 96). Australia introduced its ongoing mandatory iodine prophylaxis program in late 2009, where all bread (except organic varieties) was fortified using iodised salt (12). Since then, the current study is the only one of its kind to report the prevalence of TPOAb within a healthy, iodine replete, Australian paediatric cohort. Only three children had TPOAbs marginally greater than the reference of <60U/mL. The presence of antibodies can signify an autoimmune dysfunction of the thyroid and is usually associated with, environmental exposure (iodine), familial history and is more prevalent in females than males (170, 186, 187). Although there was no effect found between these predictors and the presence of TPOAbs in the current study, this is potentially due to lack of power due to a small sample size and a misrepresentation of the general population, especially for gender. This limitation also applies to the results reported by the separate linear regressions. As all children had normal Tg, TgAb and adequate UIC, it is unlikely the presence of these TPOAbs is related to increasing iodine status, however, without baseline data prior to iodine prophylaxis, this conclusion is only speculative. The most common form of thyroid dysfunction within the adult Australian population is autoimmunity (177), with up to 40% of pregnant women reported to have positive TPOAbs in one region of Australia (188). Thus familial history is more likely to be a plausible predictor of TPOAbs in this paediatric cohort as it was also found to be a significant predictor of TSH.

Similar to the TSH reference range debate, there are some discrepancies regarding the appropriate upper limit for TPOAbs. Other paediatric community surveys use an acceptable limit of <35U/mL (189-191). Furthermore, the Brusselton thyroid study conducted in Western Australia derived a reference of <35U/mL (Immulite2000 chemiluminescent analyser) from a community based population (ages 17-90 years) (176). As shown in figure 8.1, if a limit of <35U/mL was applied to the current study's cohort, the percentage of positive TPOAbs increases from 11% to 40%, with 63% of the group with a familial history of thyroid dysfunction. As the presence of these antibodies precedes thyroid abnormality (175), the biological significance of this finding could be important for future health. It should be noted, however, that 10-15% of adults may have detectable antibodies with no clinical significance and titres <100U/mL may not be a specific indication of thyroid autoimmunity (170). Nevertheless, this phenomenon is yet to be explored in paediatric groups.

The median Tg of children with this iodine replete Queensland group was 20.8 ug/L (IQR 16.6-44.2ug/L) for 2-3 year olds and 11.5ug/L (IQR 8.3-20.9ug/L) for 8-10 year olds was obtained. Consistent with these findings, other larger surveys in both Australia and New Zealand have reported an inverse relationship with age and Tg (83, 146). Both age groups were within acceptable range for both laboratory reference range and World Health Organisation (1-40ug/L) confirming the conclusion determined by UIC that children within this cohort were iodine replete (1).

Similar to the current study, a large New Zealand iodine nutrition and thyroid survey conducted by Skeaff and colleagues (146) found no significant effect of gender or SES on thyroid analytes (146). Like the current study they did find a significant difference in Tg between younger (5-7years) and older (8-10years) and that age was a significant predictor for this analyte (146). The current study failed to find any further effects on thyroid analytes (except for familial history on TSH), whilst the New Zealand study found significant effects of age on fT4 and UIC on Tg, and fT3 (146). Although the current study is obviously limited by power, the biological significance of its findings are somewhat similar to much larger community surveys.

This small, paediatric survey found no active overt thyroid dysfunction within a group of iodine replete children. Although limited by its small sample size, its findings are similar to larger surveys also finding no significant effect of gender, age or SES on TSH, fT4 and fT3. The conflict of acceptable limit of <35U/mL or <60U/mL may have implications to the interpretation of autoimmune tendencies within this group. With limited community-based paediatric thyroid studies in Australia, it is difficult to conclude whether thyroid autoimmunity is as much of an issue for children as it is for their adult counterparts. Understanding the trend for the presence of TPOAbs to precede thyroid abnormalities, further research should be considered.

CHAPTER 9 DISCUSSION

This chapter reviews the main discussion points presented by the papers and manuscripts in the preceding chapters and examines the findings, limitations, strengths and conclusions of the individual manuscripts in an attempt to amalgamate the key findings of this thesis.

9.1 RESEARCH PROBLEM

It is well accepted that iodine is an essential micronutrient, critical for normal growth and development, particularly for young and school aged children. This thesis discusses a number of challenges confronted when monitoring iodine nutrition within paediatric cohorts. It is important that a population remains within the acceptable parameters of iodine nutrition, avoiding both iodine deficiency and iodine excess. The overall aim of this thesis was to firstly report the current iodine status of both pre-school and school aged children residing in the previously iodine replete state of Queensland after they had been exposed to the national iodine fortification program. Secondly, this thesis attempted to identify what dietary and/or environmental exposures were of greatest influence on the iodine status of these children and thirdly, determine whether thyroid abnormalities were present within the cohort. A review of the research aims are below;

Objective 1: Evaluate the effect of collecting multiple urine samples on the variance observed in urinary iodine concentration (UIC) in an attempt to improve the reflection of iodine status.

Objective 2: Determine current iodine status of Queensland children aged 8-10 years and establish the level of significance that food consumptions, especially bread, has on the individuals UIC.

Objective 3: Document what proportion of 2-3 year old have excessive iodine status post iodine fortification of bread and report dietary determinants most influential on UIC.

Objective 4: Explore the influence of drinking water iodine concentration, discretionary use of iodised salt and supplement use on UIC in Queensland children.

Objective 5: Evaluate the thyroid function of Queensland children and identify associations between thyroid hormone analytes and UIC.

9.2 SUMMARY OF RESEARCH IN RELATION TO AIMS

The introductory literature review (Chapter 2) of this thesis described the challenges in assessing iodine status according to UIC. Within subject and between subject variability is evident, and thus the coefficient of variance is typically large within populations when single spot urine samples are used (19, 20). As discussed in Chapters 2 and 4, the spread of UIC samples within a population importantly determines what proportion of those samples fall within the various grades of iodine status according to the WHO criterion (1). Recommendations suggest (1) that large sample sizes reduce the extent of this variability. The first aim of this thesis was to evaluate the effect of collecting multiple UIC samples on the variance in attempt to improve the accuracy of interpreting iodine status in smaller cohorts. Chapter 4 shows that collecting multiple UIC samples at different times of the day can reduce the coefficient of variance by up to 40%. These results were shown to be consistent with the reduction observed in larger iodine nutrition surveys of older populations where similar statistical methods were applied (22, 23). As a result, the interpretation of iodine status according to the WHO criterion changed. Although, as expected, the median UIC remained constant in both age groups after the adjustment for four samples was made, the proportion of samples considered excessive in 2-3 year old pre-school children reduced from 25% to 20% and from 10% to 0% in 8-10 year old school aged children. Furthermore, this manuscript was the first to report results of both morning and afternoon UIC samples. The observation of higher UIC in afternoon samples reflects the circadian rhythm of UIC (19, 20, 56) and confirms the need to consider the timing of collecting UIC samples, as well as the number of samples, when assessing iodine nutrition. Therefore, Chapter 4 accepts the hypothesis that collecting multiple UIC samples improves the interpretation and classification of iodine status within populations and smaller cohorts. In response to the findings reported in Chapter 4 and considering the non-invasive ease of obtaining urine samples, together with the relatively low cost of analysis, this thesis recommends the inclusion of multiple UIC sample collection in future studies in order to obtain a better reflection of the distribution of iodine status within a group.

The second aim of the thesis was to ascertain the current iodine status of Queensland school aged children aged 8-10 years and the possible effect of the diet, especially the consumption of iodine fortified bread on UIC. Applying the method of adjusting for multiple UIC samples as discussed in Chapter 4, the manuscript in Chapter 5 concludes that the iodine status of Queensland school aged children aged 8-10 years is 'adequate' with the consumption of bread

being the most significant dietary iodine contributor to iodine status. Although the 2012 NHS observed an increase in UIC of Queensland school aged children since the implementation of iodine fortification in Australia in 2009, it did not conclude whether the increase was a direct result of the public health initiative to fortify bread with iodine. Currently, this thesis is the first to report a direct relationship between iodine fortified bread consumption and iodine status in this age group. As UIC did not increase to the level which was predicted by FSANZ, it did not cause children in this cohort to attain excessive iodine status as hypothesised by FSANZ. Therefore, it is accepted that fortified bread is a significant contributor to the iodine status of Queensland school aged children aged 8-10 years, however, consistent with the null hypothesis, it did not cause children in this cohort to have a UIC indicative of excessive iodine intake.

The third aim of this thesis was to determine the iodine status of 2-3 year olds and identify what foods usually consumed by this cohort impacted on UIC in order to assess whether concerns that iodine fortification of bread in Australia would cause this age group to become iodine excessive. Although the approach of determining iodine status via UIC was consistent with chapter 5, the interpretation of the results was adapted (see section 9.3). As this age group is often considered to be too difficult to examine, there are very few international iodine surveys that use UIC of preschool children in the evaluation of iodine nutrition. The manuscript in Chapter 6 discusses at length the limitations of applying the UIC results of 2-3 year olds to the WHO criterion as the smaller urine volume of preschool children needs to be considered. According to the extrapolated dietary iodine intake of Queensland preschool children aged 2-3 years, it was reported in Chapter 6 that almost 10% of these children were consuming iodine above the upper limit for dietary iodine. This is more than the 6% predicted by FSANZ when implementing the iodine fortification. The current thesis found no relationship between food intake, including bread, and iodine status in this 2-3 year old cohort, however, limitations discussed in the manuscript suggest an insufficient sample size and in retrospect a possibly unsuitable dietary evaluation tools may have influenced this observation. Therefore, the null hypothesis was accepted, as fortified bread was not found to be a significant contributor of dietary iodine intake amongst preschool children with an iodine status above dietary iodine requirements as determined by UIC.

The fourth aim of this thesis related to the hypothesis that variation in concentrations of iodine in drinking water may explain the geographical variations of iodine status observed across Australia. Furthermore, it evaluates the influence of discretionary salt use and iodine

supplement intake on the iodine status of both school aged children 8-10 year and pre-school children aged 2-3 years. The manuscript in Chapter 7 reports no association found between drinking water iodine concentration and UIC. This finding may be influenced by the limited geographical variability of the subjects included, as most were within 100km of Brisbane CBD and this area of Queensland is known to share similar water sources. For the purpose of this thesis, however, the null hypothesis was accepted, as drinking water iodine concentration was not found to explain differences observed between area of residence and UIC observed within the cohort. Very few children in both age groups consumed iodine supplements and thus it is concluded that supplements were unlikely to be a sustainable source of dietary iodine for the larger population. An important observation, however, was that the UIC of both age groups was greatly influenced by the household use of iodised salt on any occasion (added to meals or at the table). Thus, with the Australian public health campaign to reduce salt use by 30% by 2025, the impact of this initiative may also have some impact upon UIC in a detrimental manner.

Finally, this thesis attempted to evaluate the thyroid function of a small number Queensland children (both pre-school and school aged), in order to identify any associations between thyroid analytes and iodine status. The null hypothesis was accepted in this manuscript as no associations between UIC and any of the thyroid analytes were found to be significant. Understanding that iodine is an integral limiting factor of thyroid hormone production, although participants within this small group were iodine replete, evidence of thyroid abnormality in 1 in 5 children was still detected. The diagnosis of thyroid disease from these analytes need to be interpreted with some caution, as adult references ranges are used to determine normal parameters and the influence of familial history needs to be explored.

9.3 SYNTHESIS OF RESEARCH FINDINGS

Although easy to obtain, determining iodine status using urinary iodine concentration can be ambiguous and misinterpreted.

This thesis discusses the controversies pertaining to using UIC as a marker of iodine status, especially in young children. Chapter 6 explains the inconsistencies of comparing the iodine status of preschool children aged 2-3 years with other cohorts of similar age. The WHO criterion for various degrees of iodine status using UIC as a biomarker is only validated for school aged children with an approximated urine output of 1L/day. Whilst some investigators

compare crude 2-3 year old UIC data to the WHO criterion to categorise iodine status, others collect 24 hour urine volume adjust for creatinine or adjust for an approximate urine output of 0.5L/day. It is clear that there is inconsistency amongst investigators regarding the application of the WHO criterion and without formal guidelines for the iodine nutrition assessment of children within this age group, misclassification of iodine status is likely to continue. Whilst some national nutrition surveys report iodine intake using dietary records, these are predominantly obtained from 24 hour recalls which may not be a typical reflection of the usual habitual intake of a small child. Furthermore, without a biomarker the validation of this intake data is unsubstantiated, as dietary records may not accurately measure micronutrient intakes. With the critical window of iodine nutrition identified as being from conception to the third year of life (1), there is a need for further focus to obtain reliable UIC data from young children to better understand the iodine requirements of children within this age group and ensure appropriate dietary iodine intake. Deriving dietary iodine intake using UIC, continues to be the most comprehensive representation of iodine nutrition, however, similar to their older counterparts, the interpretation, method and timing of how the UIC is obtained impacts upon the accuracy of the iodine status obtained.

As up to 90% of dietary iodine is excreted in the urine approximately 4-5 hours after ingestion (3, 20), UIC remains to be the most practical marker of iodine status. The manuscripts in Chapters 5, 6, and 7 discuss the advantage of collecting multiple UIC samples, on different days, from each individual in the study. Twenty-four hour urine samples are accepted as the gold standard when assessing UIC because of its capability to incorporate the fluctuating circadian rhythm of iodine metabolism. This method can be cumbersome considering the schedules (school activities) and abilities (toilet trained) of children within both of the age groups studied in this thesis. This thesis showed that collecting one morning spot UIC sample (consistent with WHO guidelines), in addition to, collecting an additional afternoon spot sample incorporates the peaks of iodine metabolism similar to that observed in 24 hours urine collection (19, 20, 56). By repeating UIC samples the variability often observed within individuals and between individuals is reduced when the analysis used in Chapters 4, 5 and 6 is undertaken. Although the sample size in both age groups included in the thesis was not achieved, the application of this analysis shows a reduction in variability similar to that seen in the few older and larger population surveys who have also applied that same approach. The greatest impact of this reduction in variability was seen in the distribution of UIC across the various degrees of iodine nutrition. The WHO clearly state that the spread of iodine deficiency

with in a cohort is an important consideration when monitoring and combating insufficient iodine intake. Arguably, the same could be stated when assessing iodine excess. This thesis reports that 25% of preschool children aged 2-3 years and 10% of school aged children aged 8-10 years were considered iodine excessive when crude UIC is assessed. When adjustments are performed to reduce the impact of variability was reduced by almost 40%, reducing the number of sample outside the ideal range for optimal iodine nutrition. Despite the clear advantages of collecting multiple urine samples, very few paediatric iodine nutrition studies adopt this approach. Whilst it is argued that larger sample sizes (>100) account for varying degrees of hydration and other biological variations when collecting spot morning samples (1), the degree to what effect sample size has on the variability within a spread of UIC samples is not clearly reported (1). The application of collecting multiple UIC samples, including an afternoon sample in future studies could improve the reflection of overall iodine status within a population and thus, importantly, be the determining factor whether public health dietary iodine intervention is required or, if causing excessive consumption, should be removed.

To understand iodine status, thyroid function should also be examined.

This thesis is the first mainland Australian study to collect dietary records, UIC and thyroid analytes in the same individuals within a paediatric cohort after the implementation of iodine fortification in Australia. As the primary objective of optimising iodine nutrition is to maintain healthy thyroid function, it is important to capture how the thyroid responds to corresponding iodine intake. Whilst dietary records capture what foods children usually eat that may influence UIC, UIC reflects what amount of iodine absorbed and thyroid analytes describe how iodine is used physiologically and thus whether the recipient is consuming sufficient iodine. Although Chapter 8 reports no correlation between UIC and abnormal thyroid analytes, this may be explained by the small sample size and recruitment method i.e. predisposition to enrol because of known familial thyroid dysfunction. Nevertheless, this thesis has demonstrated that although UIC and dietary iodine remain within current acceptable limits for both age groups, thyroid dysfunction in some form exists amongst these children. What is consistent throughout the manuscripts presented in Chapters 6 and 8 is whether the references range or dietary guidelines determined for these children are appropriate. Even though these children have adequate UIC and dietary intake, thyroid abnormalities are still observed, therefore are the pre-sets for UIC and dietary intake accurate? Or, considering the thyroid references ranges provided by

laboratories are used for adults also, if paediatric reference ranges were used would 'abnormalities' decrease or increase?

Based on the principal that 90% of dietary iodine consumed is excreted in urine, an EAR of 65ug/day for school aged children (8 year olds) would convert to an approximate UIC of 59ug/L. The validated WHO criterion to ensure at least 50% of the population has adequate iodine status without any health consequence is a median UIC of 100ug. Furthermore, the UL for dietary iodine intake in this age group, is 300ug/day which converts to an approximate UIC of 270ug/L, below the excessive parameter of 300ug/L set by WHO. The development of the WHO criteria was determined by the observing UIC levels in the absence of thyroid dysfunction in school aged children, whilst dietary guidelines were derived from adult records (150, 151). It is proposed by this thesis then, that assessing iodine nutrition using UIC is a better benchmark for determining appropriate intake of iodine over EAR, RDI or UL. The dietary benchmarks of EAR, RDI and UL may be too low and not adequately assess whether a population or group is at risk of thyroid dysfunction in response to iodine intake. As previously discussed, this reiterates the need to establish an appropriate UIC criteria describing the various degrees of iodine nutrition for young children, i.e. 2-3 year old pre-schoolers.

Concerns regarding whether iodine fortification in Australia bread would cause excessive iodine intake amongst previously iodine replete populations and young children are unsubstantiated.

The purpose of introducing a national iodine fortification program into Australian bread was to counteract the various degrees of iodine deficiency observed, in particular, across the southern states (168). Exempt from such deficiency, the states of Western Australian and Queensland were already iodine replete, although the reason as to why iodine status was acceptable in these states and not in others was unknown. When considering the implementation of mandatory iodine fortification in bread, it was thought by some that the already iodine replete states of Western Australia and Queensland would consequently be at risk of attaining excessive iodine status, especially amongst young children (132). Both chapters 5 and 6 clearly show there is no evidence to show that the Queensland children recruited for this study were consuming an excessive amount of iodine under the exposure of the iodine fortification program. Furthermore, this thesis shows that fortified bread is only a significant predictor of UIC amongst school aged children aged 8-10 years but not preschool children aged 2-3 years. This finding might be explained by the fact the preschool children ate

an average of 1 serve of bread a day (Chapter 6), whilst school aged children consumed an average of 2 serves of bread a day (Chapter 5). Other than the household use of discretionary iodine salt (chapter 7), no other foods were found to be predictors of UIC. Although a significant influence of UIC, iodine fortification of bread resulted in a change in UIC lower than expected. The question could arise whether bread fortification is effective in eliminating or reducing iodine deficiency in vulnerable groups. Furthermore, as suggested previously, using EAR, RDI and UL may not be a relevant benchmark of dietary iodine for young children, and therefore are the 2-3 year olds included in this thesis actually receiving adequate amounts of iodine? And what additional strategies could be implemented to further improve this outcome? Investigations assessing iodine status of other sub-populations in Australia and New Zealand post iodine fortification have also concluded that although the iodine fortification of bread has had some impact on UIC, that impact has not reached anticipated levels (85, 136, 192).

With current public health initiatives and proposals in place to reduce Australian salt intake by 2025, the impact on UIC as a consequence of reducing salt in bread and discretionary iodised salt use needs to be considered carefully especially amongst vulnerable populations.

The influence of environmental iodine cannot be disregarded.

This thesis demonstrates that the greatest predictor of UIC is iodised salt, whether it be consumed via bread (Chapter 5) or occasionally used in cooking or added at the table (Chapter 7). Considering the cycle of iodine ecology it should be of no surprise that such primary exposure to high concentrations of iodine, salt (especially sea salt) would be such an influential dietary source. This does not explain, however, the spatial difference observed in UIC across the Australian continent. Although chapter 7 reports that Queensland children are exposed to a high use of discretionary salt, it is unconfirmed whether this is significantly different to their southern counterparts. Furthermore, the iodine fortification of salt in bread manufacturing is applicable to the entire nation and therefore exposure through this avenue may also be similar across states.

Other studies report geographical influences, especially amongst populations residing in coastal areas compared to their counterparts living in inland mountainous areas (193). The NHS noted that the iodine status of populations residing in inner regional areas of Australia had a lower UIC than their counterparts residing in coastland, major cities (119). A study from

Ireland, reported that the iodine status of populations residing in coastal areas was significantly related to the species and abundance of seaweed within proximity to that population (194). Authors Smyth and Burns et al. concluded that atmospheric exposure to iodine vapours is responsible for impacting the UIC of exposed populations (194). No such relationship has been determined in Australia, however, understanding the ecological cycle of iodine (155), and taking into account that species and abundance of seaweed is greater in warmer Australian waters compared to cooler Australian waters (195, 196). It is plausible to suggest that the warmer climates of Western Australia and Queensland may expose neighbouring populations to greater environmental iodine. Comparisons of atmospheric iodine and DWIC with UIC samples from wider regions of Australia need to be further examined to determine their level of importance to iodine status.

This thesis attempted and failed to find any association between iodine in the drinking water and corresponding UIC. The limitation regarding this aspect of the thesis is that it was conducted in one region of Queensland with shared water sources (Personal communication, SEQ Water). It should be taken into consideration, however, that the land mass of Australia is by definition a continent, and the land size of Queensland is equivalent to the United Kingdom three times over. Typically, WHO refers to national data when recording iodine status and rarely is regional data discussed within reports. With a relatively small population density (3 persons/km²) and predominately centralised production and distribution of foods, developing independent food legislation for each localised State of Australia is impractical. Nevertheless, it is important to consider the localised impact of national iodine interventions, especially in a country like Australia, where subpopulations may not share the same iodine status as the national average. Further exploration into localised iodine interventions such as the iodine concentration of drinking water needs to be considered in regions where national interventions have over or underachieved. Such explorations have been conducted in Denmark (154) and China (153).

A cross sectional survey is warranted across the various states of Australia to investigate whether this hypothesis has any merit. It is important to note, that the Australian national iodine status often refers to an average of 5 states, two sufficient, and the other three deficient.

9.4 MAIN MESSAGES

Iodine dietary guidelines may need revision as methods of determining iodine intake and excretion have improved since the initial RDI and EAR were calculated.

The EAR (65ug/day) for 2-3 year olds was derived from balance studies conducted on 12 Senegalese malnourished children aged 18-30 months, nutritionally rehabilitated after 30 days and the RDI (90ug/day) was calculated from the EAR using a coefficient variance of 20% (150). Likewise, the EAR (65ug/day) and RDI (120ug/day) for 8-10 year olds was derived from adult male balance studies and adjusted for weight (150). Both recommendations assume firstly, children worldwide exposed to a variety of dietary iodine and environmental exposures metabolise iodine the same way rehabilitated malnourished children do, and secondly, the metabolic processes and iodine requirements of children are the same as adults, just on a smaller scale. The ethical and practical concerns of balance studies in the present day in such small children means that other avenues of defining appropriate levels of dietary iodine should be explored. This thesis concurs that UIC remains the most widespread method of assessing dietary iodine intake and does not suffer from the errors that can occur with dietary records (FFQ, 24 hour recalls, 4 day weighed record) which are known to be subject to under and/or miss reporting (88). Although the most recent Australian National Health Survey (119) collected UICs from school aged children, it only collected a single morning spot sample in each child and thus the intra-variability or circadian metabolism of iodine was not accounted for. Furthermore, no biological samples were collected from the most vulnerable group of children, 2-3 year olds, and as previously discussed, dietary records collected may not accurately reflect usual dietary iodine intake. The small sample size in this thesis of course limits its ability to develop national guidelines, however, it does illustrate a paradigm that could be applied at a population level in order to fill the gaps identified in this thesis. There is an opportunity for national surveys to incorporate the methods used by this thesis, in order to 1) achieve a more accurate reflection of the distribution of iodine status within a population, 2) derive contemporary dietary guidelines for EAR, RDI and UL by assessing the spectrum of UIC levels where thyroid dysfunction exists or not. Finally as this thesis demonstrates the plausibility of collecting UIC from 2-3 year olds, there is a need develop a standardised UIC criteria for assessing iodine status in 2-3 year olds.

Determining dietary iodine intake can be challenging, regardless of dietary assessment method.

A recent publication by Condo and colleagues (48) highlighted some problems in trying to use a FFQ to assess iodine intake. Condo found a correlation between their estimate of iodine intake from their FFQ and 4 day food record of 0.349 (48). Whilst the correlation was statistically significant it means that only 12% of the variation in iodine intake captured by the weighed food record data was due to variation in the FFQ approach. They have, however, acknowledged that correlation was probably not the best way of considering the validity or accuracy of the FFQ, and rightly considered the Bland-Altman approach. The analysis showed that the limits of agreement between iodine intake calculated from the 4-day food record and the FFQ were very large (137.5ug to 105.9ug) (48). The limit of agreement should be considered in the light of the mean intake of iodine in their study being around 150ug/day (48).

Condo (48) also pointed out that to the best of their knowledge only three iodine specific FFQs had been developed and validated, none of which were specific to children. Their iodine FFQ was described as a useful tool to estimate iodine intake in pregnant women in industrialised countries that have similar dietary patterns. Clearly therefore the iodine FFQ devised by Condo et.al. could not be used for children. Indeed, Condo and colleagues concluded that their iodine FFQ could be modified to assess iodine intake in other populations.

The approach of extrapolating iodine intake from the FFQ, as demonstrated by Condo and colleagues, has its further limitations when applied to the current thesis. The current thesis did not collect a simultaneous secondary dietary record (i.e. weighed food diary) with the FFQ because evidence described by Basiotis and colleagues (54) and Bingham (55) showed that a 'true' representation of micronutrient data would require a large number of repeated records. The attempt to validate the FFQ is therefore not possible. Furthermore, Condo and colleagues only found a correlation between estimated iodine intake from the FFQ and 24hr urine estimate (correcting for creatinine) and UIE, but *not* with spot UIC. As the current thesis only collected repeated spot UIC it is expected that extrapolating an estimated iodine intake from the FFQ used in this thesis may result in no association with spot UICs and may in fact, yield a misleading representation of iodine intake due to the aforementioned limitations of quantifying micronutrients using FFQs.

Despite the importance of iodine in the maintenance of healthy thyroid function, there is little Australian documentation reporting the thyroid status of children exposed to various degrees of iodine nutrition.

The fundamental goal for establishing appropriate iodine intake for children is to develop and maintain healthy thyroid function. Consequently, if healthy thyroid function is sustained, normal growth and cognitive development during the early years of childhood is preserved. Despite the critical nature of this endocrinologic pathway, little is known about the thyroid function of children within the community. Additionally, little is known about how, or if, thyroid dysfunction is examined by the primary healthcare providers as part of a health assessment. There are perhaps two major barriers to this; 1) thyroid imaging is expensive and obtaining thyroid analytes from venepuncture is invasive and unpleasant for young children, and 2) no paediatric reference ranges are available to community practitioners and thus the evaluation of thyroid analytes using adult reference ranges may lead to misinterpretation and thus false diagnostic conclusions. This thesis found that family history was the greatest predictor of the analyte TSH, the primary indicator of thyroid function. As UIC is not indicative of individual risk of thyroid dysfunction, thyroid analytes remain to be the most immediate reflection of thyroid function. This thesis implies that if a child has a family history of thyroid dysfunction there may be merit in investigating their thyroid analytes at some point. A recommendation for thyroid screening using thyroid analytes may be premature, as evidence is lacking to support this idea, however, the exploration of alternative avenues for assessing iodine metabolism and therefore, indirectly thyroid function, may be reasonable. Emerging research has indicated that salvia maybe a valuable biomarker of iodine metabolism. The foundations of the theory relies on the biological mechanism that sodium-iodine symporter located at the site of the salivary gland, mimics the same mechanism performed at the site of the thyroid gland (197, 198). The advantage of collecting saliva over UIC and thyroid analytes is that it may provide a more comprehensive insight of how the thyroid gland responds to the exposure of iodine, without the invasive venepuncture. The sample collection process of this method would appear to be simpler than collecting multiple UICs, however, the validity of the technique is yet to be determined.

Unmodified foods may not be a reliable source of dietary iodine.

Despite the limitations of food records to determine an accurate iodine intake, this thesis attempted to uncover the relationship between usual food intake and UIC. No relationship was

determined between any food and corresponding UIC, with the exception of bread. It should be noted, however, that without iodised salt purposefully added to bread manufacturing, it is unlikely this observation would have occurred. Food typically considered to be important sources of dietary iodine for examples, dairy (especially milk), eggs and fish were not found to be of any significance in relation to UIC within this thesis. Reasons for this may include, iodophors in milk sanitation procedures are not as widely used as assumed, the iodine content of eggs and fish are not at the levels as predicted or these foods are not eaten often enough by children in order to attain sufficient dietary iodine. The limitation of small sample sizes representing the two cohorts recruited in this thesis also cannot be ignored, and thus a broader representation of usual food intake for children in both age groups is required to confirm these findings.

This thesis concludes that without the fortification of iodised salt in bread, the frequency of which other 'notable' sources of foods are consumed by children, may not be enough to meet their iodine requirements. Thus iodine fortification has an important role in the maintenance of iodine nutrition and with evidence that iodine fortification of bread is not improving UIC to expected levels in deficient populations, other vehicles of iodine fortification should be explored. As previously suggested, methods to improve the iodine status of isolated communities should be considered in order to avoid the over consumption of dietary iodine in replete regions of Australia.

9.5 RESEARCH LIMITATIONS

As expected with any research, there were a number of limitations pertaining to the investigations performed during this thesis. The research limitations of each manuscript are discussed in the corresponding chapters. The following section of this thesis integrates these limitations and discusses their influence on the overall outcomes of the study.

Recruitment methodology and Sample size

As recommended by WHO a cluster survey recruitment through government and non-government school would have been the model of enrolment preferred for this study. Unfortunately, with support from the Department of Training and Education in Queensland not forthcoming, this process was not attainable. Reasons for refusal were largely due to the fact that number of schools within south east Queensland were still in recovery stages resulting from the 2011 Brisbane flash floods. Therefore, this study was limited to recruitment via

newspaper articles, television media, community posters and social media, of which social media was the most successful. Although some criticise the scope of social media to reach appropriate members of the targeted community, the format allowed filters to specifically advertise to adults residing in Queensland, within the ages of 18-45 and have an interest in community pages that a child orientated (implying they may have children). Of 191 respondents, there were a total of 107 enrolled in the survey with 81 completing the survey as shown in figure 3.4.

Advertising and recruitment continued from November 2011 to March 2013. The influence of seasonal differences on the UIC amongst these children was not explored within this thesis, however, it would not be expected to be of great significance, as south east Queensland rarely experiences vast extremes of temperature.

Compared to sample size calculations, the study disappointingly did not reach targeted numbers. This limits the power of the findings presented and thus their application to the general population. The small sample size recruited from each age group may not have represented a diversified intake of food types/items consumed by children to make comparisons between children who consumed bread that was fortified with iodine, with those who consumed bread that was not fortified with iodine. As none of the children recruited in this thesis consumed organic or homemade bread varieties, such comparisons were not possible. Furthermore, due to the limited dietary variability, it was also considered remiss to further make associations between types of bread (flat bread, bagels, turkish bread etc.) and UIC. As all of the children who consumed bread in the thesis study, consumed bread that was fortified with iodine, focus was redirected to the crude number of bread serves consumed by the participating children. These data are reported in chapters 5 & 6. The convenience method of recruitment yielded an over representation of middle and high SES children (table 3.2), especially those aged 8-10 years. This study determined no associated between SES and UIC in either age group, which as discussed in previous chapters, is consistent with other Australian findings (86, 146). Chapter 5, table 5.1 shows that the study recruited children with similar ages and BMI as those 8-10 year olds recruited by the 2012 Australian National Health survey. There were some differences in height and weight of 2-3 year old children compared to the 2012 Australian Nation Health Survey, however, this is probably influenced by the inclusion of children up to the age of 5 years by the Australian National Health Survey. Nevertheless, biological variances such as hydration, variation in diet etc. which are accounted for in larger

populations, may not have been sufficiently offset by the small sample size of the current study. Chapter 4, however, reports that by collecting repeated measures of UIC, the variation observed within cohort was reduced by up to 40%. Furthermore, the current study does provide insight of how a small cohort of children residing in a previously iodine replete region responded to the implementation of iodine fortification. Most importantly, this thesis contributes data to only one other Australian iodine survey that includes children as young as 2-3 years. Considering the importance of iodine during the first three years of life, this thesis provides a novel examination of iodine nutrition during this vital period of the lifespan.

It is further noted that dietary iodine EAR, RDI and UL are derived from balance studies with only 12 participants, when compared to the current studies sample size, the basis of national guidelines have been formulated from much smaller sample sizes.

Determining urinary iodine

Since the commencement of the current study, Montenegro-Bethancourt and colleagues have reported convincing data suggesting that correcting UIC spot sample for creatinine can greatly improve its agreement with actual 24-hr urinary iodine excretion (45). If applied to the current study's methodology, it could have further improved the representation of UIC data presented in chapters 4,5 and especially chapter 6, as the confounder levels of hydration, would have been better accounted for. At the time of study design, however, the debate of UIC and UIC corrected for creatinine remained inconclusive. Furthermore, the WHO (1) and Institute of Medicine (33) are both of the opinion that the process of correcting for creatinine was unnecessary, the approach was not adopted by the current study. The lack of a validated cut-offs for categorising iodine status according to corrected creatinine UIC may also obscure the interpretation of the data. Hence, the approach recommended by WHO and implemented by previous Australian iodine surveys, were applied by the current study for consistency.

Dietary data collection

The food frequencies questionnaire implemented in this thesis, intended to assess usual consumption of food in relation to iodine status (UIC). At the time of study commencement, no validated food frequency questionnaires were available for the purpose of iodine surveys, so one was adapted from the Healthy Kids Queensland survey (60).

As previously discussed in chapter 2 and other parts of this discussion chapter, capturing ‘true’ micronutrient intake via dietary records can be cumbersome. In other surveys, concurrent weighed dietary records and 24-hr recalls have been used as reference models for FFQs (199).

Although weighed food records in conjunction with the FFQ may have been a useful additional reference for dietary intake in this survey, the request to perform such repeated measures was considered too burdensome when considering the other commitments. As the Australian National Health Survey uses repeated 24 hour recalls as the primary dietary record, this thesis suggests that the inclusion of a repeated 24 hour dietary recall in addition to another repeated UIC a week apart may allow the data sets to be comparable with future national surveys and may improve the validation of any associations between usual food consumed and UIC.

Single site

One of the principal objectives of this thesis was to evaluate the influence of environmental exposure (specifically DWIC) on UIC. It was expected that environmental influences may explain the geographical differences in iodine status observed across Australia. Even though this thesis found no relationship between DWIC and UIC, the findings may be limited by water samples only being collected from one region of Queensland. Further research is warranted to include UIC and corresponding DWIC samples from various regions of Australia known to differ in iodine status.

9.6 RESEARCH STRENGTHS

Contemporary data

This is the first mainland Australian study to collect three measures of iodine nutrition (UIC, dietary records and thyroid analytes) from the same individuals in two paediatric cohorts. Furthermore the data presented by this thesis is unique as it includes biological measurements of iodine nutrition from 2-3 year old Queensland children.

Multiple urine samples

This thesis is one of few Australian surveys that collected repeat UIC samples from the same individuals, and is the first to apply this method in a paediatric survey. It is also the only Australian study to include afternoon UIC samples in its analysis. As discussed throughout this thesis, the collection of multiple UIC samples, and importantly the inclusion of an

afternoon UIC sample, imitates the circadian rhythm of iodine metabolism and improves the representation of iodine status within the distribution. The application of methods performed in this thesis have the potential to improve the monitoring and reporting of iodine nutrition in larger surveys.

This is the first mainland Australian study to determine the relationship between the consumption of iodine fortified bread and UIC for both preschool children, aged 2-3 years, and school aged children, aged 8-10 years. It is also the first mainland Australian study to report the relationship between iodised salt use and UIC for both age groups. The findings presented in this thesis are important when considering the ongoing monitoring and assessment of iodine nutrition. The findings of this thesis may be useful in the development of future public health initiatives which could potentially impact the predictors of iodine nutrition identified by this thesis.

9.7 RECOMMENDATIONS AND FUTURE DIRECTIONS.

This thesis presents important considerations required for the ongoing, consistent monitoring of iodine status within a cohort.

Predominately this research provides evidence for future studies to include multiple UIC samples in the assessment of iodine status within groups to improve accuracy of iodine status representation, and highlights the necessity to develop a UIC criterion for young children. Further research incorporating these suggestions ensures the preservation of optimal iodine nutrition for future generations.

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APPENDICES

Appendix 1

PART A

SECTION 1: Estimating how often foods are eaten

Please read carefully before completing the questions about how often foods were eaten.

For each food item listed, tick (✓) the box the best represents your child's usual consumption of particular foods over the last 12 months.

Think about all eating occasions

When reading through the list of foods, think about your child's usual weekday and weekend eating patterns. We understand that this may change from time to time so please recall what would be a typical consumption of that particular food. Remember to consider all food and drink, including that eaten both at home and elsewhere.

Completing the questionnaire

Using a blue or black pen, place a tick (✓) in the appropriate boxes as instructed below. Use a ruler to work your way down the page and ensure that there is a response for all foods on each page of Section 1.

Following the instructions and examples below please answer all questions to the best of your ability.



Appendix 1

Estimating usual consumption of foods

Please indicate by placing a tick (✓) in the appropriate column to indicate how often your child would usually eat that particular food on a monthly, weekly or daily basis. Please place only one tick in each row when indicating how frequently a food item is consumed.

* PLEASE NOTE: Place only **one** tick in each row when indicating how often a food is consumed. Consider whether the usual consumption of that food is *daily* **OR** *weekly* **OR** *monthly*.



For Example: Foods consumed *daily*

- In the past 12 months, if your child usually ate 2 slices (= to one serve) of thick sliced white bread at least once a day, you would indicate this by ticking the boxes as shown below.
- In the past 12 months, if your child usually ate white bread rolls at least twice a day, you would indicate this by ticking the boxes as shown below.

Usual consumption of BREADS and CEREALS over the last 12 months										
	Daily				Weekly			Monthly or less		
	Once a day	Twice a day	3 times a day	4 or more times a day	Once a week	2-4 times a week	5-6 times a week	Never	Less than once a month	1-3 times a month
Bread slices - <i>thick sliced</i> (white, wholemeal, mixed grain)	✓									
Bread rolls/buns (white, wholemeal, mixed grain)		✓								

Appendix 1

For Example: Foods consumed weekly

- In the past 12 months, if your child usually drank cow's milk as a drink at least 2-4 times a week, you would indicate this by ticking the boxes as shown below.
- In the past 12 months, if your child usually ate ice-cream once a week, you would indicate this by ticking the boxes as shown below.

Usual consumption of DAIRY and DAIRY SUBSTITUTES over the last 12 months										
	Daily				Weekly			Monthly or less		
	Once a day	Twice a day	3 times a day	4 or more times a day	Once a week	2-4 times a week	5-6 times a week	Never	Less than once a month	1-3 times a month
Cow's milk as a drink						✓				
Ice-cream					✓					

For Example: Foods consumed Monthly

- In the past 12 months, if your child usually ate 1 small can of tuna in brine at least 1-3 times a month, you would indicate this by ticking the boxes as shown below.
- In the past 12 months, if your child usually ate 1 small fillet of salmon only occasionally or less than once per month, you would indicate this by ticking the boxes as shown below.

Usual consumption of SEAFOOD over the last 12 months										
	Daily				Weekly			Monthly or less		
	Once a day	Twice a day	3 times a day	4 or more times a day	Once a week	2-4 times a week	5-6 times a week	Never	Less than once a month	1-3 times a month
Tuna in brine – Canned										✓
Salmon - fresh									✓	



Appendix 1

For Example: Foods never consumed

- In the past 12 months, if your child *never* ate raw broccoli, you would indicate this by ticking (✓) the boxes as shown below.

Usual consumption of VEGETABLES and SALADS over the last 12 months										
	Daily				Weekly			Monthly or less		
	Once a day	Twice a day	3 times a day	4 or more times a day	Once a week	2-4 times a week	5-6 times a week	Never	Less than once a month	1-3 times a month
Broccoli – raw								✓		

Determining average food intake of seasonal foods

- In the last 12 months, if your child usually ate oranges at two meals per day on seven days a week, the average consumption of oranges would be recorded as '*twice a day*'.
- In the last 12 months, if your child usually ate peaches for breakfast twice per week for half of the year, the average consumption of peaches would be '*once a week*' for the whole year.

Usual consumption of FRUIT over the last 12 months										
	Daily				Weekly			Monthly or less		
	Once a day	Twice a day	3 times a day	4 or more times a day	Once a week	2-4 times a week	5-6 times a week	Never	Less than once a month	1-3 times a month
Orange		✓								
Peaches – canned or fresh					✓					

Now for the fun part!
Find a comfy chair with a cup of coffee and try your best to answer all the questions!

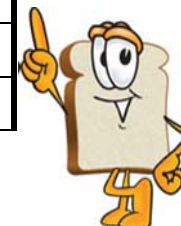


Once you have read and understood these instructions, please complete section one.

Appendix 1

SECTION 1: Estimating how often foods are eaten – the questionnaire!

Usual consumption of BREADS and CEREALS over the last 12 months										
	Daily				Weekly			Monthly or less		
	Once a day	Twice a day	3 times a day	4 or more times a day	Once a week	2-4 times a week	5-6 times a week	Never	Less than once a month	1-3 times a month
Bread slices – <i>thin sliced</i> (white, wholemeal, mixed grain, gluten free)										
Bread slices - <i>thick sliced</i> (white, wholemeal, mixed grain, gluten free)										
Bread rolls/buns (white, wholemeal, mixed grain, gluten free)										
Organic bread										
Flat breads or pita bread										
Naan bread										
Bagels (white, wholemeal, sweet)										
Topped breads, bun and rolls (e.g. cheese and bacon rolls)										
Baked English-style muffins (white, wholemeal, mixed grain and fruit)										
Sweet or iced buns										
Fruit breads – thin sliced										
Fruit breads – thick sliced										
Fruit bread – rolls or hot cross buns										



Appendix 1



Usual consumption of BREADS and CEREALS over the last 12 months .. CONTINUED										
	Daily				Weekly			Monthly or less		
	Once a day	Twice a day	3 times a day	4 or more times a day	Once a week	2-4 times a week	5-6 times a week	Never	Less than once a month	1-3 times a month
Home-made bread, made from bread mixers										
Turkish or Focaccia bread										
Breadcrumbs – home made										
Rice Crackers with nori (seaweed)										
Cooked Rice										
Cooked Pasta										
Rolled oats - cooked										
Rolled oats - uncooked										
Breakfast cereal, single grain e.g. Rice Bubbles, Cornflakes or Weat-Bix										
Breakfast cereal, mixed grain ie. Just Right, muesli										

Usual consumption of SALT over the last 12 months										
	Daily				Weekly			Monthly or less		
	Once a day	Twice a day	3 times a day	4 or more times a day	Once a week	2-4 times a week	5-6 times a week	Never	Less than once a month	1-3 times a month
Iodised salt in cooking										
Non-iodised salt in cooking										
Iodised table salt										
Non-iodised table salt										
Sea Salt										
Iodised sea salt										



Appendix 1

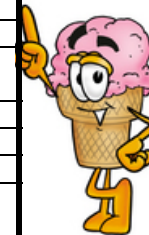
Usual consumption of NON-MILK BEVERAGES and DESSERTS over the last 12 months										
	Daily				Weekly			Monthly or less		
	Once a day	Twice a day	3 times a day	4 or more times a day	Once a week	2-4 times a week	5-6 times a week	Never	Less than once a month	1-3 times a month
Unfiltered tap water										
Filtered tap water										
Bottled water										
Sparkling Mineral Water flavoured/ unflavoured										
Orange juice										
Cordial										
Regular or diet Soft drink										
Tea or coffee										
Home-made Jelly										



Usual consumption of EGGS and EGG SUBSTITUTES over the last 12 months										
	Daily				Weekly			Monthly or less		
	Once a day	Twice a day	3 times a day	4 or more times a day	Once a week	2-4 times a week	5-6 times a week	Never	Less than once a month	1-3 times a month
Egg, whole										
Egg whites only										
Egg yolk only										
Egg substitute										

Appendix 1

Usual consumption of DAIRY and DAIRY SUBSTITUTES over the last 12 months										
	Daily				Weekly			Monthly or less		
	Once a day	Twice a day	3 times a day	4 or more times a day	Once a week	2-4 times a week	5-6 times a week	Never	Less than once a month	1-3 times a month
Flavoured cow's milk (e.g. milkshake, iced coffee, hot chocolate)										
Cow's milk as a drink										
Cow's milk on cereal										
Cream or Sour cream										
Ice-cream										
Yogurt including plain, fruit, flavoured										
Yogurt, frozen,										
Yogurt , fromage frias										
Cottage cheese										
Ricotta cheese										
Cheddar and all other hard cheeses										
Butter										
Custard, commercial										
Custard, homemade										
Soy milk or soy milk based products										



Appendix 1

Preferred milk and yogurt brands (please tick all that apply)

Pauls™	
Dairy Famers™	
Woolworths™ Home brand	
Coles™ Home brand	
Parmalat Pure Organic™	
Anlene™	
Breaka™	
Farmers Union™	
Ice Break™	
A2 Milk™	
Devondale™	



Vitasoy™	
Anchor Milk™	
Sanatarium Soy Good™	
Liddells™ Lactose Free	
Diploma Milk™ powder	
Sunshine Milk™ powder	
Coles™ home brand Milk Powder	
Woolworths™ Milk Powder	
Nestle™ milk products	
Other: (please specify)	

Jalna™	
Bulla™	
Yoplait™	
Woolworths™ home brand	
Coles™ home brand	
Easiyo™	
Nestle™	
Pauls™	
Vaalia™	
Dairy Famers™	
Connoisseur™	
Ski Yoghurt™	
Other: (please specify)	

Appendix 1

Usual consumption of SEAFOOD over the last 12 months										
	Daily				Weekly			Monthly or less		
	Once a day	Twice a day	3 times a day	4 or more times a day	Once a week	2-4 times a week	5-6 times a week	Never	Less than once a month	1-3 times a month
Tuna in brine – Canned										
Tuna in flavour/spices – Canned										
Tuna - fresh										
Salmon in brine – Canned										
Salmon in flavour/spices – Canned										
Salmon - fresh										
<i>Fresh</i> Water fish- e.g. Basa, Murray Cod, Catfish, Blue dolphin chichild										
<i>Salt</i> Water fish- e.g. Barramundi, Ocean trout, Brim, Flathead										
Sardines/Herring/Mackerel										
Shell fish and Crustaceans - Prawns, Oysters, Lobster/Crayfish										
Sushi with nori										
Sushi without nori										
Nori or other seaweed-based products										
Fish Fingers (commercial bought i.e. Birds eye)										
Fish Cakes/Patties (commercial bought i.e. Birds eye)										



Appendix 1



Usual consumption of FRUIT over the last 12 months										
	Daily				Weekly			Monthly or less		
	Once a day	Twice a day	3 times a day	4 or more times a day	Once a week	2-4 times a week	5-6 times a week	Never	Less than once a month	1-3 times a month
Preserved/glazed cherries										
Rhubarb										
Coconut, desiccated										
Orange										
Peaches – canned or fresh										
Watermelon										

Usual consumption of VEGETABLES and SALADS over the last 12 months										
	Daily				Weekly			Monthly or less		
	Once a day	Twice a day	3 times a day	4 or more times a day	Once a week	2-4 times a week	5-6 times a week	Never	Less than once a month	1-3 times a month
Broccoli – <i>raw</i>										
Broccoli – <i>cooked</i>										
Cauliflower - <i>raw</i>										
Cauliflower - <i>cooked</i>										
Brussels sprouts										
Cabbage, coleslaw - <i>raw</i>										
Cabbage- <i>cooked</i>										
Potatoes, or sweet potatoes										
Onion or leeks – cooked or used in cooking										
Carrots										
Mixed Nuts										
Brazil nuts										
Beetroot, canned										
Cucumber, raw										
Lettuce, rocket, baby spinach, other raw salad greens										
Parsley										
Spinach, cooked										



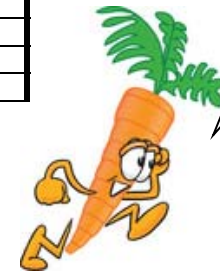
Appendix 1

Usual consumption of MEATS and MEAT SUBSTITUTES over the last 12 months										
	Daily				Weekly			Monthly or less		
	Once a day	Twice a day	3 times a day	4 or more times a day	Once a week	2-4 times a week	5-6 times a week	Never	Less than once a month	1-3 times a month
Beef – cooked e.g. steak, braised, mince/ground, sausages										
Lamb – cooked e.g. chops, mince/ground										
Pork – cooked e.g. sausage, chops, gammon steaks										
Ham										
Bacon										
Game meat (e.g. kangaroo, rabbit, emu)										
Schnitzel – bread crumbs over meat i.e. chicken, veal										
Offal (e.g. kidney, liver)										
Salami, luncheon meats (e.g. devon, pressed chicken)										
Soy-based meat substitute (e.g. TVP, soy burger, tofu)										
Baked Beans in tomato sauce										

Thank you for completing this section of the questionnaire. Please now proceed to section 2.

Appendix 1

Usual consumption of CONDIMENTS over the last 12 months										
	Daily				Weekly			Monthly or less		
	Once a day	Twice a day	3 times a day	4 or more times a day	Once a week	2-4 times a week	5-6 times a week	Never	Less than once a month	1-3 times a month
CONDIMENTS										
Tomato sauce										
BBQ sauce										
Fish Sauce in cooking										
Soy Sauce in cooking										



Nearly Finished!
Just a couple more questions to go!!!!

Appendix 1

SECTION 2: Estimating the amount of foods eaten

To complete this section, tick (✓) only one option for each question, unless otherwise specified.

Questions about amounts and types of foods over the last 12 months

1. How many serves of bread did your child usually eat each day?

(1 serve = 2 slices of bread or 1 medium bread roll or 1 medium flat bread)

- Doesn't eat bread
- Less than one serve/day
- 1 serve/day
- 2 serves/day
- 3 serves/day
- 4 serves/day
- More than 4 serves/day

2. How many serves of broccoli, cabbage, brussel sprouts or cauliflower did your child usually eat each day?

(1 serve = ½ cup of cooked vegetables or 1 cup of raw vegetables)

- Doesn't eat any of the vegetables listed above
- Less than one serve/day
- 1 serve/day
- 2 serves/day
- 3 serves/day
- 4 serves/day
- More than 4 serves/day

You only need to tick
one box for each
question....



Appendix 1

3. Including milk poured on cereal, how many serves of milk did your child usually consume each day?
(1 serve = 250 ml glass)

Doesn't consume any milk
Less than one serve/day
1 serve/day
2 serves/day
3 serves/day
4 serves/day
More than 4 serves/day

4. How many serves of eggs did your child usually eat each day?
(1 serve = 2 whole eggs)

Doesn't eat any eggs
Less than one serve/day
1 serve/day
2 serves/day
3 serves/day
4 serves/day
More than 4 serves/day

5. How many serves of yoghurt did your child usually eat each day?
(1 serve = 200g yoghurt)

Doesn't eat any yoghurt
Less than one serve/day
1 serve/day
2 serves/day
3 serves/day
4 serves/day
More than 4 serves/day



Appendix 1

6. How many serves of sushi with nori (seaweed) containing foods did your child usually eat each day?

(1 serve = 1 hand roll or 125g)

- Doesn't eat any sushi with nori
- Less than one serve/day
- 1 serve/day
- 2 serves/day
- 3 serves/day
- 4 serves/day
- More than 4 serves/day

7. How many serves of nori (seaweed) foods did your child usually eat each day?

(1 serve = 1 nori sheet)

- Doesn't eat any nori foods
- Less than one serve/day
- 1 serve/day
- 2 serves/day
- 3 serves/day
- 4 serves/day
- More than 4 serves/day

8. How many serves of salt water fish or seafood did your child usually eat each day?

(1 serve = 65-100g of cooked fish or 120g of shell fish meat)

- Doesn't eat fish or seafood
- Less than one serve/day
- 1 serve/day
- 2 serves/day
- 3 serves/day
- 4 serves/day
- More than 4 serves/day



Appendix 1

9. On average, how much water did your child usually drink each day?

(1 glass = 250ml)

- Doesn't drink water
- Less than one serve/day
- 1 glass/day
- 2 glass/day
- 3 glass/day
- 4 glass/day
- 5glass/day
- 6 glass/day
- 7 glass/day
- More than 7 glasses/day

10. From what source of water did your child normally drink?

- Doesn't drink water
- Tap Water
- Tank water
- Bore water
- Bottled water
- Filtered Tap Water
- Other (Please specify): _____

11. Which meals did your child add salt to their food?

(Tick all that apply)

- Never add salt to meals
- Every meal
- Breakfast meals
- Midday meals



Appendix 1

- Evening meals
- Only occasionally

12. To which meals was salt used in home cooked meals?
(Tick all that apply)

- Never use salt
- Every meal
- Breakfast meals
- Midday meals
- Evening meals
- Only occasionally

13. How many serves of vegetables did your child eat each day? (½ cup = 1 serve)

- Doesn't eat vegetables
- Less than one serve/day
- 1 serve/day
- 2 serves/day
- 3 serves/day
- 4 serves/day
- More than 4 serves/day

14. How many serves of fruit did your child eat each day? (1 piece of fruit = 1 serve)

- Doesn't eat fruit
- Less than one serve/day
- 1 serve/day
- 2 serves/day
- 3 serves/day



Appendix 1

- 4 serves/day
More than 4 serves/day

15. Did your child take a vitamin or other nutrient supplement (eg. Toddler milk, iron liquid, fish oil etc) over the past 12 months?

- Did not take a vitamin or nutrient supplement
Yes, 1 dose/day
Yes, 2 doses/day
Yes, 3 doses/day
Yes, more than 4 doses/day

If known, how much iodine did the supplement contain?

_____ or unknown (if unknown please state the brand of supplement used) _____



16. Did your child take a vitamin or other nutrient supplement (eg. Toddler milk, iron, fish oil etc) on the days which urine samples were collected?

- No
Yes

Appendix 1

SECTION 3: Thyroid health

To complete this section, tick (✓) only one option for each question, unless otherwise specified.

1. Prior to this questionnaire, did you know about thyroid function?

- No
Yes

2. Does your child have any known thyroid disease?

- No thyroid disease known
Yes, underactive thyroid
Yes, overactive thyroid
Yes, autoimmune Hashimotoes disease
Yes, Graves disease
If yes, do they take any medication? (please indicate type and dosage)

3. To your knowledge has your child ever been tested for thyroid disease?

- No
Yes
Unknown



Appendix 1

4. Is there any thyroid disease in your child's family history?

- No reported family history
- Yes, Mothers family history
- Yes, Fathers family history
- Unknown

SECTION 4: Demographics

To complete this section, tick (✓) only one option for each question, unless otherwise specified.

1. What is your child's place of birth?

- Australia
- Other (please specify)

2. How long has your child lived in your current residing area (postcode)?

- 1 year
- 2 years
- Since birth
- Other (please specify) _____

Appendix 1

3. What language is spoken at home?

English

Other (please specify)



4. Is your child from Aboriginal or Torres Strait Islander decent?

Yes

No

5. At the home where your child permanently lives, is there any member of the family who smokes?

Yes

No

Please place the questionnaire in the envelope provided.

WELL DONE!

Thank you very much for answering all these questions.

This information is for research purposes only and will be kept confidential.



*When you have
completed the rest of the
project... Call or email
our research team so
your contribution can be
collected!*

THANK YOU!



Appendix 2

The following are the procedures for recording anthropometric measurements.

Height

Equipment

- A seca 220 Stadiometer.
- An even, firm floor surface
- Stadiometer must be calibrated upon arrival at project site. A one meter calibrated rod must be used.

Procedure

- Engage the measuring slide in a horizontal position for measuring
- Move the measuring slide upwards according to the height of the person being measured
- When measuring from 130.5cm to 200cm read off the mark. When measuring height below 130.5 cm, release the lock and read off the mark.
- Ask participants to undo or adjust hairstyles which could interfere with measurement.
- Participant is required to stand erect under the measuring slide, in bare feet with buttocks and shoulders pressed against the stadiometer.
- Heels need to be together with arms hanging freely by the slide (palms facing thighs).
- The participants head must be positioned in the Frankfort Plane. (The Frankfort Plane is the imaginary line from the hole in the ear to the bottom of the 'orbit', i.e. bone, of the eye)
- Ensure the Frankfort Plane is parallel to the horizontal headpiece and perpendicular to the vertical back piece of the stadiometer. This is best viewed and aligned when the examiner is directly to the side and at eye level with the child.
- Ask the participant to look straight ahead and take a deep breath before taking measurement.
- Lower the head board/platform lightly to the top of the participants head until it makes firm contact with the skull (and not top of hair)

Appendix 2

Recording

- Two measurement, to the nearest 0.1cm (1.0 millimetre)
- Take a third measurement if previous two measurements differ by 0.5cm
- Include the mean of the two closest measurements in the analysis.
- Measurements that fall between two millimetres will be recorded to the nearest even millimetre.

Weight

Equipment

- Tanita (model HD316) digital bathroom scales
- Place scales on an even firm surface
- Calibrate scales using standard weights before leaving the project base each time.
Calibrate at school site before use also

Procedure

- Participants are to bare foot, waring light clothing. Participants will be asked to remove all heavy jewellery and empty pockets prior to being weighed.
- Set the scales to zero
- Ask the participant to stand evenly on the scale with feet together, arms hanging loosely at their side and head facing forward. Participants will be asked to hold that position until instructed to stand off the scales.



THE UNIVERSITY OF QUEENSLAND
Institutional Approval Form For Experiments On Humans
Including Behavioural Research

Chief Investigator: Ms Anna Walsh

Project Title: Iodine Status Of Queensland Children And Associations With Thyroid Function

Supervisor: Prof Peter SW Davies

Co-Investigator(s): Prof Peter SW Davies

Department(s): School of Medicine, Discipline of Paediatrics and Child Health

Project Number: 2011000125

Granting Agency/Degree: Childrens Nutrition Research Centre

Duration: 31st March 2015

Comments:

**Name of responsible Committee:-
Medical Research Ethics Committee**

This project complies with the provisions contained in the *National Statement on Ethical Conduct in Human Research* and complies with the regulations governing experimentation on humans.

**Name of Ethics Committee representative:-
Professor Bill Vicenzino
Chairperson
Medical Research Ethics Committee**

Date: 10 March 2011

Signature: 

CHILDREN'S INFORMATION SHEET

Project Title: "Iodine Status of Queensland Children and Associations with Thyroid Function"

Chief Investigator: Anna Walsh
Children's Nutrition Research Centre
Ph: 3365 5325

Associate Investigators: Professor Peter SW Davies – Director
Children's Nutrition Research Centre

Introduction and Background of Project

The thyroid gland is a butterfly shaped organ located in your neck that helps your brain function and your body grow. Your thyroid gland needs iodine to work properly and you eat iodine in your food.

This project will help us find out:

- How much iodine do you eat
- What foods do you eat contain iodine
- How your thyroid works
- How much iodine does your body use

Description of Project - methods and demands

If you would like to be involved, this project asks one of your parents to help you complete the following activities:

1. A short questionnaire on what foods you normally eat
2. Give 2 urine samples on 2 days
3. Give one blood sample (optional)
4. Allow someone to measure you height and weight

Withdrawing from the study

You do not have to take part in this project. Also, you can stop taking part, if you want to, at any time. If you choose to no longer take part in this project, your relationship with the Childrens Nutrition Research Centre or The University of Queensland will not be affected. If you decide to stop taking part in this project, you will not be stopped from taking part in any other projects you would like to do in the future.

Risks of participating in this project

If you agree to give a blood sample, the blood sample will be collected by a professional who is trained to do this job safely. You may feel worried about giving a blood sample and ask you parent (or guardian) to be with you. It is unlikely that harm will happen when you give a blood sample, but problems that may

happen include; bruising, fainting, bleeding and infection. You and your parent (or guardian) will be given an information sheet before you give blood, so you know how to avoid these problems.

Confidentiality

No one else will be told about the information you or your parent give us. Your name will not be recorded in the results, reports or publications.

No names will be used. If you would like information about this project when it is finished, you may ask about it when the whole project is complete.

Questions

The people organising this project must follow rules and guidelines to protect your interest, comfort and safety. If you think someone organising this project has broken any of these rules, you may call any of the project staff (contactable on (07) 3365 1981). If you would like to speak to an officer of the University not involved in the study, you may contact the Ethics Officer on 3365 3924.

PARENT/GUARDIAN INFORMATION SHEET

Project Title: “Iodine Status of Queensland Children and Associations with Thyroid Function”

Chief Investigator: Anna Walsh
Children’s Nutrition Research Centre
Ph: 3365 5325

Associate Investigators: Professor Peter SW Davies – Director
Children’s Nutrition Research Centre

Introduction

Iodine is an essential micronutrient required by the body to maintain healthy thyroid function. The thyroid gland is a component of the endocrine system which is responsible for producing hormones in the body to maintain normal growth and development. Appropriate dietary iodine intake has an important role, especially in children as it can influence brain development, metabolic rate and physical growth. Iodine intake can vary between populations and it is the purpose of this research to determine why such differences occur. The information collected by this research project will be used to develop recommendations regarding what iodine-rich foods are appropriate for children to consume to achieve adequate iodine intake and healthy thyroid function.

Background to Research Project

The aim of this research is to determine what foods children eat, and whether these foods affect thyroid function. It is well known that different foods contain different amounts of iodine. For this reason, it is important to identify which foods contribute or and which foods do not contribute to the iodine intake of children. We would like to know what your child usually eats and drinks on a daily basis to find out whether their eating behaviours are linked with how their thyroid works. To measure how much dietary iodine is being absorbed, urine samples and thyroid blood hormone levels will be assessed providing valuable information regarding how iodine is being used by the body.

Description of Research Project - methods and demands

There are two parts to this research project that require you and your child’s participation.

PART A

1. Food frequency questionnaire: You will be ask to tick boxes that represent how often your child usually eats a certain food i.e. daily, weekly, monthly. In addition to this you will be asked to complete a 21 question, multiple answers survey, regarding how much of a certain food your child may usually eat. You will also be asked to recall any known thyroid function history in the family.
2. Urine samples: Your child will be asked to provide a morning urine sample and an early afternoon sample on two consecutive days. You will be provided with specimen jars required for this part of the research project.



-
3. **Water Samples:** You will be asked to collect one water sample each day over the same two days urine samples are being collected.
 4. **Height and Weight:** Your child's weight and height will be measured upon collection of specimens by a member of the research team.

PART B (optional; you may consent to the participation of Part A (Food frequency questionnaire and urine samples) but chose not to consent to Part B (blood samples) if you or your child are not comfortable with the requirements of Part B)

1. **Blood samples:** Your child will be asked to provide one venipuncture blood sample. The blood will be retrieved into one tube and will collect no more than 8.5 mls of blood. The blood sample will be collected by an experienced phlebotomist at your local QML collection centre. A copy of the completed blood report will be forwarded to your nominated GP, if requested.

The survey will also record your child's height and weight.

Environmental Screening

(optional; you may consent to the participation of Part A (Food frequency questionnaire and urine samples) and consent to Part B (blood samples) but not chose to have the samples environmentally screened)

Children may experience different exposures to environmental pollutants. After the your child's biological samples have been analysed for iodine content and thyroid hormones, with your permission, unused leftover samples can be stored for future environmental screening. Please indicate whether you would like this to occur by circling YES or NO on the consent form.

Collection of Samples

You will be provided with clear instructions and equipment which will guide you through the appropriate procedures for the urine sampling. When you have completed the questionnaire, the urine sampling and water sampling component of the study, you will be asked to contact the principal researcher on email: kidsnutrition@uq.edu.au or a.walsh5@uq.edu.au or phone: 07 3636 9294. A member of the research team will arrange to pick up the samples from your nominated address or an approved nominated 'drop off' centre. In the instance where the research team member is collecting the samples, the research team member will measure the weight and height of the participating child.

Withdrawing from the Study

Your decision whether to participate or not to participate will not prejudice your child's future relations with the Childrens Nutrition Research Centre or the University of Queensland. If you decide to participate, you are free to withdraw your consent and to discontinue participation at any time. The decision to withdraw from the study will not affect your child's relationship with the Childrens Nutrition Research Centre of The University of Queensland and their participation in any current of future studies.

Confidentiality

All results of the study will be treated with absolute confidentiality. Participants will only be identified by number in the resultant manuscript, reports or publications. No names will be used. Information regarding the study will be available to you on completion of the data analysis period on request.

Risks of participating in this project

Blood collection will be performed by an experienced phlebotomist who is familiar with safety procedures required for obtaining blood sample. Some children, however, may feel anxious about providing a blood sample and may require reassurance from a parent or guardian. Complications during this procedure are unlikely to occur, however, risks associated with blood collection include; bruising, fainting, bleeding and infection. You will be given QML patient information sheet prior to the blood collection explaining how to minimise these risks.

Results of Blood and Urine

You will be asked to nominate a GP/Doctor on the consent form attached to this information sheet. This will enable us to forward any abnormal results to your nominated GP/Doctor, if such circumstances occur. We will notify you via letter that you should seek advice from GP/Doctor if any abnormal blood results are found.

Enquires

The investigators conducting this study abide by the principles governing the ethical conduct of research and all time avow to protect the interest, comfort and safety of all subjects. This study has been cleared by one of the human ethics committees of the University of Queensland in accordance with the National Health and Medical Research Council's guidelines. You are of course, free to discuss your participation in this study with project staff (contactable on (07) 3365 1981). If you would like to speak to an officer of the University not involved in the study, you may contact the Ethics Officer on 3365 3924

CONSENT FORM / PARTICIPANT CONSENT FORM

Project Title: "Iodine Status of Queensland Children and Associations with Thyroid Function"

Chief Investigator: Anna Walsh
Children's Nutrition Research Centre
Ph: 3365 5325

Associate Investigators: Professor Peter SW Davies – Director
Children's Nutrition Research Centre

This form and the accompanying Subject Information Package have been given to you for your own protection. They contain an outline of the experimental procedures and possible risks.

Your signature below will indicate that:

- 1) You have received the Subject Information package and that you have read its contents;
- 2) You clearly understand the procedures and possible risks involved; and that you have been given the opportunity to discuss the contents of the Subject Information Package with one of the investigators prior to the commencement of the experiment;
- 3) all results will be kept confidential, disclosed only to yourself and the chief investigators; if the results of the study are made public, all your personal details will be disguised in numerical code;
- 4) Your participation is voluntary and therefore may be terminated at any moment by you, without comment and without jeopardising your involvement with The University of Queensland and/or the Department of Education, Training and the Arts;
- 5) This study has been cleared by one of the human ethics committees of the University of Queensland in accordance with the National Health and Medical Research Council's guidelines. You are of course, free to discuss your participation in this study with project staff (contactable on (07) 3365 1981). If you would like to speak to an officer of the University not involved in the study, you may contact the Ethics Officer on 3365 3924

OFFICE USE ONLY

(Affix label here)

Identification Number:



PERSONAL DETAILS:

Name of parent/guardian: _____

Please fill out the following details applicable to the participating child. NOTE: All information will be considered confidential.

Name of participating child: _____

Date of Birth: _____

Country of Birth: _____

Gender: Female or Male

Physical address:

Postal address:

Contact number of parent/guardian: _____

Name of Participating Child: _____

CONSENT:

PART A:

1. Food frequency questionnaire: You will be ask to tick boxes that represent how often your child usually eats a certain food i.e. daily, weekly, monthly. In addition to this you will be asked to complete a 15 question, multiple answers survey, regarding how much of a certain food your child may usually eat. You will also be asked to recall any known thyroid function history in the family.
2. Urine samples: Your child will be asked to provide a morning urine sample and an early afternoon sample on two consecutive days. You will be provided with specimen jars required for this part of the research project.
3. Water Samples: You will be asked to collect one water sample each day over the same two days urine samples are being collected.
4. Height and Weight: Your child's weight and height will be measured upon collection of specimens by a member of the research team.

I consent for my child and I, under the conditions stated above, to participate in the components on PART A of this research project. I understand that we can withdraw at any time without repercussion.

Child's/patient's name (please print): _____

Name of parent or guardian: _____ Phone: _____

Address: _____

Signature of parent or guardian: _____ Date: _____

Signature of Researcher: _____ Date: _____

Do you consent for your child's sample to be stored and used for future analysis of environmental pollutants?

YES

NO

Name of Participating Child: _____

PART B:

1. Blood samples: Your child will be asked to provide one venipuncture blood sample. The blood will be retrieved into one tube and will collect no more than 8.5mls of blood. The blood sample will be collected by an experienced phlebotomist at your local QML collection centre. A copy of the completed blood report will be forwarded to your nominated GP, if requested.

I consent for my child and I, under the conditions stated above, to participate in the components on PART B of this research project. I understand that we can withdraw at any time without repercussion.

Child's/patient's name (please print): _____

Name of parent or guardian: _____ Phone: _____

Address: _____

Signature of parent or guardian: _____ Date: _____

Signature of Researcher: _____ Date: _____

For PART B, please nominate your preferred GP, if required:

Name of Dr: _____

Name of Practice: _____

Practice Address:

Contact Number:

Do you consent for your Childs' sample to be stored and used for future analysis of environmental pollutants?

YES

NO

Name of Participating Child: _____