Iodine nutrition in mothers and their infants during breastfeeding and complementary feeding

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THIS THESIS IS DEDICATED TO MY DEAREST HUSBAND DR EDGARD NGOUNDA FOR HIS UNDYING LOVE AND SUPPORT AND FOR ALWAYS BRINGING OUT THE BEST IN ME.

Do not be anxious about anything, but in everything, by prayer and petition, with thanksgiving, present your requests to God, and the peace of God, which transcends all understanding, will guard your hearts and your minds in Christ Jesus. ***Philippians 4:6-7.***

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ABSTRACT

Background

Iodine is important for normal growth and psychomotor development. Infants and lactating women are susceptible to iodine deficiency. The iodine requirements per kg of body weight of babies are higher than any other age group, while lactating women lose iodine through breast milk. Infants younger than six months of age receive iodine from breast milk or fortified infant formula, however, the introduction of complementary feeding poses a risk for deteriorating iodine status at a later age. Iodine fortification of complementary foods is therefore recommended, whilst continued breastfeeding is encouraged to ensure sufficient intake in these infants. This is the first research to investigate iodine nutrition in South African infants and lactating women.

Methods

In a cross-sectional study, urinary iodine concentrations (UIC), thyroid function and breast milk iodine concentrations (BMIC) of 100 lactating women and their two to four-month-old breastfed infants from a South African township were assessed. Potential predictors of UIC, thyroid function and BMIC, including household salt iodine concentrations (SIC) and maternal sodium excretion were further explored.

In a randomized controlled trial, baseline characteristics of infants aged six months were assessed to determine associations of iodine status with feeding practices and psychomotor milestone development. Iodine concentrations were measured in infant (n=386) and maternal (n=371) urine, as well as breast milk (n=257) and household salt (n=143). Feeding practices and psychomotor milestone development were assessed in all infants. Within the same trial, 750 infants aged six months were randomly selected to receive the following: a daily fortified small-quantity lipid-based nutrient supplement (SQ-LNS) with essential fatty acids providing 45 µg of iodine (SQ-LNS A); a daily fortified SQ-LNS with docosahexaenoic acid, arachidonic acid providing 45 µg of iodine (SQ-LNS B); or a control group receiving no SQ-LNS. Urinary iodine concentrations (UIC) were measured at baseline (n=386) and at 12 months (n=262).

Results

In breastfed infants aged two to four months and their mothers, the median (25^{th}-75^{th} percentile) UIC was 373 (202-627) µg/L and 118 (67-179) µg/L, respectively. Half (53%) of the infants had a UIC >300 µg/L. Median household SIC was 44 (27-63) ppm. Household SIC and maternal urinary sodium excretion predicted UIC of lactating mothers. Median BMIC was 179 (126-269)

µg/L; the age of infants, SIC and maternal UIC predicted BMIC. In turn, infant age and BMIC predicted UIC of infants. Infant thyroid stimulating hormone (TSH), total thyroxine (TT4) and thyroglobulin (Tg) concentrations were 1.3 (0.8-1.9) mU/L, 128±33 mmol/L and 77.1 (56.3-105.7) µg/L, respectively, and did not correlate with infant UIC or BMIC.

In infants aged six months, baseline median UIC was 345 (213-596) µg/L, and was significantly lower in stunted (302 [195-504] µg/L) than non-stunted infants (366 [225-641] µg/L). Only 6.7% of infants had a UIC <100 µg/L. Infant UIC correlated with maternal UIC (128 [81-216] µg/L) (r_s=0.218, p<0.001) and BMIC (170 [110-270] µg/kg) (r_s=0.447, p<0.0001). Most infants (72%) were still breastfed and tended to have higher UIC than non-breastfed infants (p=0.074). Almost all infants (95%) consumed semi-solid/solid foods, with commercial infant cereals (60%) and jarred infant foods (20%) being the most common foods that were first introduced. Infants who reported to frequently consume commercial infant cereals had significantly higher UIC (372 [225-637] µg/L) than those reported to seldom/never (308 [200-517] µg/L) (p=0.023) consume commercial infant cereals. There were no associations between infant UIC and psychomotor developmental scores.

Results from the intervention study showed that the geometric mean (95% CI) UIC at baseline was 333.8 (310.5, 358.9) µg/L and decreased significantly to 214.9 (189.2, 242.6) µg/L at 12 months. Non-breastfed infants had significantly lower UIC (159.6 [65.9, 397.5]) µg/L and higher odds (OR=4.9 [2.5, 9.3]) for being iodine deficient (UIC <100 µg/L; [38%]) than infants who continued to be breastfed (373.2 [202.6, 522.9] µg/L) at 12 months. Infants receiving SQ-LNS (combined group) had higher UIC (*P*=0.025) and lower odds for having a UIC <100 µg/L (OR=0.289 [0.11, 0.75]) at 12 months than infants in the control group, adjusting for maternal baseline UIC, age, sex and continued breastfeeding. In sub-group analysis, the effect of SQ-LNS for higher UIC at 12 months was only apparent in the infants who no longer received breast milk at 12 months (*P*=0.039). This effect was insignificant after adjusting for infant baseline UIC, which resulted in a smaller sample size (n=124).

Conclusion

The results of this research suggest adequate iodine intakes in lactating mothers and infants residing in peri-urban areas in South Africa. However, better monitoring of salt iodine content of the mandatory salt iodization programme in the country is required. Iodine in breast milk contributed to the adequate iodine status in infants. Commercial infant cereals potentially contributed to the sufficient iodine intakes in infants at ages six and 12 months, however, the iodine content in frequently consumed commercial infant cereals in South Africa needs to be investigated. The provision of 45 µg of iodine per day as SQ-LNS can improve UIC in non-breastfed weaning infants, but the dose is not efficacious in counteracting an overall decline in

iodine status. Therefore, it may be necessary to increase the iodine content in home fortification products such as SQ-LNS to the recommended iodine fortification level of 90 µg.

Keywords: Infants, lactating women, breast milk iodine concentration, urinary iodine concentration, salt iodine concentration, thyroid hormones, complementary feeding practices, psychomotor milestone development, commercial infant cereals, small-quantity lipid-based nutrient supplements.

OPSOMMING

Agtergrond

Jodium is belangrik vir normale groei en neuro-motoriese ontwikkeling. Babas en lakterende vroue is vatbaar vir ’n jodiumtekort. Babas se vereistes per kilogram liggaamsgewig is hoër as enige ander ouderdomsgroep, terwyl lakterende vroue jodium verloor deur borsmelk. Babas jonger as ses maande ontvang jodium deur borsmelk of gefortifiseerde poeiermelk, waarna die bekendstelling van aanvullende voeding 'n risiko vir die afname van jodiumstatus op ’n later ouderdom inhou. Aanvullende voeding wat met jodium verryk is, word dus aanbeveel, tesame met volgehoue borsvoeding om voldoende inname van jodium in hierdie babas te verseker. Hierdie is die eerste navorsing wat die jodiumstatus van Suid-Afrikaanse babas en lakterende vroue ondersoek.

Metodes

Tydens ’n dwarsdeursnit-studie is die urinêre jodiumkonsentrasie (UJK), skildklierfunksie en die borsmelk jodiumkonsentrasie (BMJK) van 100 lakterende vroue en hul twee tot vier- maande-oue babas, afkomstig van ’n Suid-Afrikaanse informele behuisingsgebied, getoets. Moontlike oorsake van UJK, skildklierfunksie en BMJK, insluitende huishoudelike sout se jodiumkonsentrasie (SJK) en moederlike natriumekskresie, is ook ondersoek.

Tydens ’n ewekansige gekontroleerde studie is babas op ses maande ondersoek vir assosiasies tussen jodiumstatus, voedingpraktyke en psigomotoriese mylpaal- ontwikkeling. Jodiumkonsentrasies is gemeet in die urine van beide die baba (n=386) en die moeder (n=371), asook in die borsmelk (n=257) en in huishoudelike sout (n=143). Die voedingspraktyke en psigomotoriese ontwikkeling is in al die babas geassesseer. In dieselfde ondersoek is 750 babas, ses maande oud, ewekansig geselekteer om een van die volgende aanvullende voedings daagliks te ontvang: ’n klein hoeveelheid lipied-gebaseerde nutriëntaanvulling (SQ-LNS) gefortifiseer met essensiële vetsure, wat 45 µg jodium bevat (SQ-LNS A); ’n SQ-LNS gefortifiseer met dokosaheksaenoësuur, aragidonsuur wat 45 µg jodium bevat (SQ-LNS B); of ’n kontrole groep wat geen SQ-LNS bevat nie. Die basislyn (ses maande) UJK is gemeet (n=386) en weer herhaal op 12 maande (n=262).

Resultate

In borsgevoede babas van twee tot vier maande, asook hul moeders, was die mediaan (25^{ste}-75^{ste} persentiel) UJK onderskeidelik 373 (202-627) µg/L en 118 (67-179) µg/L. Die helfte (53%) van die babas se UJK was >300 µg/L. Die mediaan huishoudelike SJK was 44 (27-63) dpm.

HuishoudelikeSJK en moederlike urinêre sout-ekskresie het die UJK van lakterende moeders voorspel. Die BMJK se mediaan was 179 (126-269) µg/L. Die BMJK is voorspel deur die ouderdom van die babas, SJK en die moeder se UJK. Verder was die baba se ouderdom en BMJK was 'n aanduiding van die baba se UJK. Babas se TSH, TT4 en Tg konsentrasies was onderskeidelik 1.3 (0.8-1.9) mU/L, 128±33 mmol/L en 77.1 (56.3-105.7) µg/L en het geen korrelasie met die baba se UJK of BMJK vertoon nie.

In babas ses maande oud was die basislyn UJK se mediaan 345 (213-596) µg/L en dit was betekenisvol laer in die babas met dwerggroei (302 [195-504] µg/L) as in babas wat geen dwerggroei getoon het nie (366 [225-641] µg/L). Net 6.7% van die babas het 'n UJK <100 µg/L gehad. Die babas se UJK het met die moeder se UJK (128 [81-216] µg/L) (r_s=0.218, *P*<0.001) en BMJK (170 [110-270] µg/kg) (r_s=0.447, *P*<0.0001) gekorreleer. Die meeste van die babas (72%) is steeds geborsvoed en het 'n neiging tot 'n hoër UJK as die babas wat nie meer geborsvoed is nie gehad (*P*=0.074). Byna al die kinders (95%) was blootgestel aan semi-soliede/soliede kosse, waarvan kommersiële babagraankos (60%) en gebottelde babakos (20%) die mees algemene kosse was. Die babas wat per rapportering meer kommersiële babagraankos ingeneem het, het dikwels 'n heelwat hoër UJK (372 [225-637] µg/L) gehad, as die babas wat (per rapportering) selde/nooit kommersiële babagraankos geëet het nie (308 [200-517] µg/L) (*P*=0.023). Daar was geen assosiasies tussen die baba se UJK en psigomotoriese ontwikkelingstellings nie.

Die resultate van die intervensie studie het daarop gedui dat die geometriese gemiddeld (95% CI) van die basislyn UJK (333.8 [310.5, 358.9] µg/L) betekenisvol verlaag het na 214.9 (189.2, 242.6) µg/L op 12 maande. Die babas wat nie geborsvoed is nie het 'n betekenisvolle laer UJK (159.6 [65.9, 397.5] µg/L) gehad en 'n hoër kans (OR=4.9 (2.5, 9.3]) vir 'n jodiumtekort (UJK <100 µg/L) (38%), as die babas wat aan volgehoue borsvoeding (tot op12 maande) blootgestel is (373.2 [202.6, 522.9] µg/L). Babas wat SQ-LNS ontvang het (gekombineerde groep), het 'n hoër UJK (*P*=0.025) gehad met 'n laer kans as die kontrole, om 'n UJK van <100 µg/L (OR=0.289 [0.11, 0.75]) te hê, op 12 maande, indien aangepas vir die moeder se basislyn UJK, ouderdom, geslag en volgehoue borsvoeding. Tydens sub-groep analises was die effek van die SQ-LNS vir 'n hoër UJK teen 12 maande net duidelik in babas wat glad nie meer teen daardie tyd borsmelk ontvang het nie (*P*=0.039). Hierdie effek was nie meer betekenisvol ná aangepas is vir die babas se basislyn UJK nie, wat tot 'n kleiner steekproefgrootte gelei het (n=124).

Gevolgtrekking

Die resultate van hierdie ondersoek dui daarop dat lakterende moeders en hul babas, wat woon in buitestedelike gebiede in Suid-Afrika, 'n voldoende jodium inname het. Dit is egter noodsaaklik dat die jodiuminhoud van die verpligte soutjoderingproses beter gekontroleer

behoort te word. Die jodium in borsmelk het bygedra tot voldoende jodiumstatus in babas. Alhoewel kommersiële babagraankosse waarskynlik bygedra het om voldoende jodiuminname in ses- en 12 maande-oue-babas te verseker, moet die jodiuminhoud van die populêrste graankosse in Suid-Afrika ondersoek word. Die daaglikse voorsiening van 45 µg jodium, in die vorm van SQ-LNS, kan die UJK van babas wat begin eet en gespeen word van borsvoeding, verbeter. Dit is egter nie ’n doeltreffende wyse om ’n algehele afname in jodiumstatus te bekamp nie. Daarom mag dit moontlik nodig wees om die jodiuminhoud in fortifiseringsprodukte soos SQ-LNS, wat tuis gebruik word, te verhoog na die aanbevole jodiumfortifiseringsvlak van 90 µg.

Sleutelwoorde: Babas, lakterende vroue, borsmelk jodiumkonsentrasie, urienêre jodiumkonsentrasie, sout jodiumkonsentrasie, skildklierhormone, aanvullende voedingspraktyke, psigomotoriese mylpaal-ontwikkeling, kommersiële babagraankos, klein hoeveelhede lipied-gebaseerde aanvullings.

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LIST OF ABBREVIATIONS

µg/d	Micrograms per day
µg/L	Micrograms per litre
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ARA	Arachidonic acid
BAZ	BMI-for-age Z scores
BMI	Body mass index
BMIC	Breast milk iodine concentrations
CI	Confidence Interval
cm	Centimetre
DBS	Dried blood spots
DHA	Docosahexaenoic acid
DIT	Di-iodotyrosine
ETHZ	Swiss Federal Institute of Technology Zurich
EQUIP	Ensuring the Quality of Iodine Procedures
FAO	Food and Agricultural Organization
g	grams
H_2O_2	Hydrogen peroxidase
Hb	Haemoglobin
hCG	Human chorionic gonadotropin
HREC	Health Research Ethics Council
HSRC	Human Sciences Research Council
I^-	Iodide ion
I_2	Elemental iodine
IO_3^-	Iodate ion
ICCIDD	International Council for the Control of Iodine Deficiency Disorders
ICP-MS	Inductively coupled plasma mass spectrometry
ID	Iodine deficiency
IDD	Iodine deficiency disorders
IGN	Iodine global Network
IOM	Institute of Medicine
ISPAT	Iodized Salt Program Assessment Tool
IQ	Intelligence quotient

IQR	Interquartile range
KDI	Kilifi Developmental Inventory
Kg	Kilograms
LAZ	Length-for-age Z scores
mg	Milligrams
MIT	Mono-Iodotyrosine
NFCS-FB-I	National Food Consumption Survey-Fortification baseline
NIS	Sodium-iodide symporter
NWU	North-West University
OR	Odds ratio
ppm	Parts per million
SA	South Africa
SAMRC	South African Medical Research Centre
SANHANES	South African National Health And Nutrition Examination Survey
SD	Standard deviation
SIC	Salt iodine concentration
SPSS	Statistical Package for Social Sciences
SQ-LNS	Small-quantity Lipid-based nutrient supplement
T_3	Triiodothyronine
T_4	Thyroxine
TBG	Thyroxine-binding globulin
Te	Tellurium
Tg	Thyroglobulin
TMAH	Tetramethylammonium hydroxide
TPO	Thyroperoxidase
TSH	Thyroid stimulating hormone
UIC	Urinary iodine concentration
UNICEF	United Nations Children's Emergency Fund
USA	United States of America
WAZ	Weight-for-age Z scores
WIC	Water iodine concentrations
WHO	World Health Organization

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CHAPTER 5

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CHAPTER 1: INTRODUCTION

1.1. Background and rationale

Micronutrient malnutrition, often termed “hidden hunger”, is a persisting public health concern in developing countries. A significant number of the world’s population is affected by micronutrient deficiencies with iron, zinc, vitamin A and iodine deficiency (ID) being the most common (Ramakrishnan, 2002; Kennedy *et al.*, 2003). ID and its consequences used to be a major public health problem in many countries worldwide. In 1990 it was said that about 12.0% of the world’s population exhibited goiter, whilst 11.2 million people were affected by cretinism and another 43 million individuals had some degree of mental impairment related to ID (WHO, 1994). ID is the leading single cause of preventable mental retardation in the world (Hetzel, 2005). This fact has been the primary motivation behind the worldwide drive to eliminate the deficiency (De Benoist *et al.*, 2008). Over the years great progress has been made towards eliminating the problem. With the introduction of salt iodization as the main strategy to sustainable eradication of ID, about 43 countries worldwide have optimal iodine nutrition and the number of countries with ID as a public health problem reduced drastically from 110 in 1993 to 54 in 2003 and from 47 in 2007 to 32 in 2011 (WHO, 2004; De Benoist *et al.*, 2008; Andersson *et al.*, 2012). However, despite the global improvement in the reduction of ID about 266 million children and 2 billion people worldwide are still at risk for ID (De Benoist *et al.*, 2008). Furthermore, about 321.1 million Africans have inadequate iodine intakes (Jooste *et al.*, 2014). Figure 1.1 illustrates the iodine status of the world’s nations in 2015.

Iodine is an essential substrate for thyroid hormone (thyroxine; T_4 and triiodothyronine; T_3) synthesis and plays a major role in ensuring normal brain development, growth and metabolism (WHO *et al.,* 2007). Insufficient dietary iodine intake may impair thyroid hormone synthesis leading to a myriad of developmental and functional abnormalities termed as iodine deficiency disorders (IDD) (Delange *et al.*, 2002; Zimmermann, 2008). Pregnant women, lactating women and infants are most susceptible to iodine deficiency disorders. IDD in pregnant women includes abortions, still birth and congenital anomalies (WHO, 2004; Jooste & Zimmermann, 2008). Lactating women have increased dietary iodine requirements due to the loss of iodine through breast milk (Andersson *et al.*, 2010). They are often at risk of goiter and hypothyroidism whereas their infants are susceptible to neonatal goiter, endemic mental retardation, cretinism and neonatal hypothyroidism (WHO *et al.,* 2007). It is possible that even mild ID during periods of rapid growth and brain development can cause deficits in infants (Smallridge & Ladenson, 2001). Thyroid function of breast-fed infants depends on iodine from breast milk which in turn is influenced by the mother’s iodine status. The measurement of urinary iodine concentration (UIC) is the recommended way to assess iodine status in a population since UIC directly

reflects recent dietary iodine intake. According to the WHO in lactating women and in children <2 years a median UIC of100 µg/L indicates sufficient iodine intake (WHO *et al.,* 2007). However, there is little evidence to support this cut-off in infancy, and speculations are that a median UIC cut-off of 100 µg/L for infants may be set too low, therefore, further research is required to define an estimated average requirements for iodine in infancy (Swanson *et al.*, 2012; Trumbo, 2013).

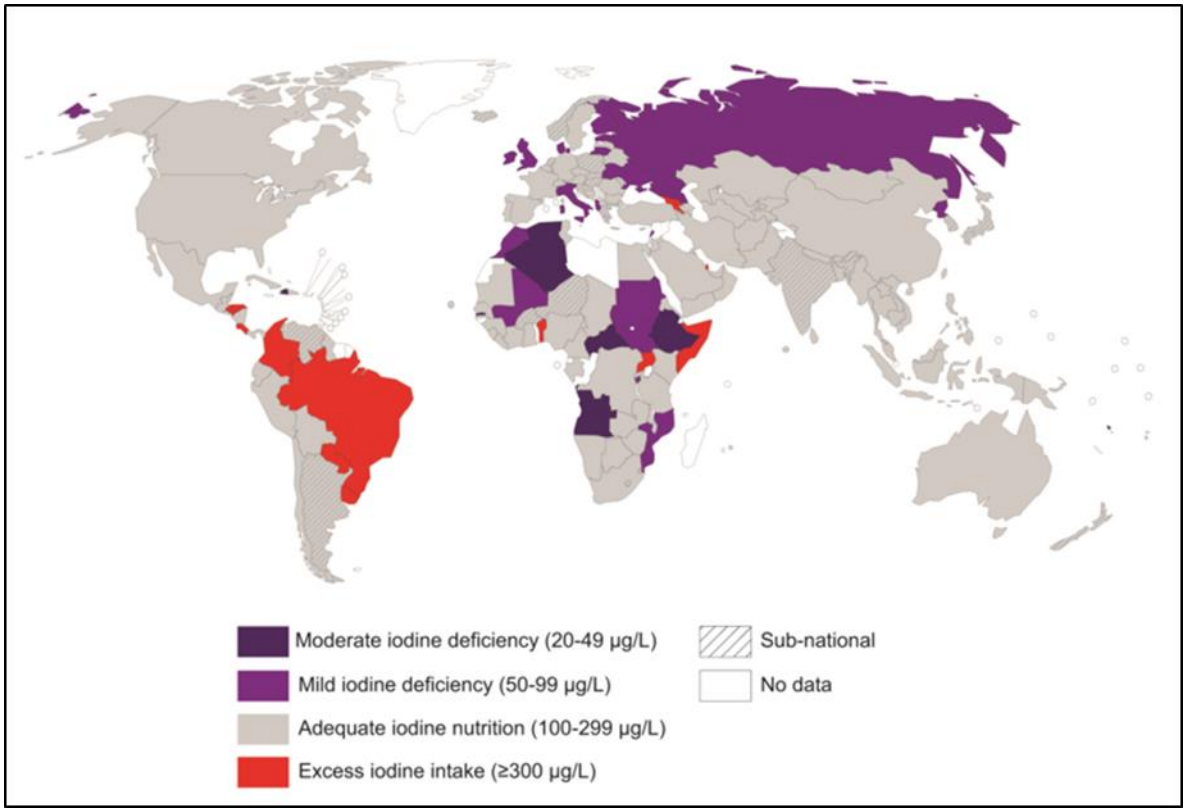


Figure 1.1: National iodine status in 2015 (source: www.ign.org)

1.1.1. Iodine nutrition in breastfed infants

Providing sufficient iodine during the first 1000 days of an infant's life from conception is a non-negotiable requirement without which brain damage may occur, therefore limiting the mental and physical development of the infant. Infants are at most risk for iodine deficiency because their iodine requirements per kilogram of body weight are the highest of any age group (Zimmermann, 2009). The period from the second trimester of pregnancy until the third year after birth is seen as the most critical for optimal brain development of the child (WHO *et al.,* 2007). By the second trimester the fetus requirements for iodide is increased as the fetal thyroid hormone production also increases to ensure adequate hormone supply after birth (Epstein *et al.*, 1994; Laurberg *et al.*, 2004). After birth the infant's thyroid actively accumulates iodine to

meet the demands for thyroid hormone production and to build up its own thyroid-iodine reserves (Delange, 1998). Breast-fed infants depend almost entirely on iodine from breast milk and the WHO recommends a daily iodine intake of 90 µg/day for infants. However, breast milk iodine concentrations (BMIC) are markedly influenced by the maternal diet and have been shown to range from five up to 892 µg/L (Azizi & Smyth, 2009). In countries with a good supply of iodine the BMIC typically ranges from 150 to 180 µg/L, while BMIC often fall to <50 µg/L in iodine-deficient areas (Mulrine *et al.*, 2010)

Iodine is transported from maternal plasma into the mammary glands of the breasts via the sodium iodide symporter (NIS) (De la Vieja *et al.*, 2000). During lactation breast milk is secreted from the mammary glands upon stimulation by various hormones (e.g. prolactin, oestradiol and oxytocin). It has been shown that iodine concentrations in human milk are 20-50 times higher than in plasma and that the expression of the NIS is increased during lactation (De la Vieja *et al.*, 2000; Tazebay *et al.*, 2000). Thus, the uptake of iodine by the mammary gland is elevated during lactation which may reduce the maternal iodine pool in mothers with inadequate iodine intake. Iodine content of breast milk has been shown to vary with dietary iodine intake and to be higher in areas where iodine prophylaxis such as salt iodization or administration of iodized oil has been introduced (Azizi & Smyth, 2009). Therefore, mothers that are at risk of having inadequate iodine intake are recommended to take iodine supplements. The recommended daily iodine intake of 250 µg (WHO *et al.*, 2007) for lactating women is meant to ensure that the iodine concentration of breast milk is sufficient to cover the infant's needs while sustaining the mother's iodine pool (Andersson *et al.*, 2007)

1.1.2. Iodine nutrition during complementary feeding

While infants below 6 months of age receive iodine from breast milk or fortified infant formula, the introduction of complementary feeding at a later stage of life poses a serious risk for deteriorating iodine status. Because of the potential harmful effect of excessive sodium intake (from salt [sodium chloride]) on the developing kidneys and blood pressure later in life, the addition of salt to home-prepared complementary foods is not recommended (Cribb *et al.*, 2012). Thus, iodized salt may not provide enough iodine to meet an infant's needs during complementary feeding unless complementary foods are fortified with iodine (Dunn, 2003; Andersson *et al.*, 2007). More studies are needed to assess iodine status during the complementary feeding period and to investigate whether the provision of iodine-fortified complementary food supplements can improve or maintain iodine status in older infants.

1.1.3. Iodine nutrition in South Africa

In South Africa (SA) iodization of table salt (40 - 60 ppm of iodine, fortified in the form of potassium iodate) was made mandatory in 1995 as a public health measure to avoid the severe consequences of iodine deficiency. It was later revised in 2006 to 35 - 65 ppm. Since then SA

has made remarkable progress in the elimination of endemic goiter and iodine deficiency (Jooste & Zimmermann, 2008). A report in 2001 showed that about 62.4% of households in the country had access to iodized table salt (Jooste *et al.*, 2001). In the 2005 National Food Consumption Survey (NFCS-FB) the median urinary iodine concentration in South African women of reproductive age and school children was 176.8 µg/L and 214.8 µg/L, respectively, indicating overall adequate iodine nutrition (Jooste *et al.*, 2007). The North-West Province was the province with the second lowest iodine status in South Africa with a median UIC in school children and women of 161.2 µg/L and 148.3 µg/L, respectively. Although South African women of reproductive age are estimated to have adequate iodine status, their requirements will increase drastically during lactation. This is because breast-fed infants depend on iodine from breast milk for the synthesis of thyroid hormones and to build up intra-thyroidal iodine stores. Also, during lactation, iodine is preferentially taken up by the mammary gland (Azizi & Smyth, 2009). In infants the median UIC is an indicator to monitor iodine nutrition. However, very few studies have been conducted in this age group and currently there are no well-established reference ranges for UIC (Andersson *et al.*, 2007). The WHO recommends a dietary iodine intake of 90 µg/day for infants (0-6 months) (WHO *et al.,* 2007). Therefore, additional to the recommended daily intake of 150 µg iodine for women of reproductive age, lactating women are recommended to increase their daily iodine intake to 250 µg/day in order to cover the additional iodine need of their breastfed infants (WHO *et al*., 2007).

Although it is assumed that South African women of reproductive age and school age children probably have adequate iodine intakes, it is uncertain whether this is also the case for lactating women as well as breast-fed and complementary fed infants. Earlier research clearly shows that low socio-economic status and people living in rural settlements in South Africa have greater vulnerability to insufficient iodine intake and status (Zimmermann *et al.*, 2008). Therefore more studies are needed to assess iodine status of infants during breastfeeding and complementary feeding. It is also necessary to investigate whether the provision of iodine-fortified complementary food supplements can improve or maintain iodine status in older infants. Despite the importance of adequate iodine status and thyroid health in lactating women and their infants, to date no data exist on breast milk iodine concentration (BMIC) or iodine status of lactating South African women and their infants. This PhD research therefore assessed the BMIC and iodine status of lactating South African women and their breast-fed infants, as well as the iodine status of complementary fed infants and associations with their feeding practices and psychomotor development. In an intervention study the iodine status of six month old complementary fed infants receiving iodine fortified small-quantity lipid-based nutrient supplements (SQ-LNS) was investigated further.

1.2. Aim and objectives

The aim of this research is to assess and improve the iodine nutrition of lactating mothers and their infants during breastfeeding and complementary feeding in the North-West Province, South Africa.

Specific objectives:

1. to assess breast milk iodine concentration (BMIC), iodine status and thyroid function of lactating women and their breastfed infants aged two to four months old;
2. to determine the iodine status of six months old infants in relation to their feeding practices and psychomotor milestone development and
3. to investigate whether the provision of novel small-quantity lipid-based nutrient supplements (SQ-LNS) fortified with iodine to infants in the age group six to 12 months will improve/maintain iodine status compared to a control group.

1.3 Research design

This PhD research is divided into two different studies. The first study was a cross-sectional analysis of iodine status, BMIC and thyroid function of lactating mothers and their breastfed infants aged two to four months. The second study was embedded within a larger research study that was a randomized, controlled trial to investigate the effects of two different novel small- quantity lipid-nutrient supplements (SQ-LNS) on linear growth in infants aged six months. Within this larger research study, a cross-sectional analysis of baseline iodine status was conducted to achieve specific objective 2. The efficacy of SQ-LNS in maintaining adequate iodine status during complementary feeding was assessed to achieve specific objective 3.

1.4 Ethical approval

The first cross-sectional analysis of iodine status in infants and lactating mothers was approved by the Health Research Ethics Committee (HREC) of the North-West University (NWU-00016-13-A1). Permission was also granted from the Provincial and District Health Departments in the North West Province to recruit mother-infant pairs for this study at local health clinics. The randomized controlled trial was approved by HREC (NWU-00001-11-A1) of the North-West University and the Ethics Committee of the South African Medical Research Council (SAMRC) (EC-01-03/2012). The trial was also reviewed by the North West Provincial Department of Health and Social Development, and registered with the Directorate for Policy, Planning and Research. The trial is registered as a clinical trial at Clinicaltrials.gov registry (NCT01845610).

1.5 Research team and contributions

The following people contributed to the success of the PhD research:

Team members	Role
Dr. Jeannine Baumgartner	Promoter of the PhD thesis. Principal Investigator of the cross-sectional study to assess breast milk iodine concentration (BMIC), iodine status and thyroid function of lactating women and their breastfed infants aged two to four months old. Responsible for design, quality control of laboratory analysis and overall execution of the study. Guidance on statistical analysis, interpretation of results and writing of the thesis. Co-author of all manuscripts.
Prof. C. Marius Smuts	Co-promoter of the PhD thesis. Principal Investigator of Tswaka trial. Responsible for design and overall execution of Tswaka trial. Guidance on interpretation of research results. Co-author of all manuscripts.
Jennifer Osei	PhD student. Responsible for protocol development, execution and data management of the cross-sectional study. Responsible for on-site laboratory blood and urine aliquots for the Tswaka randomized controlled trial. Data analysis, interpretation of results and full responsibility of writing thesis. First author on all manuscripts.
Prof. Mieke Faber	Co-Principal Investigator of Tswaka trial. Questionnaire development, fieldworker training, data coding and analysis for dietary data. Guidance regarding interpretation of results and dietary data on second manuscript. Co-author of two manuscripts (Chapters 4 and 5).
Dr. Namukolo Covic	Questionnaire development and fieldworker training for psychomotor assessment. Guidance regarding interpretation of results on second manuscript. Co-author on one manuscript (Chapter 4).
Dr. Maria Andersson	Provided expert guidance on interpretation of results and writing of cross-sectional study in chapter 3. Co-author of one manuscript (Chapter 3).
Susanne Dold	Responsible for training on BMIC analysis and standardization of the analytical methods. Co-author of one manuscript (Chapter 3).
Olivia van der Reijden	Data collection and analysis of the cross-sectional study in Chapter 3. Co-author of one manuscript (Chapter 3)
Sr. Chrissie Lessing	Registered Nurse: Overall responsibility for clinical procedures and blood sample collection in both studies.
Dr. Marinel Rothman	One of the study coordinators of Tswaka trial. Involved in questionnaire development and fieldworker training. Supervision of data collection and quality control of dietary data, feeding practices and psycho-motor development. Co-author of two manuscripts (Chapter 4 and 5).

Tonderayi M. Matsungo	One of the study coordinators of Tswaka trial. Supervision of data collection and quality control of anthropometric data. Co-author of two manuscripts (Chapter 4 and 5).

The following is a statement from the co-authors confirming their individual role in the research and the three manuscripts.

I declare that as a co-author I have approved the above-mentioned article(s), that my role in the research, as indicated above, is a representation of my actual contribution and that I hereby give consent that the manuscript(s) may be used for the PhD thesis of Jennifer Osei.

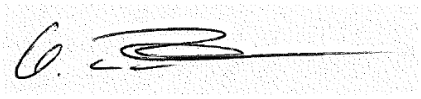
Prof. C. Marius Smuts

Dr. Jeannine Baumgartner

Prof Mieke Faber

Dr. Maria Andersson

Dr. Namukolo Covic



Olivia van der Reijden

Susanne Dold

Tonderayi M. Matsungo

Dr. Marinel Rothman

1.6 Thesis outline

The thesis is presented in article format and divided into six chapters. The format and referencing style of the manuscripts (chapters 3 – 5) are in accordance with the recommendations stipulated by the respective journals' guidelines to which the manuscripts were or will be submitted.

Chapter 1 provides the background information, aim and objectives, research design, research team and thesis outline.

Chapter 2 gives an overview of relevant literature on iodine nutrition, the impact of iodine deficiency and excess intakes and strategies to ensure adequate iodine nutrition during infancy and lactation.

Chapter 3 presents the first article manuscript titled **Breast milk iodine concentrations, iodine status and thyroid function of breastfed infants aged 2-4 months and their mothers residing in a South African township**. This manuscript documents for the first time in South Africa BMIC and UIC of lactating mothers and UIC, thyroxine (T_4), thyroid stimulating hormones (TSH) and thyroglobulin (Tg) of breastfed infants. The manuscript was submitted to *Journal of Clinical Research in Paediatric Endocrinology (JCRPE)*. A content and style guideline for *JCRPE* is given in Addendum 1.

Chapter 4 presents the second article manuscript titled **Iodine status and associations with feeding practices and psychomotor milestone development in 6 months old South African infants.** This manuscript describes the iodine status of infants at 6 months and associations with feeding practices and psychomotor development. The manuscript will be submitted to *Maternal and Child Nutrition.* A content and style guideline *for Maternal and Child Nutrition* is given in Addendum 2.

Chapter 5 presents the third article manuscript titled **Efficacy of novel small-quantity lipid-based nutrient supplements in maintaining adequate iodine status during complementary feeding: A randomized controlled trial in South African infants.** The manuscript documents the impact of iodine-fortified novel small-quantity lipid-based nutrient supplements on the iodine status of complementary fed infants. The manuscript will be submitted to *Maternal and Child Nutrition.* A content and style guideline for *Maternal and Child Nutrition* is given in Addendum 2.

Chapter 6 gives a summary of the main research findings and provides recommendations for future research.

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CHAPTER 2: LITERATURE REVIEW

2.1. Introduction

Iodine deficiency falls within the top four most prevalent micronutrient deficiencies worldwide. Iodine deficiency is the leading cause of preventable and irreversible intellectual disability (Semba & Delange, 2001), and during pregnancy it has been associated with adverse birth outcomes, such as spontaneous abortions, still births and increased perinatal and infant deaths (Dunn & Delange, 2001). The median urinary iodine concentration (UIC) is often used to classify population iodine status and the distribution of UIC is used to approximate the number of individuals with low iodine intakes (WHO *et al.,* 2007). The estimation of the household penetration of adequately iodized salt has also become a common approach, used together with UIC to determine iodine nutrition (Zimmermann & Andersson, 2012b). Before 1990, only a small portion of the world's population was iodine-sufficient, and these were mostly inhabitants of Switzerland, the Netherlands some of the Scandinavian countries, Australia, the United States and Canada (Zimmermann, 2013).

Over the years, through the introduction of universal household salt iodization, remarkable progress has been made towards eliminating iodine deficiency globally. About 71% of the world's population is now said to have access to iodized salt and the number of iodine-deficient countries has decreased from 54 to 32 (Andersson *et al.*, 2012). Despite this improvement, about 29.8% of school-going children have insufficient iodine intake worldwide, with Southeast Asia and Africa being hardest hit (Andersson *et al.*, 2012; Zimmermann & Andersson, 2012b). On the contrary, due to over-iodization of salt and/or poor monitoring of salt iodization, about 10 countries worldwide are faced with excessive iodine intake (Zimmermann, 2013). Excess iodine intake is now also emerging as a public health concern. Both iodine deficiency and iodine excess can be detrimental to human health (Zimmermann, 2009a) and therefore iodine status of populations need to be surveyed on a continual basis.

The iodine status of a population is often generalized to be sufficient if school-aged children and pregnant women have adequate iodine intake determined by urinary iodine. The iodine status of infants, however, is often ignored because their iodine status are assumed to be sufficient if the general population has a sufficient status (Zimmermann, 2014). Iodine deficiency during infancy may lead to irreversible impaired development and increased mortality, and despite the importance of iodine nutrition during infancy, to date very little is known about the iodine status of infants in countries where salt iodization programs are established and iodine intake is adequate (Zimmermann, 2014). According to the global iodine nutrition scorecard, the general iodine status in South Africa is considered more than adequate with the national median UIC of 215 µg/L (Andersson *et al.*, 2012; Zimmermann & Andersson, 2012b). The national data is,

however, primarily focused on iodine intake by school-aged children. This chapter will provide background on iodine as a nutrient and review the current literature on the importance of iodine during infancy and lactation, with focus on the periods of breastfeeding and complementary feeding.

2.2. Iodine

Iodine, just like oxygen and hydrogen, is a chemical element and has an atomic weight of 126.9 g per atom. The most important chemical forms of iodine are elemental iodine (I_2), the ions iodide (I^-) and iodate (IO_3^-) (Eastman & Zimmermann, 2009). Iodine can be found in water, air and soil (Jooste & Zimmermann, 2008). The iodine content in soil varies with region and it is often prone to leaching away into the sea by erosion, therefore, making it rich in seawater and more scarce in soil (Venturi, 2011). A healthy adult body may optimally contain about 15-20 mg of iodine of which 70-80% is stored in the thyroid gland (Zimmermann, 2012). In human nutrition, iodine is obtained from what is consumed, although the native content of iodine in food and beverages is said to be generally low (Zimmermann *et al.*, 2008). Sea food is the richest natural source of iodine. Plants and animal foods produced in iodine rich soils may contain iodine, however, these amounts may be insignificant (Zimmermann *et al.*, 2008; Swanson *et al.*, 2012). Thus, to improve iodine intake and nutrition, fortification of household salt with iodine was introduced. The natural form of edible salt (sodium chloride) does not contain iodine (WHO, 2001). The level of fortification varies according to region; however, the WHO recommends that table salt be fortified at a level of 15-40 ppm (WHO *et al.,* 2007).

Iodine is an integral component of the chemical structure of the hormones thyroxine (T_4) and triiodothyronine (T_3) produced by the thyroid gland and is crucial for the synthesis of these hormones (Eastman & Zimmermann, 2009). Thyroid hormones are essential as they play a vital role in regulating various physiological processes in target tissues in the liver, kidneys, heart, muscles and brain by acting through specific thyroid receptors (Zimmermann, 2009a). They are involved in human metabolism and are essential for normal growth and physical and mental development (Zimmermann, 2011). In the central nervous system, thyroid hormones regulate cell migration, differentiation and myelination. They are also involved in regulating micronutrient metabolism and basal metabolic rate (Dunn, 1998; Eastman & Zimmermann, 2009).

2.3 Iodine metabolism and homeostatic control

Iodine metabolism coupled with thyroid hormone synthesis are mechanisms regulated by complex interactions between the brain, pituitary gland, thyroid and iodine intake (Semba & Delange, 2001). Dietary iodine, in an inorganic salt form of iodide (I^-), is directly absorbed in the

stomach and duodenum, with an absolute bioavailability of >90% (Alexander *et al.*, 1967; Zicker & Schoenherr, 2012). Iodide absorbed from the gastrointestinal tract is transported to the bloodstream, where it is rapidly taken up by the thyroid gland and the kidneys. Iodide is transported by the sodium-iodide symporter (NIS) which is down regulated as the concentration of iodide from food increases (Nicola *et al.*, 2009; Zimmermann, 2009a). Through an active transport mechanism known as the iodine pump, the thyroid traps iodine and this trapping is regulated by the thyroid stimulating hormone (TSH), or thyrotrophin, which is released by the pituitary gland (Semba & Delange, 2001). An increase in TSH concentration stimulates thyroglobulin proteolysis, which eventually leads to a subsequent release of thyroid hormones into the blood circulation (Zimmermann, 2009a; Zicker & Schoenherr, 2012).

In adults, the thyroid gland secrets 80 micrograms (µg) of iodine in the form of T_3 and T_4 hormones per day and this mechanism is regulated by the pituitary gland through TSH (Ahad & Ganie, 2010). A decrease in T4 levels stimulates the pituitary gland to increase the secretion of TSH, which in turn stimulates the thyroid gland to release T_4 into the blood circulation (Ahad & Ganie, 2010). About 65% and 59% of the weight of the thyroid hormones T_4 and T_3, respectively, comprises of iodine, (Zimmermann *et al.*, 2008). T_3 and T_4 are metabolized in the liver which releases about 60 µg of iodine per day, of which 40 µg appear in extracellular fluid whilst the remaining 20 µg are released into the bile to be excreted in stools (Ahad & Ganie, 2010). In case of sufficient dietary iodine intake, about 90% of ingested iodide is excreted in the urine and the rest in faeces (Zimmermann, 2009a). The pathway of iodine within the thyroid cell is illustrated in Figure 2.1. In populations where iodine is sufficient, the thyroid is capable of trapping up to 60 µg of iodine per day to control losses and maintain thyroid hormone synthesis (Zimmermann *et al.*, 2008).

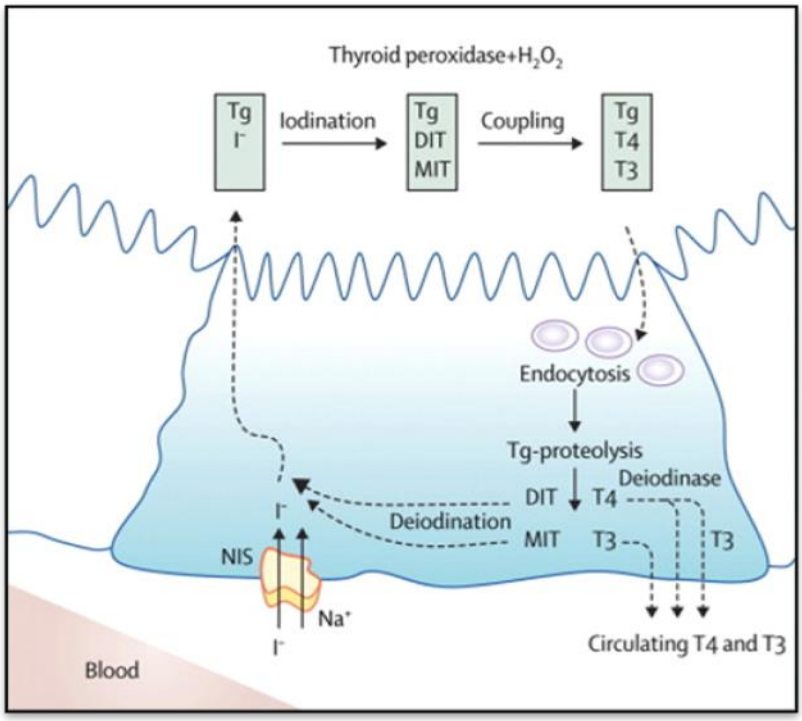


Figure 2.1: The pathway of iodine in the thyroid cell. The sodium/iodide symporter (NIS) at the basal membrane transports iodide (I^-) into the thyrocyte where it further migrates into the apical membrane. The oxidation of I^- by the enzymes thyroperoxidase (TPO) and hydrogen peroxidase (H_2O_2) cause I^- to attach to tyrosyl residues in thyroglobulin (Tg) leading to the production of hormone precursors iodotyrosine (MIT) and di-iodotyrosine (DIT). Residues then couple to form thyroxine (T_4) and triiodothyronine (T_3) within the Tg molecule in the follicular lumen. Through a process known as endocytosis, Tg enters the cell and is digested. Thyroid hormones (T_4 and T_3) are released into the circulation and iodine on MIT and DIT is recycled within the thyrocyte or excreted by the kidneys. (Source: Zimmermann *et al*., 2008a).

2.3.1. Iodine metabolism and thyroid function during the foetal period and infancy

During pregnancy, the maternal thyroid gland is responsible for ensuring that there is adequate thyroxine production for normal growth and development of the foetus, provided that the mother's iodine intake is sufficient (Eltom *et al.*, 2000). Thyroid hormones thyroxine (T_4) and triiodothyronine (T_3) produced by the mother are transferred to the foetus through the placenta, and can be detected in the foetal serum by 11 to 12 weeks of gestation (Brown, 2000). By the second trimester the foetus' requirements for iodide is increased as the feotal thyroid hormone production also increases to ensure adequate hormone supply after birth (Epstein *et al.*, 1994; Laurberg *et al.*, 2004) (Figure 2.2). Over the entire period of pregnancy until birth, the thyroid hormones continue to slowly increase (Thorpe-Beeston *et al.*, 1992; Brown, 2000; Williams *et al.*, 2004). It is not clear how much T_4 is transferred from the mother to the developing foetus,

although 40% of measured T_4 in cord blood at delivery is said to be that of the mother (Delange, 2007).

Not much is known about iodine metabolism in infancy and most human studies on iodine metabolism have been carried out in adults (Hays, 1984; Cavalieri, 1997). However, at the time of birth, a full term baby has drastic changes to the thyroid physiology, one being an abrupt increase in serum TSH within the first 30 minutes of birth (Brown, 2000). Infants are born with very small intra-thyroidal iodine stores, namely 0.3 mg compared to 15-20 mg in adults (Zimmermann, 2009b). The infant now starts to build up iodine stores and also produce the thyroid hormones T_3 and T_4, and for this iodine is required from either breast milk or formula milk. As the infant matures, the concentrations of T_4, free T_4, T_3 and TSH slowly decrease (Zurakowski *et al.*, 1999). By the age of six months, the infant's serum Tg levels also drop to concentrations similar to that of adults (Brown, 2000).

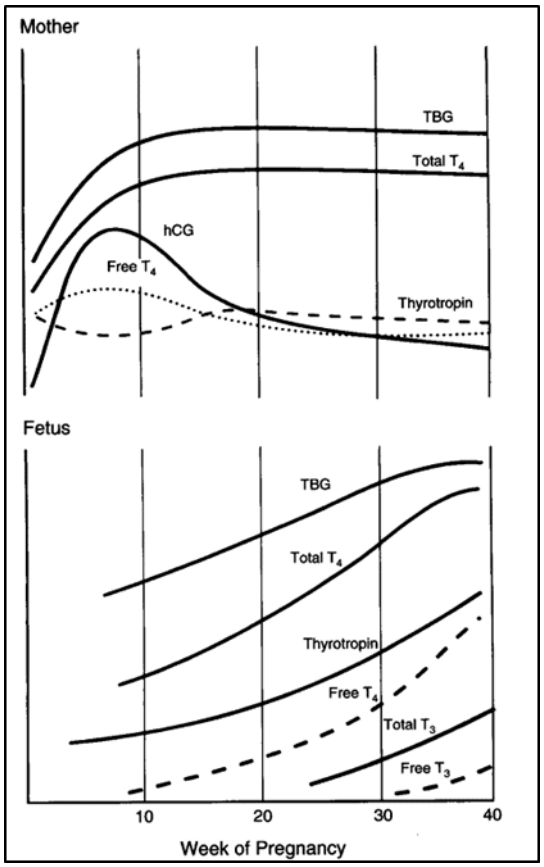


Figure 2.2: Maternal and foetal thyroid function changes during pregnancy (source: Epstein, *et al.,* 1994)

2.3.2. Iodine metabolism during lactation

During lactation, the mammary gland concentrates iodide with the help of increased expression of the sodium iodide symporter (NIS) (Etling *et al.*, 1986). This concentrating mechanism enables an adequate supply of iodine into the breast milk for the new born infant (Figure 2.3). In human milk, the concentration of iodine is 20-50 times higher than in plasma (De la Vieja *et al.*, 2000; Tazebay *et al.*, 2000). It has been previously observed that in the first few days of lactation (two to five days), iodine concentrations in breast milk are highest with approximately 200 to 400 µg iodine per litre in colostrum (Azizi & Smyth, 2009), and gradually declines until they stabilize to normal levels in mature milk (Etling *et al.*, 1986).

Uptake of iodine by the milk ducts in the breast during lactation can be decreased or hindered by the anion perchlorate which has been detected in fertilizers, drinking water, lettuce and wheat in the United States (Pearce *et al.*, 2007). Although the study by Pearce *et al.*, (2007) of lactating women in Boston did not find any correlations between breast milk iodine and perchlorate concentrations, perchlorate can block iodine intake by inhibiting the function of the sodium/iodide symporter (NIS) on the basolateral surface on lactating breast cells (Pearce *et al.*, 2007).

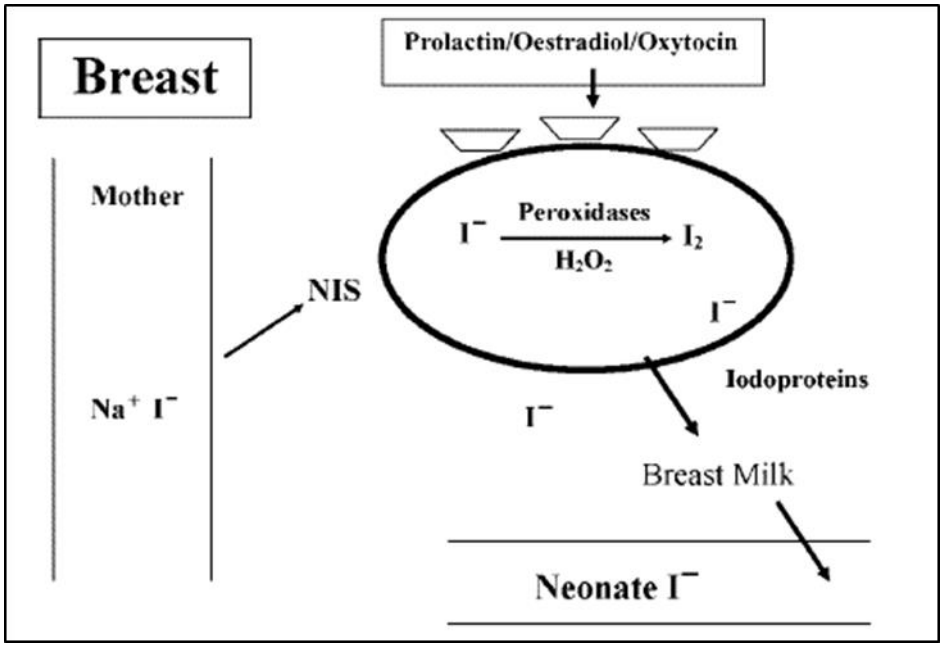


Figure 2.3: Secretion of iodine in human milk and the functions of the sodium iodide symporter (NIS). The NIS facilitates and controls the transport of iodide from the blood of the mother into the breast, and iodine appears in the breast milk, which is secreted into milk ducts upon influence of various hormones (Source: Azizi & Smyth, 2009).

2.4. Assessment of iodine status in populations

In populations, salt iodine content, dietary assessment, urinary iodine concentrations (UIC), breast milk iodine concentrations (BMIC), serum thyroid stimulating hormone (TSH) concentrations, serum thyroglobulin (Tg) concentrations, thyroxine (T_4)/triiodothyronine (T_3) ratios and goitre rates can be used as biomarkers for the assessment of iodine nutrition (Eastman & Zimmermann, 2009). These indicators are also complementary (Semba & Delange, 2001; Zicker & Schoenherr, 2012). For the purpose of this research, the use of UIC, BMIC, and markers of thyroid function (T_3/T_4, TSH Tg and goitre rate) in the assessment of iodine status in populations will be further discussed.

2.4.1. Urinary iodine concentrations (UIC)

Urinary iodine is an excellent indicator of recent iodine intake because 90% of iodine absorbed by the body is excreted in the urine, although a few traces can be seen in faeces as well (WHO *et al.,* 2007). Iodine in urine can be expressed as a concentration (UIC; µg/L), in relationship to creatinine excretion (mg iodine/g creatinine), or as a 24-hour excretion (mg/day). Iodine in the urine of an individual can have diurnal and day-to-day variations. There are two main ways in which urinary iodine can be assessed, namely either by collection of a 24 hour sample, or by collection of a spot urine sample (Vejbjerg *et al.*, 2009). In population studies, it is not practical to collect 24 hour urine samples. Therefore, urinary iodine can be measured in spot samples from a representative sample in a target population and expressed as the median (König *et al.*, 2011). In larger samples, variations in individual hydration levels is generally evened, hence making the median UIC in spot samples correlate well with that of 24 hour urine samples (WHO *et al.,* 2007).

For lactating women and children <2 years old, a median UIC of 100 µg/l is currently being used to define adequate iodine status. Table 2.1 is an Illustration of indicators and criteria used to assess iodine deficiency in populations using median UIC. There are no established cut-offs for excessive UIC in infants, however, a median urinary iodine greater than 300 is considered excessive in older children (Table 2.1) (WHO *et al.,* 2007).

Although, the measurement of UIC is a good indicator for current iodine intake and thyroid hormone catabolism, on its own, it is not a direct measure for thyroid function. Therefore, populations with current normal ranges of UIC, may still in the long term experience serious functional consequences of iodine deficiency (van den Briel *et al.*, 2001). A low UIC value indicates that a population is at high risk of developing thyroid disorders (Andersson *et al.*, 2007; Zimmermann, 2010).

Table 2.1: Epidemiological indicators and criteria for assessing iodine deficiency*

Criteria and population group	Iodine intake (iodine nutrition status)
Median UIC in children aged ≥6 years and adults (µg/L)	
<20	Insufficient (severe iodine deficiency)
20-49	Insufficient (moderate iodine deficiency)
50-99	Insufficient (mild iodine deficiency)
100-299	Adequate (adequate iodine nutrition)
≥300	Excessive (risk of adverse health consequences)
Median UIC in pregnant women (µg/L)	
<150	Insufficient
150-249	Adequate
250-499	Above requirements
≥500	Excessive
Lactating women (µg/L)	
≥100	Adequate (optimal iodine status)
Infants <2 years old (µg/L)	
≥100	Adequate (optimal iodine status)

Abbreviations: UIC, urinary iodine concentration
*Adapted from Li & Eastman, 2012 and WHO *et al.,* 2007 (WHO, UNICEF & International Council for the Control of Iodine Deficiency Disorders. Assessment of iodine deficiency disorders and monitoring their elimination: A Guide for Programme Managers 3rd edition.)

Urine collection from infants

Collecting urine from young infants may be a challenge. Recently, a simple method of collecting spot urine samples using an absorbent pad was developed (Dorey & Zimmermann, 2008). This pad is non-invasive and free of iodine (Figure 2.4). It can be inserted into the diapers of infants to absorb urine which is then extracted by either using a syringe or squeezing the pad. A median UIC can be used to assess iodine intake of infants. This is done by assuming that the average urine volume of an infant is 300–500 ml day^{-1}; then, of every 100 µg of iodine ingested, 90 µg is excreted in urine at a concentration of 60 µg l^{-1} (Andersson *et al.*, 2007).

Figure 2.4.: SteriSets Uricol urine collection pack (source: Amazon.com, 2015)

Determination of urinary iodine concentration: The Pino modification of the Sandell-Kolthoff method

The Pino modification of the Sandell-Kolthoff microplate method is one of the most commonly used methods for measuring urinary iodine concentration (WHO *et al.,* 2007). It is based on the Sandell-Kolthoff reaction, which is a quantitative colorimetric method in which iodide is used as a catalyst in the presence of arsenious acid to reduce ceric ammonium sulphate (yellow) to a colourless cerous form (Gnat *et al.*, 2003). The Pino modification of the Sandell-Kolthoff microplate method is a more simplified, convenient and economical version of the Sandell-Kolthoff method in which urine is digested in 1 mol/L ammonium persulphate instead of chloric acid. Chloric acid unlike ammonium persulphate is potentially hazardous requiring the use of an explosive-proof hood among other precautions (Pino *et al.*, 1996).

The Centres for Disease Control and Prevention has a laboratory quality assurance standardization program for iodine procedures known as the Ensuring the Quality of Iodine Procedures (EQUIP), which is used as an international external quality control for the measurements of UIC (WHO *et al.,* 2007).

2.4.2. Breast milk iodine concentration (BMIC)

Amongst all the trace elements in milk, iodine is said to be unique because it is avidly concentrated by the mammary gland, therefore making it exclusively available for breast fed infants (Semba & Delange, 2001). Iodine and thyroid hormones in breast milk are known to be well absorbed by infants (Tenore, 1986). The concentration of iodine in maternal milk is said to be at higher concentrations than what is found in maternal serum (Semba & Delange, 2001). Furthermore, iodine levels in maternal milk has day to day variations and can also vary from one population to the other (Kirk *et al.*, 2007). In areas where iodised salt is consumed, BMIC tend to be higher (Azizi & Smyth, 2009). BMIC were also found to be higher in women who

consumed iodized salt when compared to those ingesting iodine-containing multivitamin supplements (Kirk *et al.*, 2012). There is no established optimal level of concentration for breast milk. Although Brazil has regions of iodine deficiency and excess, in an iodine sufficient area de Lima *et.al*., (2013) reported BMIC ranging from 51-560µg/l. China, USA and Iran also reported median BMIC of 146.7, 155 and 58.23 µg/l, respectively (Yan *et al.*, 2005; Pearce *et al.*, 2007; Mobasseri *et al.*, 2014).

BMIC is negatively affected by maternal smoking. A study by Laurberg *et al.* (2004) showed that maternal smoking impaired iodine transport and reduced breast milk iodine by half, therefore, exposing infants to reduced iodine intake and increased risk of deficiency. In Thailand, the median BMIC in 100 lactating mothers was 129.7 µ/L (IQR = 81.0, 205.7), and factors that were significantly related to breast milk iodine content were baby's age (*P* =0.004), mother's age (*P* =0.035) and mother's consumption of iodine fortified eggs (*P* =0.030) (Mekrungcharas & Kasemsup, 2014).

2.4.3. Markers of thyroid function

Thyroxine (T_4), triiodothyronine (T_3) and Thyroid stimulating hormone (TSH)

Determination of T_3/T_4 rations and TSH concentrations are useful in certain populations, however, they are not reliable indicators of iodine status, therefore, there is a need to complement it with other indicators (Zicker & Schoenherr, 2012; Rohner *et al.*, 2014). In populations with iodine deficiency, serum T_4 usually decreases, whilst serum T_3 increases or remains unchanged and these changes are often within the normal thyroid hormone ranges (Zimmermann, 2008). Certain compounds found in food like thiocyanate, perchlorate and soy goitrogen may interfere with iodine usage by inhibiting the NIS and interrupting the organification process respectively. Therefore it is probably most useful to perform multiple measurements over time with well-defined dietary constituents to get the most accurate assessment of iodine status (Zicker & Schoenherr, 2012).

In neonates' serum TSH is the only indicator that enables the prediction of possible impairment of mental development caused by iodine deficiency (Delange, 1998). Elevated serum TSH is an indication of inadequate supply of thyroid hormones to the developing brain, and compared to adults, neonates seem to be more hypersensitive to the effects of iodine deficiency (Delange, 1998). Under normal circumstances, the prevalence of neonatal TSH above 5 mU/L whole blood is less than 3%. However, in the presence of iodine deficiency, the prevalence is increased. A prevalence of 3%-19.9% indicates mild iodine deficiency disorders (IDD). Prevalences of 20%-39.9% and above 40% indicate moderate and severe IDD, respectively (Delange, 1998).

Thyroglobulin (Tg)

Thyroglobulin is a reflection of an intermediate response to changes in iodine intake over periods of weeks and even months (Zicker & Schoenherr, 2012). Elevated Tg values reflect increased TSH stimulation due to iodine deficiency therefore making it a sensitive marker of iodine status and thyroid function (Federal Commission for Nutrition, 2013). The WHO recommends that Tg should be measured to complement UIC as biomarker to monitor iodine nutrition in children (WHO *et al.,* 2007; van den Briel *et al.*, 2001), adults and pregnant women (Laurberg *et al.*, 2007; Vejbjerg *et al.*, 2009). In large population studies the measurement of Tg in dried blood spots (DBS) has been developed as a simple method in the surveillance of iodine status (Federal Commission for Nutrition, 2013). However, normal range reference values for DBS-Tg are only available for school-age children (4–40 µg/L), but not for young children and infants (Sobrero *et al.,* 2007; Zimmermann *et al.*, 2006b).

Goitre rate

The goitre rate is a reflection of long-term iodine status (months to years). The total goitre rate in school-aged children is usually measured in order to define the severity of iodine deficiency in populations as established by the WHO. According to this method, total goitre rates of <5%, 5.0–19.9% , 20.0–29.9 % and >30% are classified as iodine sufficiency, mild deficiency, moderate deficiency and severe deficiency, respectively (WHO *et al.,* 2007). Goitre can be measured by a neck inspection and palpation or by conducting an ultrasonography on the thyroid (Zimmermann, 2008).

In areas of endemic goitre, it may take months or even years for thyroid size to return to normal after increased intakes of iodine (Zimmermann *et al.*, 2003), therefore, making it difficult to interpret goitre rates in populations especially after correction of deficiency (Zimmermann, 2008). In South Africa, after one year mandatory salt iodization, goitre rates remained unchanged, whilst UIC and household salt iodine concentration improved (Jooste *et al.*, 2000).

2.5. Consequences of iodine deficiency

Habitual inadequate iodine intake below recommended levels results in deficiency (WHO *et al.,* 2007). Deficiencies of selenium, iron and vitamin A can also worsen iodine deficiency by interfering with normal thyroid function. Selenium, for instance, forms part of the deiodinase enzymes required for the activation of T_4 to T_3 (Swanson *et al.*, 2012). In chronic and severe cases, Iodine deficiency leads to thyroid dysfunction (Zimmermann & Andersson, 2012a). Iodine

deficiency is the leading single cause of potentially preventable and irreversible mental retardation especially in early childhood (Delange *et al.*, 2001).

2.5.1. Iodine deficiency disorders

Iodine deficiency (ID) leads to a spectrum of adverse effects on growth, mental and physical development, collectively termed as iodine deficiency disorders (IDD) (WHO *et al.,* 2007) (Table 2.2). These disorders occur as a result of inadequate thyroid hormone production and their health consequences depend on the time in life a deficiency occurs (Zimmermann *et al.*, 2008). Goitre is the most visible effect of ID, however, cognitive impairment caused by the deficiency is the most serious (Zimmermann & Boelaert, 2015). Pregnant women, lactating women and infants are most susceptible to IDD. Congenital hypothyroidism is one of the most devastating effects of maternal iodine deficiency, which may lead to cretinism in severe cases (Levander & Whanger, 1996).

Endemic cretinism is said to be the most serious IDD. Under severe circumstances, iodine deficiency during pregnancy may lead to endemic cretinism in the infant (Delange, 2001). Cretinism occurs in two types, namely neurological cretinism and myxedematous or hypothyroid cretinism. Neurological cretinism is characterized by mental and physical retardation and a high prevalence of deafness. It usually occurs due to maternal ID that affects the foetus before its own thyroid is functional. Myxedematous or hypothyroid cretinism, on the other hand, is characterized by short stature and mental retardation. This form of cretinism is known to be associated with selenium deficiency and with the presence of goitrogens in the diet that hinder thyroid hormone production (Levander & Whanger, 1996). Figure 2.5 shows the characteristics of Neurologic cretinism. The photograph was taken in western China in 2007 of a nine year old girl (Zimmermann, *et al.* 2008). Figure 2.6 illustrates characteristics of Myxedematous cretinism. The photograph shows a tall and normal male and three short statured females all aged 15-20 years old, taken in the Democratic Republic of Congo. The males have severe longstanding hypothyroidism coupled with severe mental retardation, dwarfism and retarded sexual development (Eastman & Zimmermann, 2009; Zimmermann, 2009a).

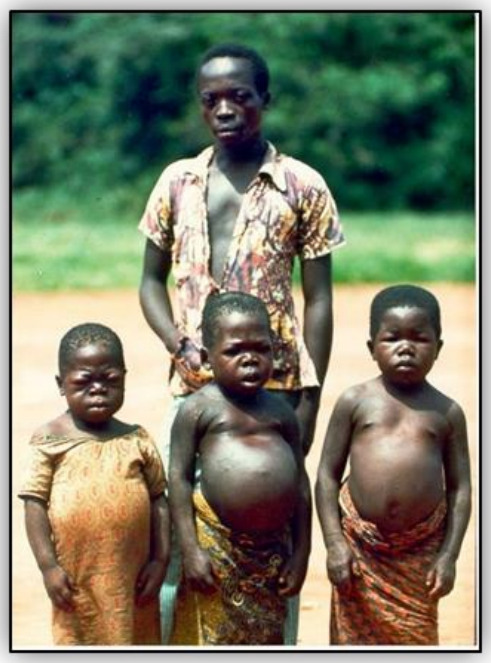
Table 2.2: The spectrum of iodine deficiency disorders and their health consequences[††]

Physiological groups	Health consequences of iodine deficiency
All ages	Goitre
	Hypothyroidism
	Increased susceptibility of the thyroid gland to nuclear radiation
Fetus	Spontaneous abortion
	Stillbirth
	Congenital anomalies
	Perinatal mortality
Neonate	Infant mortality
	Endemic cretinism including mental deficiency with a mixture of mutism, spastic diplegia, squint, hypothyroidism and short stature
Child and adolescent	Impaired mental function
	Delayed physical development
	Iodine-induced hyperthyroidism (IIH)
Adults	Impaired mental function
	Iodine-induced hyperthyroidism (IIH)

[††]Adapted from WHO *et al.,* 2007 and Zimmermann & Boelaert, 2015

Characteristics
- mental retardation
- abnormal gait
- deaf-mutism
- motor spasticity of the arms and legs

Figure 2.5: Neurologic cretinism (source: Zimmermann, *et al.* 2008)



Characteristics

- severe mental retardation
- short stature
- profound hypothyroidism
- incomplete maturation of the face with wide-set eyes

Figure 2.6: Myxedematous cretinism (source: Eastman & Zimmermann, 2009; Zimmermann, 2009a)

2.5.2. Role of iodine nutrition in motor milestone development

Thyroid hormones play a very crucial role during the period of rapid growth and development in the foetus and also in young infants (Semba & Delange, 2001). During pregnancy, especially towards the end of the second trimester, there is a crucial period where thyroxine is required for brain development, therefore making adequate iodine and thyroid hormones vital for optimal brain development (O'Donnell *et al.*, 2002). Development of the brain at the postnatal stage depends on the thyroid hormone produced by the neonate, and lack of these hormones causes an impairment to the cytoarchitecture of the neocortex and the cerebellum (Bernal & Nunez, 1995). Reports from a study on rats indicated that small amounts of thyroid hormones are transferred to the foetus from the mother before the start of foetal thyroid function, however, if the new-born thyroid gland is absent or unable to produce hormones, these hormones may not be adequate at birth (Calvo *et al.*, 1990). Maternal thyroid hormone status, and therefore iodine status, plays a critical role for proper in utero brain development. Previous studies have observed that low maternal serum or plasma fT4 levels during pregnancy may impair

psychomotor and visual-motor development in their offspring (Haddow *et al.*, 1999; Pop *et al.*, 1999; Klein & Mitchell, 2002). Leneman *et al*., (2001) also reported permanent or selective effects on visuospatial processing in adolescents with congenital hypothyroidism in adolescents who experienced a brief postnatal period of hypothyroidism. Evidence points out that early maternal iodine supplementation during pregnancy (before end of the second trimester) is more beneficial to the psychological development of the offspring, as compared to supplementation later in pregnancy or early in childhood (O'Donnell *et al.*, 2002).

Children born to severely iodine deficient mothers are at high risk for cognitive disability, especially cretinism manifested by delayed motor and mental development (Federal Commission for Nutrition, 2013). During the foetal and neonatal period, severe iodine deficiency associated with critically low levels of thyroid hormones, cause neurological brain damage (Bougma *et al.*, 2013). Even mild to moderate deficiency of iodine has resulted in delayed mental development (Zimmermann, 2007). Although it is less certain whether mild to moderate iodine deficiency during childhood distorts cognition and learning, recent randomised controlled trials have reported beneficial effects of iodine repletion on children's performance on cognitive tests (Zimmermann *et al.*, 2006a; Gordon *et al.*, 2009).

2.5.3. Role of iodine nutrition on child growth

Thyroid hormones are crucial for the secretion of growth hormone (Giustina & Wehrenberg, 1995) and they regulate bone cell growth and differentiation. A dysfunction in the thyroid may lead to severe disturbances of bone metabolism (Klaushofer *et al.*, 1995). Severe iodine deficiency during pregnancy may cause dwarfism (WHO *et al.,* 2007). Maternal iodine supplementation (either early or later in pregnancy) and iodine supplementation later in childhood may not have different effects on linear growth (height) in school-aged children. However, maternal iodine supplementation during pregnancy, particularly in the first or second trimester may improve the head-circumference of the offspring (O'Donnell *et al.*, 2002).

It is not well known how iodine deficiency affects child growth at the postnatal stages. Although some early studies have not been able to show any effects of iodine supplementation on child growth, Zimmermann *et al*., (2007) reported that iodine repletion improved somatic growth in moderately to severely iodine deficient children

2.6. Iodine excess

Under normal circumstances a human can tolerate chronic iodine excess of up to 2g of iodide per day, without any obvious clinical symptoms or signs of thyroid dysfunction or goitre (Bürgi, 2010). This is because the thyroid gland has intrinsic regulatory mechanisms that maintain normal thyroid function even in the presence of iodine excess (Roti & Uberti, 2004; Bürgi, 2010).

This is known as the acute Wolff–Chaikoff effect. The mechanism behind the acute Wolff–Chaikoff effect is not fully understood, but is thought to be at least partially explained by reduced intrathyroidal deiodinase activity and decreased thyroid hormone synthesis caused by iodine overload (Pramyothin *et al.*, 2011; Leung & Braverman, 2014). Iodine supplementation, high iodine diets, multivitamin supplements and topical iodine (povidone iodine) are some of the common sources of excess iodine intake (Leung & Braverman, 2014). Potential consequences of excess iodine intake include iodine-induced hypothyroidism and iodine-induced hyperthyroidism (Rhee *et al.*, 2011).

Iodine-induced hypothyroidism occurs when the thyroid gland fails to adapt to the presence of excess iodine. The primary mechanism behind iodine-induced hypothyroidism is unclear, however, it has been attributed to failure to adapt to the acute Wolff–Chaikoff effect, because of a malfunctioned thyroid (Leung & Braverman, 2014). Hypothyroidism can be overt or subclinical. In overt hypothyroidism the thyroxine levels are below normal, whereas in subclinical hypothyroidism the blood level of thyroxine remains in the normal range, but the level of the thyroid stimulating hormone (TSH) is elevated (Pluta *et al.*, 2010). Because of their immature thyroid gland, infants are more susceptible to iodine-induced hypothyroidism, as they are unable to escape from the acute Wolff-Chaikoff effect (Connelly *et al.*, 2012). In a mildly iodine deficient population, iodine supplementation to a level that is more than adequate can escalate the development of subclinical hypothyroidism to overt hypothyroidism (Teng *et al.*, 2006).

In the presence of excess iodine, the thyroid may produce too much thyroxine leading to iodine-induced hyperthyroidism (Leung & Braverman, 2014). In a previously deficient population, an increased intake of iodine can lead to iodine-induced hyperthyroidism (Bourdoux *et al.*, 1996; Delange *et al.*, 1999). Overt hyperthyroidism is characterised by low serum thyroid-stimulating hormone (TSH) concentrations and raised serum concentrations of thyroid hormones i.e. thyroxine (T_4), tri-iodothyronine (T_3), or both. Subclinical hyperthyroidism is characterised by low serum TSH, but normal serum T_4 and T_3 concentrations (De Leo *et al.*, 2016).

In South Korea and Japan continuous ingestion of supplements which contained seaweed or seaweed itself lead to excessive intakes and was linked with thyroid disorders such as goiter, hypothyroidism and autoimmune thyroiditis (Konno *et al.*, 1994; Kim & Kim, 2000). Teng *et al.,* (2011) also studied two populations in China and found that the population that had median UIC of 261 µg/l was at increased risks of developing autoimmune thyroiditis and subclinical hypothyroidism. In this study, pregnant women, women who had given birth within the past year and those on oral contraceptives were excluded; persons receiving glucocorticoids, dopamine, or anti-epileptic drugs as well as persons diagnosed with renal insufficiency or adrenocortical hypofunction, were also excluded (Teng *et al.*, 2011).

Both hyperthyroidism and hypothyroidism caused by excessive iodine intakes were observed in lactating women in the Saharawi refugee camps in Algeria (Aakre *et al.*, 2015). In these women the median urinary iodine concentrations and breast milk iodine concentrations (BMIC) were 350 µg/L and 479 µg/L respectively, and BMIC was associated with thyroid function in women (Aakre *et al.*, 2015).

There is no clear evidence to define the cut-off for excess iodine intake during infancy. However, recommendations set by the World Health Organization/International Council for Control of Iodine Deficiency Disorders (WHO/ICCIDD) stipulate that a median UIC >300 µg/L and UIC >500 µg/L in school-aged children and pregnant women, respectively, are excessive (WHO *et al.,* 2007).

2.7. Dietary iodine requirements

Adequate iodine intake is particularly important during pregnancy, lactation and infancy because of the crucial role thyroid hormones have in brain development of the growing foetus and infant (Bath, 2014).

2.7.1. Recommendations during infancy

Dietary iodine requirements during infancy are not well defined. The Institute of Medicine (IOM) of the US National Academy of Sciences recommends a dietary adequate iodine intake of 110 µg/day during the first six months of life to maintain adequate iodine status (Institute of Medicine *et al.*, 2001). The WHO recommends a lower daily iodine intake of 90 µg/day (WHO *et al.,* 2007). The IOM recommendation is an adequate intake (AI) which was set based on the multiplication of the median breast milk iodine concentration of 146 µg/L measured in U.S women, by an average milk intake of 0.78 L/day (0-6 months) (WHO, 1998). This was conducted during a period of excessive iodine intake in the US (Gushurst *et al.*, 1984), and therefore it is not clear whether it can be generalized to other populations. In contrast, the WHO recommendation was based on a small balance study of one month old infants fed daily on an average of 20 ± 1.9 µg iodine/kg body weight (WHO *et al.,* 2007). Infants retained 7.3 ± 1.0 µg/kg/day and excreted 11.4 ± 1.8 µg/kg/day. From this outcome, the researchers concluded that the iodine intake of infants should be at least 15 µg/kg/day. Therefore, a six month old infant with an average body weight of six kg would require 90 µg of iodine per day (WHO *et al.,* 2007).

The discrepancy between the two recommended levels of iodine intake and the proposed UIC cut-off (100 µg/L) to define optimal iodine status in infancy can be misleading as the measures derive two completely different conclusions with regard to infant iodine nutrition. Assuming mean daily urine volume of approximately 0.5L in early infancy (0-6 months) (WHO & FAO,

2004) and iodine bioavailability of approximately 92% (Institute of Medicine *et al.*, 2001), a median UIC of 100 µg/L would lead to a mean daily iodine intake of approximately 55 µg, which is half the current adequate intake. Therefore, more research is needed to clearly define the estimated average requirement for infants (Swanson *et al.*, 2012; Trumbo, 2013)

Currently there is no cut-off limit to define excessive intake for iodine during infancy, however, according to the IOM, the highest average daily iodine intake (upper intake level) that is likely not to pose adverse health risks, is set at 200 µg iodine/day for children aged between 1 and 3 years (Institute of Medicine *et al.*, 2001).

2.7.2. Recommendations during lactation

Recommendations from the World Health Organization/Iodine global network/United Nations Children's Emergency Fund (WHO/IGN/UNICEF) indicates that lactating women should consume 250 µg of iodine per day, and that a median of UIC of 100 µg/L or higher indicates sufficiency (WHO *et al.,* 2001; WHO *et al.,* 2007). For lactating women, the values for adequate median UIC are lower than their iodine requirements because of iodine excretion via breast milk (WHO *et al.,* 2007). The recommendation for iodine intake during lactation is also higher than that of women of reproductive age (Table 2.3).

Unlike during pregnancy, in lactation the physiology of the thyroid hormone production and urinary iodine excretion return to normal and iodine is concentrated into the mammary gland for excretion into the breast milk (Andersson *et al.*, 2007). Colostrum has approximately 200-400 µg/l concentration of iodine, which is higher compared with mature milk (Etling *et al.*, 1986). Breast milk iodine concentrations have substantial diurnal and day-to-day variations, and it may also vary based on the age of the infant and the stage of lactation (Kirk *et al.*, 2007).

To date, there is no set optimal level for iodine concentrations in breast milk. A review by Azizi and colleagues reported that in areas of varying iodine sufficiency, there was a wide range (13-155 µg/L) of mean or median breast milk iodine concentrations (Azizi & Smyth, 2009). Studies, although contradictory, show that supplementation may increase iodine concentration in breast milk in iodine deficient areas. A recent study reported that in regions without effective salt iodization and moderate-to-severe iodine deficiency, supplementation with one dose of oral iodized oil (400mg) to lactating mothers immediately after delivery provided sufficient iodine to their breastfed infants for at least 6 months (Bouhouch *et al.*, 2014). Although previously Mulrine *et al.,* (2010) observed that supplementation with 75 or 150 µg of iodine per day in iodine-deficient lactating women over a period of 6 months increased BMIC however, it did not provide adequate iodine for the mother or breastfed infant.

Table 2.3: Recommended iodine intakes by age or population group*

	Iodine intake (µg per day)
US Institute of Medicine recommendations	
Infants[1]	
0-6 months	110
7-12 months	130
Children[2]	
1-8 years	90
9-13 years	120
≥14 years & adults	150
Pregnancy	220
Lactation	290
WHO, the IGN and UNICEF recommendations	
Children[3]	
0-5 years	90
6-12 years	120
≥12 years & adults	150
Pregnancy	250
Lactation	250

[1] Adequate intakes. [2] Recommended Daily Allowance. [3] Recommended Nutrient intake
Abbreviations: IGN, Iodine Global Network (formerly ICCIDD, International Council for the Control of Iodine Deficiency Disorders); UNICEF, United Nations Children's Fund; WHO, World Health Organization.
*Adapted from Zimmermann et al., 2008, Eastman & Zimmermann, 2009 and Li & Eastman, 2012.

2.8. Strategies to address iodine deficiency (iodine fortification and supplementation)

A community based strategy that can be used to address iodine deficiency in infancy is the promotion of breastfeeding for the first six months and continued breastfeeding for older infants (WHO *et al.,* 2007). However, this strategy can only be effective if lactating mothers have adequate iodine status. Other strategies that are employed are supplementation and fortification. Supplementation of iodine is a technical approach often employed as a short term measure to immediately target populations that are at high risk of deficiency, for example, during pregnancy/lactation or in acute food shortages (Kennedy *et al.*, 2003). Iodine supplements can be delivered in the form of syrups or tablets (for example iodised oil). Supplementation is often replaced by a more sustainable and long-term measure such as fortification (Kennedy *et al.*, 2003). Universal salt iodization has been the best fortification approach to alleviate IDD (Zimmermann, 2004). This approach is further discussed in section 2.8.1.

In 2007, the WHO Secretariat published the consensus reached by the Technical Consultation on issues regarding the prevention and control of iodine deficiency in pregnant and lactating

women and in children younger than two years old, summarized in Table 2.4 by Andersson *et al.* (2007). The report emphasises that in populations with access to and consumption of adequately iodized salt, it can be assumed that the iodine needs of women of child-bearing age as well as pregnant and lactating women are met by their diet and breast milk provides sufficient iodine for infants younger than 6 months (Andersson *et al.*, 2007). However, children starting on complementary foods may need to consume iodine fortified foods (together with continued breastfeeding), as iodized salt may not provide sufficient iodine to meet their needs (Andersson *et al.*, 2007) and it is not recommended to add salt to infants foods (Cribb *et al.*, 2012).

Home-prepared complementary foods often lack adequate iodine content (Alexy *et al.*, 2009; Andersson *et al.*, 2010). These foods can be fortified with iodine through micronutrient powders or small-quantity lipid-based nutrient supplements (SQ-LNS) (WHO, 2011; Hess *et al.*, 2015). For the purpose of this research the focus will be on SQ-LNS as a vehicle for iodine fortification which will be further discussed in section 2.8.3. For fortification strategies to be successful, many issues such as improvement of infrastructure to increase access and mechanisms of quality control need to be put in place, more especially in developing countries (Kennedy *et al.*, 2003).

Table 2.4: Recommended strategies to control iodine deficiency in pregnant and lactating women and in children from birth to 24 months of age as proposed by the WHO Secretariat[1]

Country/region salt iodization status	Recommended interventions		
	lactating women	***Children 0–6 months old***	***Children 7–24 months old***
Effective and sustained salt iodization (more than 90% of the households consumed iodised salt)	Universal Salt Iodization	Exclusive breastfeeding	Universal Salt Iodization and ensure iodine fortified complementary foods.
Uneven or lapsed iodized salt distribution (between 20 and 90% of households consume iodized salt)	Supplement with an oral daily dose of potassium iodide (250µg) or give a single annual oral dose of 400mg of iodine as iodised oil	1. Exclusive breast-feeding. 2. Measure should be taken to ensure the mother received iodine supplements during pregnancy and lactation	1. Give additional iodine as soon as possible after 6months through complementary foods fortified with iodine. 2. Breast-feeding should be sustained.
Weak or negligible iodised salt distribution (less than 90% of the households consumed iodised salt)	1. Give iodine supplement: As a single annual oral dose of 400mg of iodine as iodised oil or if feasible, as a daily oral dose of iodine as potassium iodide so that the total iodine intake is 1 RNI or 250g of iodine per day, either alone or combined with other minerals and vitamins. 2. Iodine supplements should not be given if the mother has already received iodised oil during her current pregnancy or up to 3months before her pregnancy started.	1. Exclusive breast-feeding. 2. Measure should be taken so that the mother received iodine supplements during pregnancy and lactation	1. Give additional iodine as soon as possible after 6months through complementary foods fortified with iodine. 2. Breastfeeding should be sustained.

[1] Adapted from Andersson, *et al.* 2007.

2.8.1. Iodine in salt

Salt has been identified has the most appropriate vehicle for iodine fortification and universal salt iodization, although a challenge, has been the most simple and cost effective way of combating iodine deficiencies in the general population, because it can be easily monitored in most countries (Zimmermann, 2009a; Horton & Miloff, 2010). The World Health Organization has designed a monitoring tool for assessing a country's progress in universal salt iodization programs, known as the Iodized Salt Program Assessment Tool (ISPAT). Most countries with salt iodization programs fortify from 10-40 ppm of iodine per gram of salt. The WHO considers household salt to be adequately iodized when it contains 15-40 ppm iodine (WHO *et al.,* 2007). Despite the global effort to ensure high coverage of iodized salt, some nations still have poor coverage of household access to iodized salt. Figure 2.7 below shows the household coverage with iodized salt in many countries around the world as reported by the iodine global network (IGN).

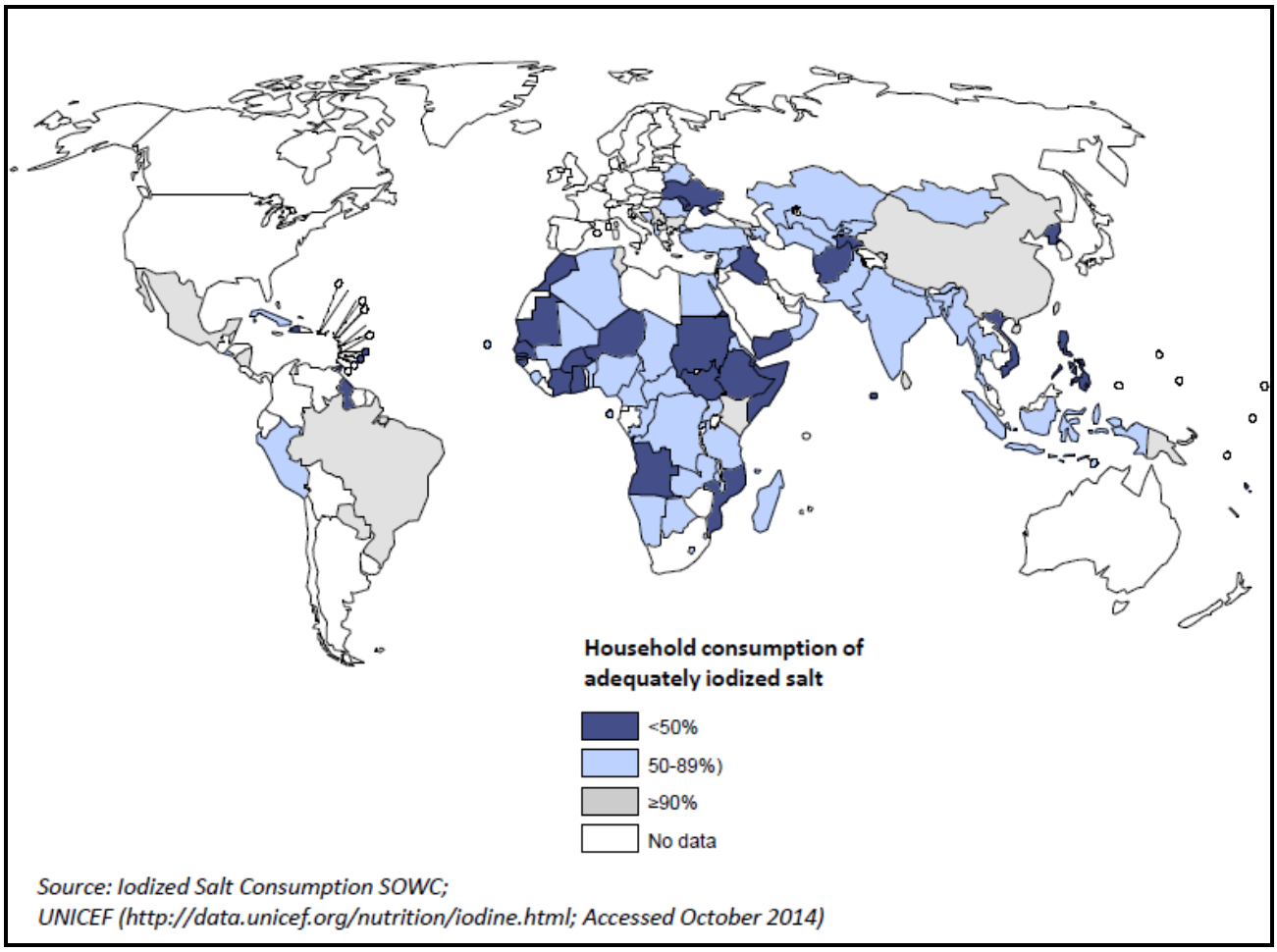


Figure 2.7: Household consumption of adequately iodized salt 2014 (source: www.ign.org)

A recent national report on salt iodization coverage in Cambodia found that in 2014 62% of household salt samples were non-iodized and subsequently more than 60% of mothers and their children were iodine deficient (Laillou *et al.*, 2016). The reported median urinary iodine concentrations were 63 µg/L and 72 µg/L in mothers and children, respectively (Laillou *et al.*, 2016).

Although it is not recommended to add salt to infants foods (Cribb *et al.*, 2012), iodized salt can contribute indirectly to the iodine status of infants via breast milk in areas with successful salt iodization programme (Andersson *et al.*, 2007; Hess *et al.*, 2015).

Salt intake and public health efforts to reduce sodium intake

As more and more countries move through a nutrition transition, there is a growing global concern on excessive salt intakes that has led to the rise of non-communicable diseases such as hypertension, which is the leading cause of 9.4 million annual deaths (Lim *et al.*, 2012). Therefore, global efforts to increase the consumption of iodized salt need to work in collaboration with salt reduction programs to ensure the effective implementation of salt reduction policies (WHO, 2014).

The mean salt consumption in South Africa is 6-12 g per day per person, which is higher than the WHO recommendation of ≤5 g of salt (<2000 mg sodium) per day per person (Wentzel-Viljoen *et al.*, 2013). Increased salt intake may lead to high blood pressure which is a risk factor for hypertension, cardiovascular disease, stroke and coronary heart disease (Wentzel-Viljoen *et al.*, 2013). In 2000 hypertension caused 9% of all deaths in the country (Norman *et al.*, 2007). Based on this, development of policies are currently on-going to reduce the salt intake of the general population. Salt intake as low as 5 g per day are known to provide an adequate amount of iodine, as far as the salt is sufficiently iodized, therefore, planned efforts to lower population sodium intake in the country will not interfere with salt iodization regulations (Charlton *et al.*, 2013). Moreover, these are two significant public health policies that can be complementary, but it is important that the messages that are conveyed are integrated to avoid confusion (Federal Commission for Nutrition, 2013).

2.8.2. Iodine fortification of complementary foods

It is important that the nutritional needs of infants are met in their complementary foods (Monte & Giugliani, 2004). From the age of six months, infants should be introduced to nutritionally adequate, safe and appropriately-fed complementary foods, whilst breastfeeding is continued until two years of age or more (WHO, 1998; Lutter & Dewey, 2003). In order to meet the WHO/UNICEF/ICCIDD recommendations for daily iodine intake, complementary foods must be fortified with 90 µg of iodine (Dunn, 2003; WHO *et al.,* 2007). Iodized salt may not directly provide for an infant's iodine needs as the addition of salt to home-prepared complementary

foods is not encouraged (Andersson *et al.*, 2007; Cribb *et al.*, 2012). Furthermore, the developing kidneys of infants may not tolerate the potential detrimental effects of excessive sodium intakes (Cribb *et al.*, 2012).

Alexy *et al*. (2009) reported on the iodine concentration of certain complementary food products of ten manufacturers in Germany. Of all the surveyed, products 64% were fortified with iodine (100% of formula and 51% of complementary foods) (Alexy *et al.*, 2009). Although infant formula and follow-on formula had a minimum iodine content of 7µg/ 100 g, follow-on formula had a higher variation of iodine content with median and maximum content above the infant formula. Commercial cereals were fortified in varying degrees, with the highest variation in fortified milk-cereal-porridges (Alexy *et al.*, 2009). In order to ensure that infants fed on home-made complementary cereals have access to adequate iodine intake, the food industry needs to be encouraged to produce whole iodine fortified infant cereals (Alexy *et al.*, 2009).

In Switzerland, Andersson *et al*., (2010) reported that, infants who were not receiving iodine-fortified infant formula milk during the weaning period, had insufficient iodine intake. Furthermore, breast fed infants who did not receive any iodine fortified infant formula milk, were at higher risks of lower iodine intake compared to breastfed infants who also received iodine fortified formula milk. Breastfed infants, who received home-prepared complementary foods without added salt or with little salt, were at the highest risk of low iodine intakes. In this study lactating mothers had low iodine intakes despite the 80% of the households that had access to adequately iodized salt (>15 ppm) (Andersson *et al.*, 2010). According to WHO (2011), micronutrient powders and small-quantity lipid-based nutrient supplements (SQ-LNS) can be potential vehicles for adding iodine into home-prepared complementary foods for infants (WHO, 2011). As far as could be established, only one study to date has assessed the iodine status of complementary fed infants receiving iodine fortified SQ-LNS (Hess *et al.*, 2015). Findings from this study showed that in an area with a working salt iodization programme and a high breastfeeding rate, median UIC in infants indicated adequate iodine status. The daily provision of 90 µg iodine in the form of SQ-LNS to these infants, did not affect measured indicators of iodine status (urinary iodine concentration (UIC), thyroid stimulating hormone (TSH) or thyroglobulin (Tg) (Hess *et al.*, 2015).

2.8.3. Iodine supplementation in infancy and lactation

Iodine supplementation was designed as a temporary measure to address iodine deficiency in regions where salt iodization could not be implemented or was unsuccessful (Untoro *et al.*, 2010).

Current recommendations by the WHO/UNICEF/ICCIDD state that in iodine deficient populations, where access to iodized salt is inadequate (<90%), pregnant and lactating women

and young children should be supplemented with iodine and breastfeeding should be sustained (Table 2.4) (Andersson *et al.*, 2007; De Benoist *et al.*, 2008). For exclusively breastfed infants, the lactating mother should be supplemented with either a daily dose of potassium iodide to meet the total iodine intake requirement of 250 µg/day, or be provided with an annual dose of 400 mg iodine as iodized oil. In case an infant is not exclusively breastfed and in the absence of iodine fortified complementary foods, infants should be supplemented with 90 µg iodine daily or an annual dose of 200 mg (Andersson *et al.*, 2007). In a moderate-to-severe iodine deficient area without effective salt iodization, supplementation of one dose of oral iodized oil (400 mg) in lactating mothers soon after delivery, was more effective in improving iodine status of breastfed infants, as compared to direct supplementation of infants (Bouhouch *et al.*, 2014). These results support the recommendations by WHO/UNICEF/ICCIDD for lactating women in iodine deficient areas; however, the results regarding supplementation in infants cannot be adopted, because of the lower dose (100 mg) used in the study as compared to what is recommended by the WHO/UNICEF/ICCIDD (200 mg) and also the high rate of exclusive breastfeeding in the trial, which is not comparable to some countries (Bath, 2014).

Previously Cobra *et al.*, (1997) demonstrated that in an iodine deficient area, maternal iodine supplementation and also supplementing infants with 100 mg of iodine at six weeks of age, may reduce the risks of infant mortality. In New Zealand, iodine supplementation improved cognition in previously mildly iodine deficient school children (Gordon *et al.*, 2009). Supplementation with 150 µg of iodine daily for 28 weeks increased median UIC from 16.4 µg/L to 145 µg/L. Supplementation also resulted in significantly improved performance in the iodine group compared to the placebo group in two of the four subtests, after confounding for sex, method of recruitment, cohort, ethnicity and household income (Gordon *et al.*, 2009).

Although effective, the down fall of iodine supplementation is that it is less likely to reach high coverage in a fast, cost effective and sustainable way (Untoro *et al.*, 2010). Furthermore implementation of supplementation programs requires political commitment, continuous education and a comprehensive monitoring system, therefore making it less sustainable in eliminating iodine deficiency as compared to universal salt iodization (Untoro *et al.*, 2010; Speeckaert *et al.*, 2011).

2.9. The situation of iodine nutrition in South Africa

In South Africa table salt iodization (40 to 60 ppm of iodine, fortified in the form of potassium iodate) was made mandatory in December 1995 through a revised legislation as a public health measure to avoid the severe consequences of iodine deficiency (Jooste *et al.*, 1995a). This legislation meant that the availability of iodized salt in supermarkets will increase by more than

30% within a period of 6 months (Jooste *et al.*, 1995b; Jooste *et al.*, 2000). This legislation was further revised in 2006 to the levels 35 to 65 ppm. Since then South Africa has made remarkable progress in the elimination of endemic goiter and iodine deficiency (Jooste & Zimmermann, 2008). In 1998, about 62.4% of households in the country had access to adequately iodized table salt (salt containing more than 15 ppm of iodine) (Jooste *et al.*, 2001). This percentage increased to 77% in 2005 (Jooste *et al.*, 2007) The 2005 South African Food Consumption Survey (NFCS-FB) indicated adequate overall iodine nutrition in women of reproductive age and school children. The distribution of median urinary iodine concentration was 176.8 µg/L and 214.8 µg/L in women and children, respectively (Jooste *et al.*, 2007). School children are often targeted for the monitoring of iodine nutrition in population studies because they are easily available as subjects and they are often more vulnerable to the adverse effects of iodine deficiency (WHO *et al.,* 2001).

2.9.1. Iodine nutrition in infants and young children in South Africa

The iodine status of infants and their lactating mothers during the breastfeeding and weaning periods, have not been previously researched in South Africa, hence the relevance of this PhD study. During infancy breast milk is the most important source of iodine for breast fed infants, provided the lactating mother consumes adequately iodized salt and iodine containing foods (Azizi & Smyth, 2009). It is, therefore, important to ensure that the lactating mother has a sufficient iodine status to cater for her own needs as well as that of her infant. Research findings from other countries that have effective salt iodization programs like South Africa, have found adequate, inadequate or excessive iodine status in infants and lactating women (Pearce *et al.*, 2007; Wang *et al.*, 2009; Andersson *et al.*, 2010).

The WHO recommends exclusive breastfeeding for the first six months of life for optimal growth, health and development (WHO, 2010). Breast milk is well endowed to meet all the nutrient requirements for infants younger than six months. At the age of six months infants should be introduced to age appropriate complementary foods, together with continued breastfeeding up to two years, in order to meet the expanding needs of the growing infant (WHO *et al.,* 2001; WHO, 2010). South Africa adopted these WHO feeding guidelines in an effort to improve infant and young child nutrition (Department of Health, 2013). However, the infant and young child feeding practices in the country remain sub-optimal. Faber et al. (2014) reported a continued breastfeeding rate of 14.4% in infants aged 18-24 months (Faber *et al.*, 2014). According to the South African National Health And Nutrition Examination Survey (SANHANES-1), the rate of breastfeeding is 75.1% with exclusive breastfeeding rates of only 7.4%, although nationally 83.0% of children younger than two years received breast milk within an hour of birth (HSRC, 2013). On average infants under the age of two years are breastfed for only 5.9 months (HSRC, 2013). When these data were collected, the WHO recommendations with regards to infant

feeding and HIV was that women should exclusively breastfeed for six months unless replacement feeding is acceptable, feasible, affordable, sustainable and safe (WHO & UNICEF, 2007; HSRC, 2013). A previous study in the country reported that mothers intentionally made decisions not to breastfeed already before giving birth (Doherty *et al.*, 2012) and these decisions were either linked to HIV stigmatization or lack of education on how to breastfeed and the importance of exclusive breastfeeding (Doherty *et al.*, 2012; Ijumba *et al.*, 2014).

The complementary feeding period for infants is a transition to foods other than breast milk or formula milk (Fein *et al.*, 2008). The South African national data report that infants are introduced to semi-solid or solid foods on average at the age of 4.5 months regardless of locality, province or race (HSRC, 2013). Commercial infant cereal (51.2%) is reported to be the most common food first introduced to infants, followed by homemade (29.0%) cereal/porridge, pureed vegetables/fruits and traditional baby food (3.1%) and other foods (HSRC, 2013). This finding is contrary to what was reported by earlier individual studies within the country that found maize-meal porridge to be the most commonly used complementary food for infants (Mamabolo *et al.*, 2004; Faber, 2005; Mushaphi *et al.*, 2008). The maize meal porridge in the country has been mandatorily fortified with iron, vitamin A and zinc (Department of Health, 2003), however, not with iodine. Pelto *et al.,* (2013), suggested that home fortification through the use of micronutrient powders or small-quantity lipid-based nutrient supplements, could be a potential way to address micronutrient decencies in the country.

2.10. Study site

The current research was conducted in two districts situated approximately 40 km apart in the North-West Province, South Africa (Figure 2.8). The first study site was in two peri-urban settlements (Ikageng and Promosa) situated on the fringes of Potchefstroom in the Kenneth Kaunda District municipal area. The second study site was a peri-urban settlement (Jouberton) situated in the greater Matlosana municipality in Klerksdorp. Jouberton is 200 km from Johannesburg which is the nearest metropolitan area. The site has a relatively stable population migration.

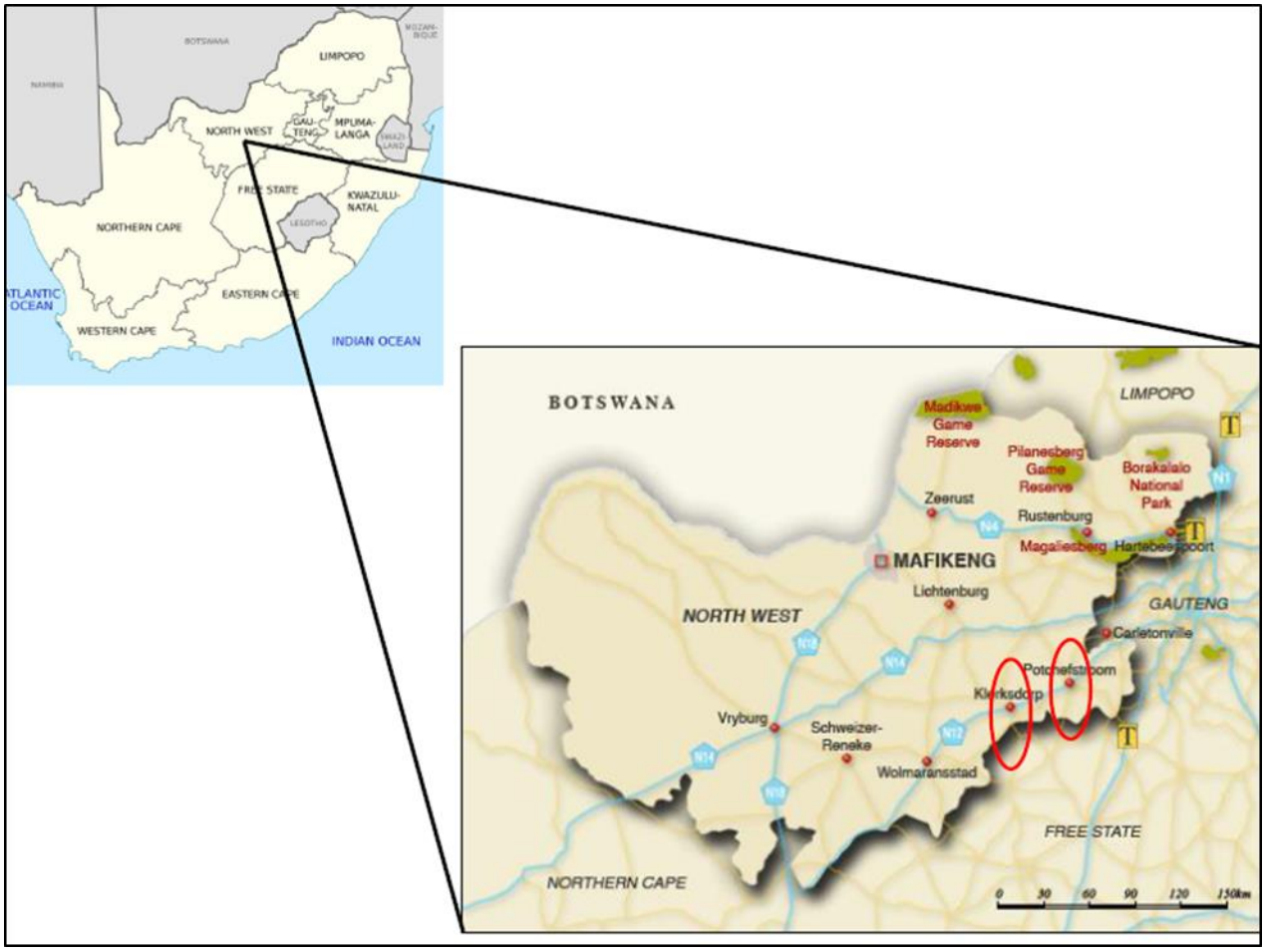


Figure 2.8: Location of North-West Province and the two study areas, Potchefstroom and Klerksdorp within South Africa (source: courtesy of Google images)

2.11. Conclusion

Iodine is an essential micronutrient required for the production of thyroid hormones (Eastman & Zimmermann, 2009). The primary source of iodine for humans in the diet is through consumption of iodine-fortified foods, including salt (WHO *et al.,* 2007). For breastfed infants living in iodine sufficient areas, breast milk can provide enough iodine provided the lactating mother has adequate iodine intake. Complementary fed infants in the same regions need to also have access to iodine fortified complementary foods, whilst breastfeeding is continued (Andersson *et al.*, 2007). Both Iodine deficiency and excessive iodine intake can lead to the malfunction of the thyroid gland (Zimmermann *et al.*, 2008; Leung & Braverman, 2014). Iodine deficiency, especially in infancy, leads to irreversible impairment of mental development and growth (Zimmermann, 2009a; Zimmermann, 2011). Infants are more susceptible to iodine deficiency because their iodine and thyroid hormone requirements per kg of body weight are higher than any other age group (Zimmermann, 2009a). South African women of reproductive age and school children have adequate iodine status (Jooste *et al.*, 2007); however, no data exist on the iodine status of lactating mothers and infants who are breastfed and infants receiving complementary foods.

2.12. References

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CHAPTER 3

Breast milk iodine concentrations, iodine status and thyroid function of breastfed infants aged 2-4 months and their mothers residing in a South African township

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Abstract

Background: Lactating women and infants are susceptible to iodine deficiency and iodine excess. In South Africa, no data exist on the iodine status and thyroid function of these vulnerable groups.

Methods: In a cross-sectional study, we assessed urinary iodine concentrations (UIC), thyroid function and breast milk iodine concentrations (BMIC) of 100 lactating women and their 2-4 month-old breastfed infants from a South African township, and explored potential predictors of UIC, thyroid function and BMIC, including household salt iodine concentrations (SIC) and maternal sodium excretion.

Results: The median (25^{th}-75^{th} percentile) UIC was 373 (202-627) µg/L in infants and 118 (67-179) µg/L in mothers. Median household SIC was 44 (27-63) ppm. Household SIC and maternal urinary sodium excretion predicted UIC of lactating mothers. Median BMIC was 179 (126-269) µg/L; Age of infants, SIC and maternal UIC predicted BMIC. In turn, infant age and BMIC predicted UIC of infants. Forty-two percent of SIC fell within the South African recommended salt iodine fortification level at production of 35-65 ppm, whilst 21% of SIC were >65 ppm. Infant dried whole blood spot (DBS) TSH, TT4 and Tg concentrations were 1.3 (0.8-1.9) mU/L, 128±33 mmol/L and 77.1 (56.3-105.7) µg/L, respectively, and did not correlate with infant UIC or BMIC.

Conclusion: Our results suggest that the salt fortification programme in South Africa provides adequate iodine to lactating women, and indirectly to their infants via breast milk. However, monitoring of salt iodine content of the mandatory salt iodization program in South Africa is important to avoid “over-iodization” of salt.

Introduction

Dietary iodine is an essential substrate for thyroid hormone (thyroxine; T_4 and triiodothyronine; T_3) synthesis and is as such required for normal brain development, growth and metabolism (1). Both low and high iodine intake can lead to thyroid dysfunction (2). Infants may be particularly vulnerable to iodine deficiency and iodine excess as the fetal and newborn thyroid has limited iodine stores and adapts poorly to high intakes (3-5). Acute iodine excess from for example maternal iodine supplements (6) and iodine containing skin disinfectants (7) may cause hypothyroidism in newborns. Recent data indicate that older infants may be able to adapt to high iodine intakes and maintain euthyroidism (8). However, little is known about the effects of habitual high iodine intake on thyroid function in breastfed infants.

Programs of universal salt iodization have made remarkable progress in improving iodine status worldwide, but in a handful of countries salt iodine fortification is poorly monitored and the iodine intake is excessive (1, 5).

In South Africa, iodization of table salt to a concentration of 35-65 ppm at the point of production was revisedin 2006/2007 (9) in order to achieve a level of 30 ppm at retail and 15 ppm in households (10). The legislation does not involve fortification of agricultural salt or salt for processed foods. The introduction of universal salt iodization remarkably improved the iodine status in school children and women of reproductive age. The 2005 South African National Food Consumption Survey (NFCS-FB-I) reported a median urinary iodine concentration (UIC) in South African school children and women of reproductive age of 215 µg/L and 177 µg/L, respectively, indicating overall adequate iodine intake (11). However, more recent data point gaps in iodine nutrition of South Africans, as more than a third of the population still lacks access to adequately iodized salt (9, 12). Furthermore, no data exist on iodine status in lactating women and infants.

The iodine requirements as recommended by WHO increase to 250 µg during lactation: additional to the recommended daily intake of 150 µg iodine for women of reproductive age, lactating women should consume 100 µg/day extra in order to cover the additional iodine need of their breastfed infants (10). Breastfed infants depend on iodine from breast milk for the synthesis of thyroid hormones and to build up intra-thyroidal iodine stores (13, 14). Breast milk iodine concentrations (BMIC) are determined by the maternal iodine intake; population medians have been shown to range from 9-32 µg/L in iodine deficient goitrous areas to 146 µg/L in iodine sufficient Chinese women (15, 16). The WHO recommends a dietary iodine intake of 90 µg/day for infants (0-6 months) (10).

Population iodine status is assessed by UIC, as 90% of ingested iodine is excreted through the renal system and median spot UIC directly reflects recent dietary iodine intake (1, 10). In

lactating women and in children <2 years, a median UIC <100 µg/L indicates insufficient iodine intake (10).

Measurement of serum or dried blood spot thyroglobulin can be an additional useful biomarker of iodine status to accompany UIC measurements. Zimmermann *et al.*, showed that Tg is a sensitive marker for both low and high iodine intakes in school aged children (17). Tg is also a sensitive indicator for iodine deficiency in adults (18, 19).

Despite the importance of adequate iodine status and thyroid health in lactating women and their breastfed infants, to date, no data exist on BMIC or iodine status of infants and lactating South African women. This study therefore assessed iodine status, BMIC and thyroid function of breastfed infants and their lactating mothers living in a township located in the North-West Province of South Africa, and further explored potential predictors of UIC, thyroid function and BMIC.

Methods

Participants

This study included a convenient sample of 100 apparently healthy infants aged 2 to 4 months and their lactating mothers residing in two peri-urban settlements (Ikageng and Promosa) on the fringes of Potchefstroom in the Kenneth Kaunda District municipal area, in the North West Province of South Africa. The majority of residents in these townships are of Black African descent, the socio-economic status is low and unemployment is high. Recruitment of mother-infant pairs was done at local health clinics in Ikageng and Promosa. Infants included in the study were: 1) generally healthy; 2) singletons; 3) had no history of thyroid disease; 4) currently being breastfed and; 5) not using any iodine containing supplements. Mothers included in the study were: 1) generally healthy; 2) had no history of thyroid disease; 3) currently breastfeeding and; 4) not using any iodine containing supplements.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Health Research Ethics Committee of the North-West University (NWU-00016-13-A1). Permission was also granted from the Provincial and District Health Departments in the North West Province to recruit mother-infant pairs for this study at local health clinics. The study protocol was fully explained by a trained study assistant fluent in the local language (Setswana or Afrikaans) and written informed consent was obtained from participating women (Addendum 3).

Data collection

The study design was cross-sectional. Lactating mothers and their infants were invited to the metabolic clinic at the North-West University, South Africa, where the study procedures were conducted between 0800hrs and 1200hrs. Mothers were asked to bring samples of salt (10 g) and water (10 ml) from their homes. Upon arrival the study protocol was fully explained to the mothers in their home language (Setswana or Afrikaans) and they signed informed consent. A detailed questionnaire was used to collect information on socio-economic characteristics, use of iodized salt, consumption of iodine containing foods, use of iodine containing supplements (currently and during pregnancy) and breastfeeding practices (Addendum 4). Breastfeeding practices were divided into three categories, namely; 1) *Exclusive breastfeeding*; 2) *Predominantly breastfeeding*; 3) *Partial breastfeeding* (20).

Height and weight of mothers and weight, length and head circumference of infants were measured using standard anthropometric techniques (21). For the measurements, mothers removed their shoes, emptied their pockets, and wore minimal clothing. Height measurements were done using a rigid stadiometer and recorded to the nearest 0.5 cm. Weights of the women were measured on a high capacity electronic flat scale (seca 813; Germany) and recorded to the nearest 0.1 kg. Measurements of infants were done using an infant scale (seca 334; Germany) to the nearest 2g with no clothing or nappy. To measure length, infants wore only their nappy, and measurements were taken to the nearest centimeter on a ShorrBoard portable height-length measuring board with auto-lock sliding foot piece (Weigh and measure, LLC; USA). Head circumference was also measured using a head circumference measuring tape for infants (seca 212; Germany) to the nearest centimeter. BMI-for-age z-scores (BAZ) were calculated using the WHO (2006) growth standards. Wasting was defined as BAZ <-2, normal weight as BAZ ≥-2 and ≤2, risk for overweight as BAZ >1, and overweight as BAZ >2 (22).

A standard breakfast was served to the mothers at arrival at the metabolic clinic and before collection of biological samples. Spot urine samples (5 ml) were obtained from the mothers (within a maximum of 30 minutes after breakfast consumption), aliquoted, and stored at -80ºC until analysis. Breast milk samples (5 ml of fore milk) were obtained by manual expression. To obtain fore milk, mothers were asked to express milk from the breast that was not used at the last feed. The baby was then allowed to suckle the breast until fully satisfied. Breast milk samples were aliquoted and stored at -20ºC until analysis. Spot urine samples were collected from infants using a urine collection pad (SteriSets Uricol Set), aliquoted and stored at -80ºC until analysis. Whole blood obtained via venipuncture or foot prick was spotted onto filter paper (Whatman 903; GE Healthcare) and allowed to dry at room temperature for 24 hours. They were then stored at -20ºC in sealed low-density polyethylene bags containing desiccant packets until analysis of thyroid hormones.

Laboratory analyses

UIC in spot urine samples from infants and mothers was measured in duplicate at the North-West University in Potchefstroom using the Pino modification of the Sandell-Kolthoff reaction with spectrophotometric detection (10, 23). The laboratory successfully participates in the Program to Ensure the Quality of Urinary Iodine Procedures (EQUIP, U.S. Centers for Disease Control and Prevention, Atlanta GA, USA) (24). Iodine in spot urine samples from infants and lactating mothers were expressed as median concentrations (µg/L). A median UIC greater than 100 µg/L was considered to indicate adequate iodine intake in lactating women and infants (10).

In lactating mothers we additionally determined the iodine:creatinine ratio (µg iodine/g creatinine) to reduce the intra-individual variation in daily urine volume and also to adjust for fluid intake (25, 26). Urinary creatinine and sodium concentrations in spot urine from mothers were analyzed using the UniCel® DxC800 System (Beckman Coulture) at a commercial pathological laboratory (Ampath Johannesburg).

BMIC was analyzed at the Laboratory of Human Nutrition of ETH Zurich, Switzerland (27). Iodine was extracted from the samples using a modified TMAH extraction procedure (28, 29). The iodine content in filtered TMAH extracts was measured using a multicollector inductively coupled plasma mass spectrometer (MC-ICP-MS [Finnigan NEPTUNE, Thermo Scientific™ Waltham, MA, USA]). Quantification was done using isotope dilution analysis (IDA) with ^{129}I (SRM 4949C, National Institute of Standards and Technology NIST, Gaithersburg, MD, USA). Tellurium (AppliChem, Darmstadt, Germany) was used for mass bias correction of the measured ^{127}I/^{129}I intensity ratio according to Russell's law. The iodine concentrations of the milk samples were calculated using the dilution factors applied to each milk sample. Standard reference material (SRM 1549a, Whole Milk Powder, National Institute of Standards and Technology, Gaithersburg, MD, USA) was analysed as external control with each ICP-MS run (30). The method was recently validated at the Human Nutrition Laboratory of ETH Zurich, Switzerland. The mean (±SD) iodine content for the NIST SRM1549a reference material was 3502 (±89) ng/g (n = 16), well within the certified acceptable range (3040–3640 ng/g). The total-assay variability of the method is 2.6%. The within-assay variability is 1.1% and the between-assay variability is 1.3%. The limit of detection of the method (LOD) is 0.26 ng/g.

Dried whole blood spots were analyzed for TSH (DELFIA NeoTSH kit, PerkinElmer Life Sciences, Turku, Finland) and TT4 (Delfia Neonatal T4 kit, PerkinElmer Life Sciences, Turku, Finland) using automated fluoroimmuno assay (31). Analysis of DBS-Tg was done by a new sandwich ELISA assay that was recently developed and validated at the Human Nutrition Laboratory of ETH Zurich, Switzerland (32). Serum control samples (Liquicheck Tumor Marker Control, Bio-Rad, Hercules, CA, USA) were used as standards for the DBS-Tg assay. Normal

reference ranges for TSH and T4 as supplied by the manufacturer were as follows: TSH of 0.1-4.5 mU/L and 0.1-3.7 mU/L for 60-155 day-old infants and for subject 1-99 years of age, respectively; TT4 of 80-165 nmol/L and 65-165 nmol/L for 60-155 day-old infants and of for subject 1-99 years of age, respectively. Normal range reference values for DBS-Tg are only available for school-age children (4–40 µg/l), but not for young children and infants (33, 34).

Salt iodine concentrations (SIC) and water iodine concentrations (WIC) were determined by using the Pino modification of the Sandell-Kolthoff reaction with spectrophotometric detection (23). Household SIC was expressed as median and classified into three categories, according to ranges in ppm based on the 2006/2007 South African mandatory fortification level for table salt at the point of production (9). Salt samples were considered inadequately, adequately and "over-iodized" when SIC was <35 ppm, 35-65 ppm and >65 ppm, respectively. Household SIC were also classified into the three fortification categories indicating inadequately (SIC <15 ppm), adequately (SIC 15-40 ppm) or excessively (SIC >40 ppm) iodized salt at household level as recommended by WHO (35).

Data on the intake of potentially iodine-rich foods in lactating mothers were collected using an unquantified food frequency questionnaire and presented as the number (%) of mothers who consumed specific iodine-rich foods.

Statistical analysis

All data processing and analysis was done using IBM SPSS statistics version 20. Data were checked for normality using Q-Q plots and the Shapiro-Wilk test. Normally distributed data were presented as mean ± SD. Non-normally distributed data were presented as median (25^{th}- 75^{th} percentiles) values. For between-group comparisons the Mann-Whitney or Kruskal-Wallis tests were used for non-parametric data. Spearman correlations were performed to determine associations between variables. Multiple linear regression analyses were used to explore whether household SIC, salt intake of mothers (urinary sodium excretion), UIC of mothers (only for BMIC and UIC of infants as dependent variable), age of mothers and infants, and BMIC (only for UIC of mothers and infants as dependent variables) are predictors of BMIC and UIC in lactating mothers and breast fed infants. Other dietary and maternal factors (e.g mode of breastfeeding and delivery, smoking habits, HIV status, etc.) were also tested using a stepwise procedure, but none of those were significant predictors of BMIC or UIC of mothers and infants and were therefore not included in the final regression models. Non-parametric dependent variables were transformed prior to analysis. Furthermore, we examined the odds ratios (OR) for infants to have abnormal thyroid hormone concentrations with excessive or inadequate iodine intake using binary logistic regression analyses, adjusting for age of mothers and infants, as well as HIV status of mothers. *P*-values < 0.05 were considered significant.

Results

One hundred mother-infant pairs participated in the study. Characteristics of the infants and mothers are shown in **Table 3.1**. Infants were aged 3-4 months (mean ± SD: 3.0 ± 1.1); 54 were females and 46 were males. Of all the infants, 67% were exclusively breastfed, whilst 9% and 24% were predominantly and partially breastfed, respectively.

The median (25^{th}-75^{th} percentiles) UIC of infants (n=92) was 373 (202-627) µg/L **(Table 3.2)**. The median UIC of mothers was 118 (67-179) µg/L and the iodine:creatinine ratio was 126 (86-207) µg/g. **Figure 3.1** illustrates the frequency distribution of infant and maternal spot UIC, and BMIC. Thirty-nine percent of mothers had UIC <100 µg/L, whereas, only 4% of infants had UIC <100 µg/L. Fifty-three per cent of infants had a UIC >300 µg/L and 26.1% >600 µg/L. UIC of mothers were positively correlated with the UIC of infants (r_s= 0.425, *P* <0.001) **(Figure 3.2A)**. Both, the UIC of infants and mothers were positively correlated with BMIC (infants: r_s= 0.552, P <0.001; mothers: r_s= 0.593, P <0.001) **(Figure 3.2B,C).** Infants of obese mothers had higher UIC (495.3 [141.8-1060.9] µg/L; p=0.04) than infants of mothers that were normal weight (n=39). We found no other association of UIC in infants and mothers with participant characteristics and frequency of iodine-containing foods consumed by mothers. Generally, cow's milk was consumed either every day or sometimes by 82% of mothers. Whilst 60% consumed fish sometimes and 69% consumed meat always **(Table 3.3)**.

Median BMIC was 179 (126-269) µg/L. Median SIC (n=85) was 44 (27-63) ppm. The majority of women (90%) used adequately iodized salt in the household (≥15 ppm) as defined by WHO, 42% consumed salt that was within the South African recommended salt iodine fortification level at production (35-65 ppm), whilst 21% of households consumed salt that was iodised above 65 ppm. Iodine in water collected from the different households was below detection limit (<10 µg/L).

Table 3.1: Characteristics of breastfed infants and their lactating mothers

Infants (n=100)	%	Mean	SD
Males (%)	46		
Females (%)	54		
Age (months)		3.0	1.1
Length (cm)		58.3	3.4
Weight (kg)		5.7	1.0
BMI-for-age z-scores [BAZ] (%)			
Wasted (BAZ < -2)	3		
Normal (-2 ≥ BAZ ≤ 1)	80		
Risk for overweight (BAZ >1)	11		
Overweight (BAZ >2)	6		
Mothers (n = 100)			
Age (years)		27.7	6.8
Number of children		2.2	1.2
HIV positive (%)	22		
Smoking now (%)	10		
Smoking before pregnancy (%)	8		
Education (%)			
Primary	5		
Secondary	90		
Tertiary	4		
Other	1		
Employed (%)	11		
Mode of delivery (%)			
Vaginal delivery	79		
Caesarean	21		
Height (m)		1.57	0.05
Weight (Kg)		66.5	14.7
BMI (%)			
Underweight (BMI < 18.5)	3		
Normal weight (BMI 18.5 - 24.9)	39		
Overweight (BMI 25.0 - 29.9)	31		
Obese (BMI ≥ 30)	27		
Breastfeeding practice (%)			
Exclusive breastfeeding	67		
Predominant	9		
Partial	24		

Table 3.2: Urinary iodine and thyroid hormone concentrations in South African lactating mothers and breastfed infants

Infants	n	Median	25th percentile	75th percentile
Urinary iodine concentration (µg/L)	92	373	202.0	627.0
Estimated 24h iodine intake from breast milk (µ/day)[2]	97	140	97.9	209.4
TSH (mU/L)	96	1	0.8	1.9
TSH > 4.5 mU/L (n [%])		1 [1]		
T4 (mmol/L)	97	128.0 (32.8)*		
T4 < 80 nmol/L (n [%])		5 [5.2]		
80 ≥ T4 ≤ 165 nmol/L (n [%])		81 [83.5]		
T4 > 165 nmol/L (n [%])		11 [11.3]		
Tg (µg/L) (n = 66)[1]		77	56.3	105.7
Subclinical hypothyroidism (n [%])		0 [0]		
Overt hypothyroidism (n [%])		0 [0]		
Hypothyroxinemia (n [%])		5 [5.2]		
Mothers				
Urinary iodine concentration (µg/L)	100	118	67	179
Iodine-creatinine ratio (µg/g)	100	126	86	207
Breast milk iodine concentration (µg/L)	100	179	126	269
Urinary sodium excretion in spot samples (mmol / g creatinine)	88	154	99	220
TSH (mU/L)	100	0.8	0.6	1.0
TSH > 3.7 mU/L (n [%])		0 [0]		
T4 (mmol/L)	100	69.6 (15.9)*		
T4 < 65 nmol/L (n [%])		43 [57]		
Tg (µg/L)	96	22.2 (14.4-30.7)	14.4	30.7
Tg > 40 µg/L (n [%])		16 [17]		
Subclinical hypothyroidism (%)	100	0		
Overt hypothyroidism (%)	100	0		
Hypothyroxinemia (%)	100	43		

Abbreviations: T4- Thyroxine; TSH-thyroid-stimulating hormone; Tg-Thyroglobulin

Subclinical hypothyroidism defined as elevated TSH (relative to age specific cutoffs) and normal T4; overt hypothyroidism defined as elevated TSH (relative to age specific cutoff) and low T4 (relative to age specific cutoffs); and hypothyroxinaemia defined as T4 less than age-specific cutoff and normal TSH.

[1]Eight values were above measuring range of 150 µg/L.

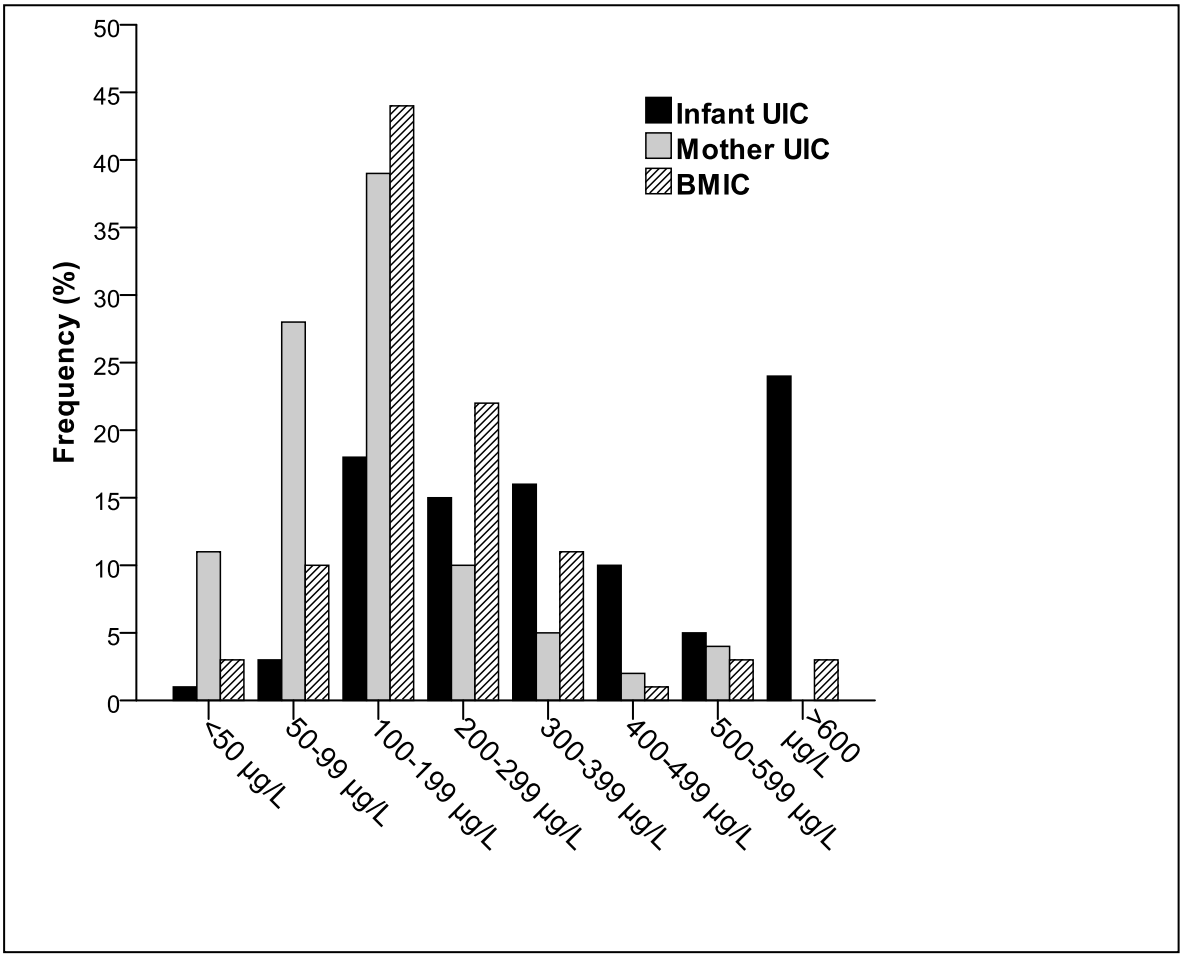

Figure 3.1: Frequency distribution of spot urinary iodine concentrations (UIC) of lactating mothers (n = 100) and their breastfed infants (n = 92), and breast milk iodine concentrations (BMIC) in µg/L. The UIC range indicating sufficient iodine intake in lactating women and infants is highlighted.

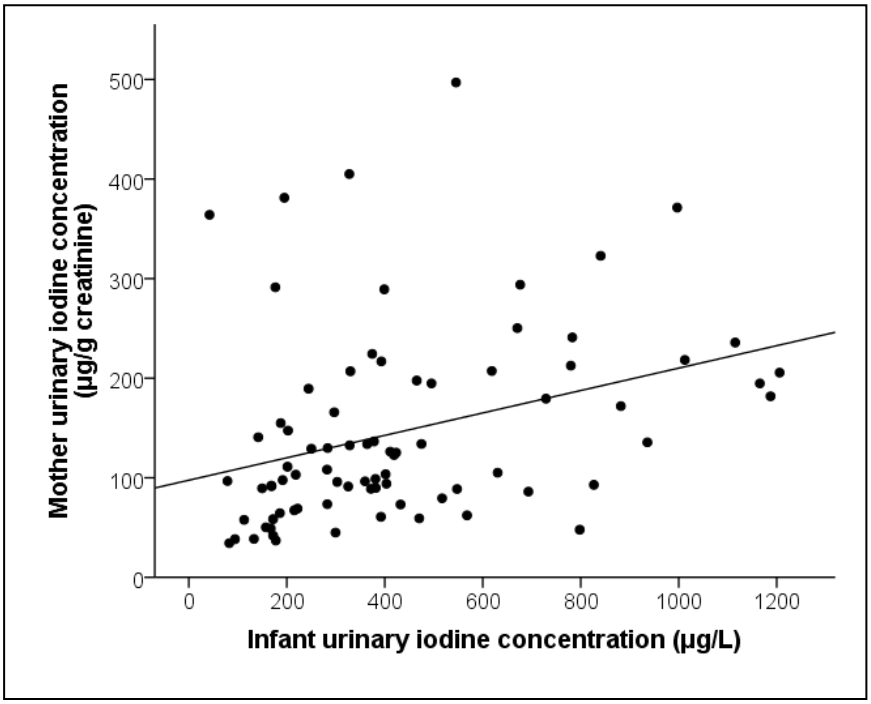

Figure 3.2 (A): Scatter plots indicating the spearman correlations between urinary iodine concentrations of South African lactating mothers and their breastfed infants (Spearman correlations: r_s = 0.425 and P <0.001)

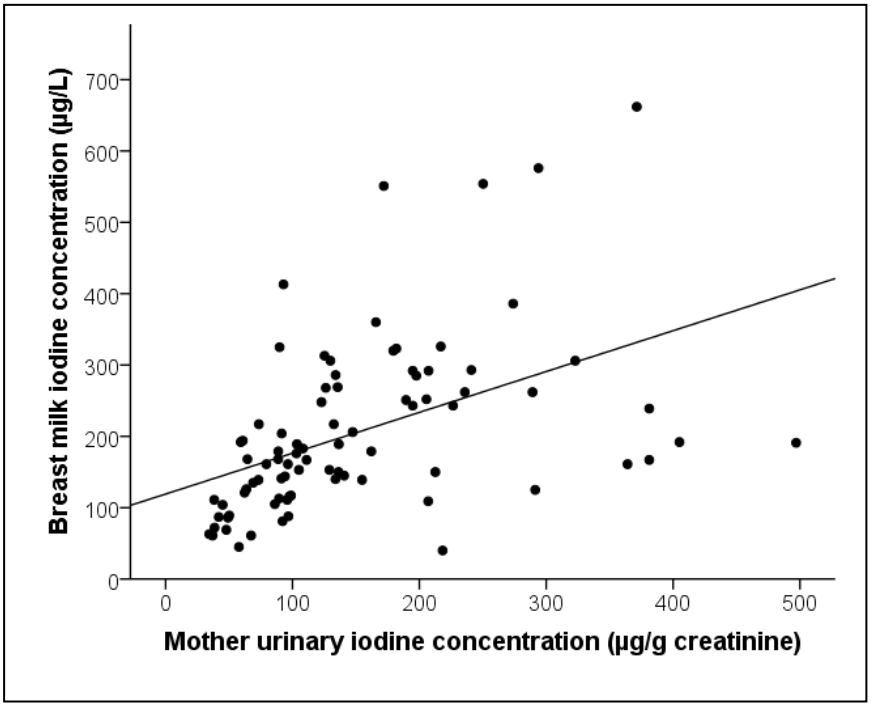

Figure 3.2 (B): Scatter plots indicating the spearman correlations between urinary iodine concentrations of lactating mothers and breast milk iodine concentrations (Spearman correlations: r_s = 0.593 and P <0.001)

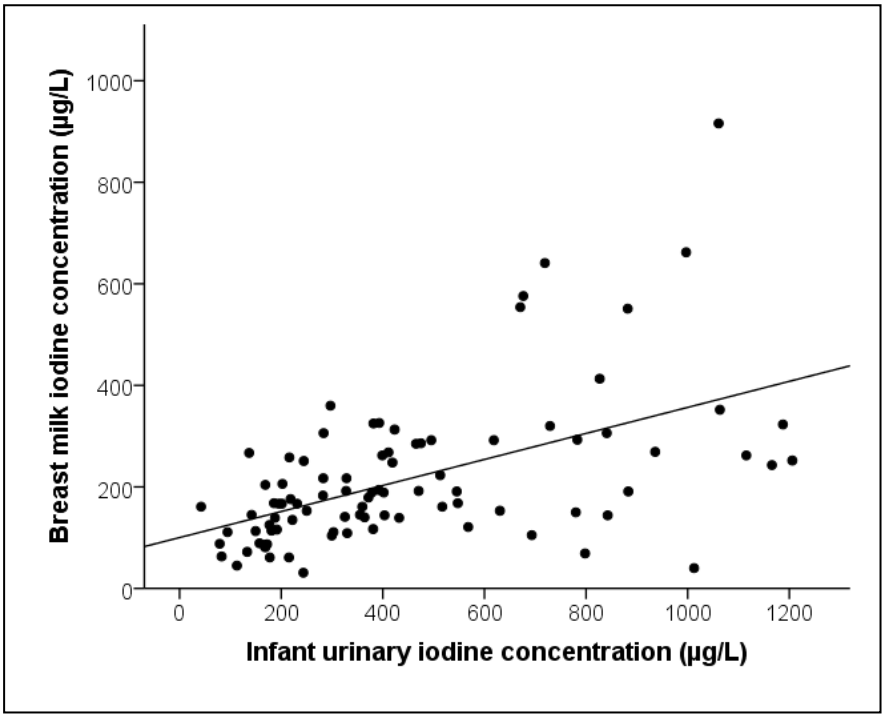

Figure 3.2 (C): Scatter plots indicating the spearman correlations between urinary iodine concentrations of infants and breast milk iodine concentrations (Spearman correlations: r_s = 0.552 and P <0.001).

The UIC of infants and their mothers were positively correlated with household SIC (infants: r_s= 0.341, P <0.001; mother: r_s= 0.252, P <0.001). The median UIC of mothers from households with SIC above 65 ppm (185 [117-411] µg/L) was higher than of mothers from households with iodized salt containing 35-65 ppm (105 [68-163] µg/L; P = 0.024), and households with SIC below 35 ppm (117 [62-136] µg/L; P = 0.004). Likewise, the median UIC of infants from households with SIC above 65 ppm (719 [478-911] µg/L) was higher than of infants from households with iodized salt at 35-65 ppm (346 [194-530] µg/L; P = 0.006), and with SIC below 35 ppm (250 [177-411] µg/L; P <0.001).

Table 3.3: Consumption patterns of iodine-containing foods

General frequency of consumption (%)	Foods				
	Cow's milk (n=100)	Fish (n=99)	Seafood (n=100)	Eggs (n=100)	Meat (n=99)
Never	4.0	16.2	89.0	7.0	1.0
Rarely	9.0	20.2	0	32.0	1.0
Sometimes	52.0	60.6	11.0	46.0	10.1
Often	5.0	3.0	0	6.0	18.2
Always	30.0	0	0	9.0	69.7

Using multiple linear regression analysis, household SIC and maternal urinary sodium excretion significantly predicted UIC of lactating mothers (**Table 3.4**). Household SIC, maternal UIC, and age of infants significantly predicted BMIC. In turn, BMIC as well as infant age significantly predicted UIC in infants.

Thyroid hormone concentrations in lactating mothers and their infants are shown in **Table 3.2**. Infant TSH, TT4 and Tg concentrations were 1.3 (0.8-1.9) mU/L, 128±33 mmol/L and 77.1 (56.3-105.7) µg/L, respectively. Mother TSH, TT4 and Tg were 0.8 (0.6-1.0) mU/L, 69.6±15.9 mmol/L and 22.2 (14.4-30.7) µg/L, respectively. We found that 99% of infants had TSH concentrations within the normal range. No associations of UIC were found with Tg, TSH and thyroid hormone concentrations in infants or mothers. However, median TSH concentrations were significantly higher in HIV positive (0.95 [0.0-1.7] mU/L) than HIV negative (0.7 [0.0-2.2] mU/L) mothers (P = 0.021). Further, maternal TT4 concentrations were associated with TT4 concentrations of their infants (r = 0.236, P = 0.020). TT4 concentrations were significantly lower in HIV positive (61.0 ± 15.3 nmol/L) than HIV negative (72.1 ± 15.3 nmol/L) mothers (P = 0.004). In turn, the odds for having low TT4 concentrations were significantly higher in HIV positive (TT4 < 65 nmol/L = 63%) than HIV negative (TT4 < 65 nmol/L = 37%) mothers (OR= 2.95, 95%CI: 1.11-7.90). We did not observe any significant differences in Tg concentrations by HIV status. Furthermore, the thyroid hormone status of the infants did not differ in regards to maternal HIV status.

Table 3.4: Predictors of BMIC and UIC in South African lactating mothers and breastfed infants

	UIC (µg/g creatinine) in mothers		BMIC (µg/L)		UIC (µg/L) in infants	
Multiple linear regression	**β**	***P***	**β**	***P***	**β**	***P***
Salt iodine concentrations (µg/g)	0.340	0.003	0.329	0.005	0.120	0.349
Sodium excretion (mmol Na/g creatinine)	0.461	<0.001	0.174	0.115	0.215	0.061
Age infant (months)	-0.084	0.455	-0.386	<0.001	0.281	0.019
Age mother (years)	0.123	0.241	0.001	0.989	0.126	0.216
BMIC (µg/L)	-	-	-	-	0.522	<0.001
Maternal UIC (µg/g creatinine)	-	-	0.325	0.005	-0.117	0.338
Adjusted R^2		0.243		0.273		0.347

Dependent variables were log-transformed to perform multiple linear regression analysis. UIC- urinary iodine concentration; BMIC- breast milk iodine concentration; Na- sodium.

Discussion

To our knowledge, this is the first study to report iodine status, breast milk iodine concentrations (BMIC), and thyroid function of breastfed infants and lactating mothers in South Africa. Our findings suggest adequate iodine status in both lactating women and their breastfed infants in this convenience sample. Based on a BMIC of 179 µg/L and a breast-milk consumption of 0.78 L at 3 months (36), infants consumed 140 µg iodine/day, well above the recommended daily iodine intake of 90 µg and 110 µg for infants below 6 months of age by WHO and the IOM, respectively (10, 36). WHO applies the threshold of ≥100 µg/L for the median UIC to determine iodine sufficiency in children less than 2 years of age. This cut-off sharply disagrees with the intake recommendations from both WHO and IOM. By assuming a urine volume in infants of approximately 500 ml/day and 90% bioavailability and excretion (10, 36), the UIC corresponding to the recommended dietary iodine intake of 90-110 µg/day would be in the range of 160-240 µg/L. Although, the median UIC in the infants in our study is more than 3 times higher than the WHO UIC threshold it should be noted that no range for median UIC reflecting optimal iodine nutrition during infancy has been defined. Considering the small urine volume in infants a wide UIC range is expected. Previous studies in Niger and Algeria also reported high median UICs in breastfed infants of 220 µg/L and 728 µg/L, respectively (33, 37, 38).

Lower median UICs were observed in exclusively breastfed infants from the Boston area in the U.S. (204 µg/L) and Switzerland (82 µg/L), which are both considered iodine sufficient populations (39, 40). The scientific basis for the dietary iodine requirements during infancy is weak. The IOM intake recommendation is an Adequate Intake (AI) because there were insufficient data to establish an Estimated Average Requirement (EAR) for this age group and no upper intake level has been defined (10, 36). Work is on-going to add data to this knowledge gap (ClinicalTrials.gov: Project NCT02045784).

The median Tg concentrations of 77.1 µg/L is six times higher than reported in iodine sufficient school aged children (17). Pediatric reference ranges for serum-Tg assays indicate physiologically elevated Tg concentrations during the first two years of life (41, 42). The Tg concentration gradually decline with age. Infant reference values are lacking for DBS-Tg as well as data on DBS-Tg in iodine sufficient breastfed infant populations. However, the magnitude of the elevated DBS-Tg concentration in infants in our study compared to median DBS-Tg concentration observed in iodine sufficient school children suggests that Tg production may increase in response to a marginally high iodine intake in infants. However, none of the infants had subclinical hypothyroidism, and we found no associations of infant UIC with Tg, TSH and thyroid hormone (T4) concentrations, indicating a possible adaptation of the thyroid gland.

Based on median UIC, lactating women in the present study have adequate iodine status. The iodine intake in the mothers is estimated to 320 µg/day; 140 µg iodine is excreted in the breast milk and 180 µg in the urine (assuming a urine volume of 1.5 liter/day). All women have normal TSH concentrations. The high prevalence of hypothyroxinaemia should be interpreted with caution; we applied the TT4 thresholds defined for women of reproductive age in the absence of normal reference ranges for maternal TT4 in breastfeeding women. Cross-sectional data suggest lower TT4 concentrations during early lactation (43, 44). In addition, we observed higher TSH and lower TT4 concentration in HIV positive women than in HIV negative women. Abnormalities in thyroid function of HIV patients have been previously described (45, 46). Most HIV positive mothers in our study reported to be on the highly active antiretroviral therapy (HAART). Increased prevalence of sub-clinical hypothyroidism is known to be found in HIV treated patients, especially those on HAART (47, 48). Madeddu *et al.* emphasized the need to sequentially check thyroid function in HIV patients on HAART after they observed elevated TSH levels in these patients as compared to naive patients and controls (49). This might be particularly crucial in lactating women, considering the positive associations that we observed between TT4 concentrations in mothers and their infants.

Confirming the influential role of maternal iodine intake on the iodine status of breastfed infants (15), we found maternal UIC to be a predictor for BMIC, which in turn was a predictor for UIC in infants. Most infants included in the present study, were exclusively breastfed (67%), but some mothers reported to occasionally feed their infants with commercial infant formula or other foods (for example, maize meal porridge and commercial infant cereals). We did not observe any differences in infant UIC based on feeding practices. Our findings, however, show that infant age is a strong negative predictor for BMIC, confirming that BMIC may decline within the first six months of lactation (50). Furthermore, discrepancies in literature exist regarding the relationship between maternal and infant UIC. In agreement with our study, various authors have found positive correlations between infant and mother UIC (38, 51, 52), while others did not (53, 54).

Our results indicate that household salt was a major source of iodine for mothers; SIC predicted both BMIC and maternal UIC. The majority (90%) of women consumed adequately iodized salt (>15 ppm) and our data indicates that the iodized salt coverage in the Potchefstroom area is high and meets the WHO criteria for a successful salt iodization program (10). In 2005, a national study reported 77% of households in South Africa had access to and consumed adequately iodized salt (9). The median iodine concentration in the household salt was 44 ppm, ranging from 0-153 ppm, slightly above the upper level of 40 ppm recommended by WHO (10). Iodine is a volatile micronutrient (55) and the iodine fortification level in the South African program (35-65 ppm) has been set to account for possible losses in salt iodine concentrations before salt reaches households. However, our data also show that 21% of households

consumed salt iodized above the upper level of 65 ppm. It has been previously documented that poor implementation and insufficient monitoring of universal salt iodization programs worldwide have resulted in inadequate and even excess intakes of iodine in several countries (56). In South Africa, the median iodine concentration in household salt has previously been reported to range from 6 ppm to 42 ppm across all provinces and 30 ppm nationwide (57).

The daily quantity of salt consumed by mothers was beyond the scope of this study and sodium excretion was measured only in spot urine samples, which is not recommended for estimating individual sodium intake. However, 90% of mothers indicated that they used salt every day for food preparation. The South African population is known to have high salt intakes. The mean salt consumption in the country is 6-12 g per day per person, which is higher than the WHO recommendation of ≤5 g of salt (<2000 mg sodium) per day per person (58). Currently, policies are being implemented to reduce the salt intake of the general population. Salt intakes as low as 5 g per day are known to have adequate amounts of iodine, provided the salt is sufficiently iodized (12). Our results indicate that the amount of iodine added to salt by some producers may be to too high and that the compliance with the current legislation (35-65 ppm) is not properly monitored and may therefore lead to over-iodized salt in the market.

In South Africa, fortification of salt is only mandatory for table salt and not for salt used in processed foods. In this study sodium excretion and household SIC were independent predictors of maternal UIC, indicating that iodized household salt may not have been the sole source for iodine. Thus, the possibility of obtaining iodine from other food sources cannot be over-ruled. For example, there was a reported frequent consumption of cow's milk, and the assessment of various brands of milk available in the local market showed that the iodine content in milk ranged from 116-366 µg/L (unpublished results). Only few studies have determined whether processed foods in South Africa contain iodized salt. Bread is the major source of dietary salt intake for adults, especially urban black dwellers, who are said to obtain 49-54% of their salt intake from bread and cereal food groups (59). Several manufacturing companies have previously reported to use salt containing substantial amounts of iodine (39-69 ppm), especially for food items that were frequently distributed countrywide (60). These reports are, however, old and it is likely that more food manufacturing companies now use iodized salt.

A limitation of this study is the small sample size. However, the vast amount of data collected in the study provides a complete picture of the iodine status in the studied infants and their mothers. We did not collect data on the use of any iodine containing disinfectants applied for maternal wound disinfection or continuous umbilical care of the infants and acknowledge this limitation (61). Based on oral information received from clinics and the local hospital, the most commonly used disinfectant in theatre during caesarean delivery is HibiTane® (containing chlorhexidine) in either alcohol or iodine, and water in chlorhexidine is also being used for

perineal laceration. Iodine is preferably used over alcohol as it was said to cause less irritations in patients. However, we did not observe any significant difference in UIC between infants born via vaginal delivery or caesarean, therefore ruling out possible contamination from maternal wound disinfectants. Furthermore, in clinics mothers are mainly advised to use alcohol (surgical spirit) for their infant's umbilical care and hence iodine contamination is unlikely.

Conclusions

Our results suggest that iodized salt is a major contributor to iodine status in lactating mothers and their infants. It also confirms that the salt iodization program in South Africa not only supplies sufficient iodine for children and women of reproductive age, but also for lactating mothers and breastfed infants. However, salt iodine levels appear to be poorly monitored. There is a dire need for on-going monitoring and surveillance of salt fortification at production, to avoid over-iodized salt and ensure sustenance of optimal iodine status in vulnerable population groups.

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Conflicts of Interest

The authors have no conflict of interest.

Authorship

JB, CMS, MA and JO conceptualized and designed the study; JO, OvdR and JB executed the study and collected data; JO, OvdR and SD performed biochemical analyses; JB and JO performed statistical analyses. JO wrote the first draft of the manuscript and all authors read and edited the manuscript.

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CHAPTER 4

Iodine status and associations with feeding practices and psychomotor milestone development in six-month-old South African infants

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Abstract

Iodine is important for normal growth and psychomotor development. While infants below six months of age receive iodine from breast milk or fortified infant formula, the introduction of complementary foods poses a serious risk for deteriorating iodine status. Therefore, this cross-sectional analysis assessed the iodine status of 6-month-old complementary fed South African infants from a peri-urban community and its associations with feeding practices and psychomotor milestone development. Iodine concentrations were measured in infant (n=386) and maternal (n=371) urine (UIC), as well as breast milk (n=257) (BMIC) and household salt (n=143). Feeding practices and psychomotor milestone development were assessed in all infants. The median (25^{th}-75^{th} percentile) UIC in infants was 345 (213-596) µg/L, and was significantly lower in stunted (302 [195-504] µg/L) than non-stunted infants (366 [225-641] µg/L). However, only 6.7% of infants had a UIC <100 µg/L. Infant UIC correlated with maternal UIC (128 [81-216] µg/L) (r_s=0.218, *P*<0.001) and BMIC (170 [110-270] µg/kg) (r_s=0.447, *P*<0.0001). Most infants (72%) were still breastfed, and tended to have higher UIC than infants not receiving breast milk (*P*=0.074). Almost all infants (95%) consumed semi-solid/solid foods, with commercial infant cereals (60%) and jarred infant foods (20%) being the most common solid foods first introduced. Infants who reported to consume commercial infant cereals every day to most days had significantly higher UIC (372 [225-637] µg/L) than those reported to consume commercial infant cereals only once a week to never (308 [200-517] µg/L) (*P*=0.023). No association between infant UIC and psychomotor developmental scores or parental rating scores were observed. The results suggest that iodine intake in six-month-old peri-urban South African infants is adequate. Iodine in breast milk and commercial infant cereals potentially contributed to this adequate intake.

Keywords: Iodine status, complementary feeding practices, psychomotor milestone development

Introduction

Despite the remarkable progress made over the last several decades, iodine deficiency remains a major public health problem that is not solely unique to developing countries but equally affects developed nations (Pearce *et al.* 2013). Detrimental effects of iodine deficiency on mental and cognitive development in children are well documented (Zimmermann *et al.* 2008), and even moderate iodine deficiency during infancy can have negative effects on growth and development (Choudhury & Gorman 2003).

The World Health Organisation (WHO) recommends exclusive breastfeeding during the first six months of life for optimal growth (WHO 2001). From six months onward infants should be introduced to nutritionally adequate, safe and appropriately-fed complementary foods, whilst breastfeeding is continued until two years of age or older (Lutter & Dewey 2003). Because complementary feeding is the transition from a diet of breast milk and/or infant formula to a diet that includes solid foods and other beverages (Fein *et al.* 2008), it is associated with major changes in both macronutrient and micronutrient intake by infants (Agostoni *et al.* 2008). The nutritional adequacy of complementary foods especially in iodine content is thus essential to ensure optimal infant growth and neurodevelopment.

Median urinary iodine concentration (UIC) is used as the primary indicator for iodine status in populations, and a median UIC ≥100 µg/L in infants indicates adequate intake (WHO *et al.* 2007). For children younger than 60 months of age, the WHO, the International Council for the Control of Iodine Deficiency Disorders (ICCIDD) and UNICEF recommend a daily iodine intake of 90 µg (WHO *et al.* 2007), whereas the US Institute of Medicine (IOM) recommends 110–130 µg of iodine daily for children aged between 0 and 12 months and 90 µg/day for children between one and eight years of age (Institute of Medicine *et al.* 2001). According to the South African Regulations Relating to Foodstuffs for Infants and Young Children published by the South African Department of Health, the set Nutrient Reference Value (NRV) for iodine is 130 µg/day for infants aged six to 12 months (Department of Health 2012)

Infant feeding practices in South Africa are found to be substandard, where mixed feeding is the most common practice even before six months of age (Doherty *et al.* 2012, Siziba *et al.* 2015). The South African National Health And Nutrition Examination Survey (SANHANES-1) of 2012 reported the exclusive breastfeeding rates for the first six months of life to be very low at 7.4% nationwide, despite the high rates (83.0%) of breastfeeding initiation (HSRC 2013). Additionally, the complementary foods received by infants, especially those living in low socio-economic areas, were reported to be of poor nutritional quality (Faber 2005).

Like in many other African countries, grain-based porridges are a staple food for South African infants (Mamabolo *et al.* 2004). Although the maize flour in the country is fortified with other

micronutrients such as iron, vitamin A and zinc (Department of Health 2003) it is not fortified with iodine, therefore, not contributing to iodine status of infants. Furthermore, despite the successful salt iodization programme in South Africa (Jooste & Zimmermann 2008), the addition of salt to home prepared complementary foods is not recommended (Cribb *et al.* 2012); hence commercial complementary foods are fortified with iodine (Dunn 2003, Zimmermann 2012). Various inappropriate infant feeding practices like the early introduction of salty snacks to young infants have been reported to be common in South Africa (Faber *et al.* 2014). This may have an impact on iodine status as several food manufacturing companies in the country have previously reported to use salt that contained substantial amounts of iodine (39-69 ppm) (Harris *et al.* 2003).

There is limited information on the iodine status of weaning infants in countries with established iodized salt programmes, of which the general population also has adequate iodine intake. Furthermore, associations of iodine status with infant feeding practices have not been previously investigated in South Africa, where infant feeding practices are reported to be sub-optimal (HSRC 2013). Therefore, this cross-sectional study assessed the iodine status of peri-urban South African infants receiving complementary foods, and associations with feeding practices and psychomotor milestone development.

Methods

Participants and study site

The present study was a cross-sectional analysis of baseline data from the TSWAKA trial, which was designed as a randomized, controlled trial to investigate the effects of two different small-quantity lipid-based nutrient supplements (SQ-LNS) on linear growth in infants aged six months. The trial was conducted from September 2013 to January 2015 in the Jouberton area of the greater Matlosana Municipality in the North West Province, South Africa. This study site is a peri-urban and low socio-economic settlement situated approximately 200 km from Johannesburg, which is the nearest metropolitan area. The population migration in this area is relatively stable.

To be included in the study, infants had to be six months of age, and were excluded if they had 1) not received any breast milk previously; 2) severe obvious congenital abnormalities; 3) severe anaemia (haemoglobin < 7 g/dL); 4) severe malnutrition (weight-for-length Z-score < -3.00 SD); 5) other diseases referred for hospitalization by clinic staff; 6) plans to move out of the study area in the next seven months; 7) known allergies/intolerances to peanuts and/or soy, milk and/or lactose and fish; 8) received special nutritional supplements as part of feeding

programmes; 9) known HIV+; and 10) not been born as a singleton. Infants with severe malnutrition or severe anaemia were referred for treatment.

Sample size

The sample size for the main TSWAKA trial was 750 (250 infants per treatment group), which was based on expected difference in growth achieved during the six months of active intervention and also accounting for an expected drop-out rate of 25%. For the iodine status assessments, all infants in the study had an equal chance of being included and only infants that successfully provided urine samples at baseline were included in this study.

Data collection and measurements

During enrollment, the infants' age was confirmed by using their health cards and a detailed questionnaire was used to collect and record information on family socio-economic characteristics (Addendum 5). Haemoglobin (Hb) concentrations were determined in an aliquot of whole blood obtained via antecubital venepuncture of the arm. If this was not successful, a capillary blood sample was obtained via a finger prick. Hb concentrations were determined by using Hemocue (Hemocue 201+; HemoCue® AB). Anaemia was defined as Hb <11 g/dL. Weight and recumbent length were measured in duplicate according to WHO standardized techniques (WHO 1995). Infants were weighed without clothing to the nearest 0.01 kg using a digital baby scale (Seca model 354, GmbH & Co. KG., Hamburg, Germany, maximum 20 kg). Recumbent length was measured to the nearest 0.1 cm (Seca 416 infantometer; Seca GmbH & Co. KG., Hamburg, Germany).

Spot urine samples were collected from all infants in the morning between 08:00 am and 12:00 noon, using urine collection pads (SteriSets Uricol Set), aliquoted and stored at -80ºC until analysis. Mothers also provided a midstream spot urine sample (10-40 mL). Breast milk samples (5 ml of fore milk) were obtained by manual expression from mothers that still breastfed their infant at six months. To collect fore milk, mothers were requested to express milk from the breast that was not used at the last feed. Breast milk samples were aliquoted and stored at -20ºC until analysis. All mothers were requested to provide 10 g of salt used in the household during home visits by field workers.

Feeding practices

A structured questionnaire that was developed based on WHO guidelines for assessing infant and young child feeding practices (WHO 2010) was used to collect retrospective information on the infants' complementary feeding practices (Addendum 6). Indicators that were of particular interest for this present study analysis were whether the infant was currently breastfed, and whether the infant had already been introduced to any liquids, milk-feeds and semi-solid or

solid foods. A set of unquantified food frequency questions was used to obtain descriptive qualitative information on the usual consumption of foods by the infants over the past seven days (Faber *et al.* 2014, Smuts *et al.* 2005). The mother made a choice out of options to describe the infant's usual intake of listed foods. These options were (i) every day; (ii) most days (not every day but at least four days per week); (iii) once a week (at least once a week, but less often than four days a week); and (iv) never. For each food item, infants were grouped according to the usual consumption. Infants who ate the food at least four days in a week were categorised as "frequent consumption", whereas infants that ate the food less than four days in a week were categorised as "seldom/never".

Psychomotor milestone development

The Kilifi Developmental Inventory (KDI) and parent rating scale were used as assessment tools for psychomotor milestone development. These tools were developed based on the assessment of psychomotor activities of children aged six to 35 months (Abubakar *et al*. 2008). However, for the purpose of this study, the tools were evaluated by a qualified psychologist, and only those activities applicable for 6-month-old infants were included on the score sheet. The KDI questions were translated into the local language (Setswana) by a team member with experience in translating similar research tools and fluent in the language. The translated questions were then pre-tested for verification using volunteers who were not part of the study and corrections were adapted where necessary. The administration of the KDI was done by trained assessors. The training process involved both role play and practice sessions with infants not part of the study. A trained assessor observed all practice sessions and provided feedback.

The KDI was used to calculate scores for locomotor skills, eye-hand coordination and combined psychomotor development (combination of locomotor skills and eye-hand coordination). Locomotor skills assessed, included the infants' movement in space, static and dynamic balance and motor coordination. Eye-hand coordination assessments included infants' ability to manipulate objects and coordinate fine motor movement. These activities were scored on a dichotomous scale which ranked from 0 to 2, where 0 = Infant not able to perform the task; 1 = Infant can perform the task, but not fluently/partially successful and 2 = Infant can perform the task fluently/successfully (Abubakar *et al.* 2008). For parental rating assessments, a parent rating of motor development questionnaire was used. The questionnaire enabled the caregiver to provide a rating on the gross motor developmental milestones of the infant. Activities were scored on a dichotomous scale which ranked from 1 to 4, where 1 = yes, infant was able; 2 = yes, but caregiver not able to tell when infant started; 3 = infant is learning; 4 = Infant was not able (Abubakar *et al.* 2008). Both the KDI and parent rating have been validated in a study that

was developed by Dr Jane Kalsvig with financial and technical support from UNICEF and the South African Department of Basic Education.

Laboratory analyses

Iodine status was determined based on urinary iodine concentration (UIC). UIC were determined in duplicate at the North-West University in Potchefstroom by using a modification of the Sandell-Kolthoff reaction with spectrophotometric detection (Jooste & Strydom 2010). The laboratory successfully participates in the Program to Ensure the Quality of Urinary Iodine Procedures (EQUIP, U.S. Centres for Disease Control and Prevention, Atlanta GA, USA) (Caldwell *et al.* 2005). Iodine in spot urine samples were expressed as median concentrations (µg/L). A median UIC of 100 µg/L and more was considered to indicate adequate iodine intake in infants and lactating mothers (WHO *et al.* 2007). Salt iodine concentrations were also determined using a modification of the Sandell-Kolthoff reaction with spectrophotometric detection (Jooste & Strydom 2010). Breast milk iodine concentrations (BMIC) were analyzed at the Laboratory of Human Nutrition of ETH Zurich, Switzerland. Iodine content in filtered TMAH extracts was measured using a multicollector inductively coupled plasma mass spectrometer (MC-ICP-MS [Finnigan NEPTUNE, Thermo Scientific™ Waltham, MA, USA]) as described previously by Dold *et al.* (2016). BMIC were expressed as median concentrations (µg/L).

Data management and statistical methods

All data processing and analysis was done using IBM SPSS statistics version 23. Data on infant feeding practices were entered manually into an EpiInfo data base. Anthropometry data were entered using EpiData 3.1. Anthropometric indices, Length-for-age Z-score, weight-for-age Z-score and BMI-for-age Z-scores (BAZ) were calculated using the SAS macros for the WHO Child growth standards (WHO 2006). Stunting was defined as LAZ <-2 SD, wasting was defined as BAZ <-2 SD, normal weight as BAZ ≥-2 and ≤2 SD, overweight as BAZ >2 SD and obese as BAZ >3 SD (WHO 2006).

The Shapiro-Wilk test and Q-Q plots were used to check for normality of data. For continuous data, normally distributed data were presented as mean ± SD whereas not normally distributed data were presented as median (25^{th}-75^{th} percentiles) values. Categorical data were reported as frequencies. For non-parametric data, the Mann-Whitney or Kruskal-Wallis tests were used for between-group comparisons. Associations between continuous data were determined using Pearson's correlation coefficient.

BMIC were categorised into quartiles and presented as the proportion of infants within the different quartiles; Q1 (BMIC <110), Q2 (BMIC ≥110 <170), Q3 (BMIC ≥170 <269.9) and Q4 (BMIC ≥269.9). Dietary intake of infants was expressed as the proportion of infants in the

different frequency of consumption categories. Salt iodine data were presented as the proportion of households in the different categories by salt iodization level (<15 ppm, 15-39.9 ppm, 40-65 ppm or >65 ppm) and the proportion of infants in the categories of daily addition of salt to infant food. The categorised salt iodization levels were based on the WHO recommendations for adequately iodized salt (WHO *et al.* 2007) and the mandatory salt fortification levels in South Africa (Jooste & Zimmermann 2008).

KDI scores were calculated by summing up the scores recorded by the fieldworkers (0 = unable to perform task; 1 = partially able to perform task; 2 = able to perform task). Parent rating scores as recorded by the fieldworkers, were re-coded and grouped accordingly (1 = infant was able; 0 = infant was not able). Analysis of covariance (ANCOVA) was used to determine differences in mean psychomotor development and parental rating scores between infant UIC categories; adjusting for Hb and LAZ. Hb and LAZ were added as covariates, as a previous analysis in this study population found significant associations of anaemia status and stunting with psychomotor development scores and parental rating scores respectively (Rothman 2015). Significance was set at $P < 0.05$.

Ethical considerations

The randomized controlled trial was conducted according to the guidelines laid down in the Declaration of Helsinki involving human subjects and was registered as a clinical trial at Clinicaltrials.gov registry (NCT01845610). The trial was approved by the Health Research Ethics Committee (HREC) (NWU-00001-11-A1) of the North-West University and the Ethics Committee of the South African Medical Research Council (SAMRC) (EC-01-03/2012). The trial was also reviewed by the North-West Provincial Department of Health and Social Development and registered with the Directorate for Policy, Planning and Research.

All parents/legal guardians of infants received information letters and completed informed consent forms (Addendum 7). The study and its implications were explained to parents/guardians/caregivers in a one-on-one setting and only infants whose parents/guardians signed informed consent were included in the study. In case of illiterate parents/guardians the fieldworker read the information out to them and the parents/guardians used their thumb print as a signature for consent. Parents or legal guardians had the right to withdraw their child from the study at any time, without being obliged to give reasons and without penalty or loss of benefits they were entitled to.

Results

Out of the 750 infants that were enrolled in the Tswaka trial, urine from a total of 386 infants was obtained and included in this present study. Mean age of infants was 6.2 ± 0.2 months, including 51% males and 49% females. Table 4.1 gives an overview of the infants' characteristics and further illustrates infants' UIC distribution in relation to these characteristics. The prevalence of stunting in infants was 27%, and median UIC was significantly lower in stunted compared to non-stunted infants (*P*=0.008).

The median (25^{th}-75^{th} percentile) UIC in infants was 345 (213-596) µg/L. Only 6.7% of infants had a UIC less than 100 µg/L, and 14% had a UIC between 100 and 199 µg/L. The majority (78.9%) of infants had a UIC above 200 µg/L. Of these, 22.5% and 33.9% had a UIC greater than 300 µg/L and 500 µg/L respectively (Figure 4.1). Median (25^{th}-75^{th} percentile) maternal UIC (n=371) was 142 (83-225) µg/L and more than 30% of mothers had a UIC less than 100 µg/L. Infant UIC correlated positively with maternal UIC (r_s= 0.218, *P* <0.001). Median (25^{th}-75^{th} percentile) BMIC was 170 (110-270) µg/L and correlated positively with infant UIC (r_s = 0.447, *P* <0.0001). Moreover, a significant difference was observed in infant UIC between different BMIC quartiles (Q) (*P* <0.0001), with infants of Q1 having significantly lower UIC than infants of Q3 (*P* =0.003) and Q4 (*P* <0.0001), while UIC of infants of Q2 and Q3 were significantly lower than of Q4 (*P* <0.0001 and *P* =0.001 respectively) (Table 4.1).

Table 4.1: Urinary iodine concentrations (UIC) in relation to participant characteristics in peri-urban South African infants aged six months

Characteristics			Infant UIC (µg/L)			
	n	%	Mean ± SD/ Median (25^{th} - 75^{th})	Median (25^{th}-75^{th})	*P*	r_s
Males	197	50.9	-	313 (204-634)	0.842	
Females	189	49.1	-	358 (224-574)		
LAZ	386	-	-1.38 ± 1.05	-	0.034	0.108*
WLZ	386	-	0.53 ± 1.17	-	0.345	0.048
Stunting						
Stunted (LAZ <-2 SD)	104	26.9	-	302 (195-504)	0.008	
Not stunted (LAZ ≥-2 SD)	282	73.1	-	366 (225-641)		
BAZ	386	-	0.4 ± 1.2	-	0.211	0.064
Wasted (< -2 BAZ < -3)	9	2.3	-	344 (249-581)	0.897	
Normal (> -2 BAZ <2)	345	89.4	-	347 (212-596)		
Overweight/Obese (>2 BAZ >3 SD SD)	32	6.5	-	326 (237-610)		
Haemoglobin (Hb) [g/dL]	386	-	11.3 ± 1.36	-	0.974	-0.002
Anaemic (Hb <11 g/dL)	138	35.8	-	341 (206-601)	0.966	
Non-anaemic (Hb ≥11 g/dL)	248	64.2	-	346 (216-596)		
Caregiver age (years)	386	-	27.9 ± 7.8	-	0.218	-0.007
Caregiver education						
Lower than grade 10	71	18.7	-	357 (209-549)	0.563	
Higher than grade 10	308	81.3	-	333 (213-603)		
Maternal UIC (µg/L)[a]	217	-	128 (81-216)	-	0.001	0.218**
<100	71	32.7	-	319 (151-584)	0.189	
100-200	86	39.6	-	358 (241-553)		
≥200	60	27.6	-	383 (209-746)		
Maternal BMIC (µg/L)	205	-	170 (109-270)	-	<0.0001	0.477**
Q1 (BMIC <110)	52	25.4	-	229 (127-354)[b]	<0.0001	
Q2 (BMIC ≥110 <170)	51	24.9	-	341 (229-499)[b,c]		

Characteristics			Infant UIC (µg/L)			
	n	%	Mean ± SD/ Median (25^{th} - 75^{th})	Median (25^{th}-75^{th})	*P*	r_s
Q3 (BMIC ≥170 <269.9)	51	24.9	-	357 (263-636)[c]		
Q4 (BMIC ≥269.9)	51	24.9	-	653 (451-864)[d]		

Abbreviations: BMIC =Breast milk iodine concentration; Hb =Haemoglobin; LAZ =Length-for-age Z-score; Min =Minimum; Max =Maximum; UIC =Urinary iodine concentration; WLZ =Weight-for-length Z-score.

Caregiver relationship to child (other): father, grandmother, grandfather, uncle, aunt, not related

[Total] number of mothers who had corresponding infant UIC values

[b,c,d] Q1 significantly lower than Q3 and Q4, while Q2 and Q3 significantly lower from Q4

P value for Krustall Wallis test with significance level set at p=0.05

* Pearson Correlation test with significance level set at 0.05

**Pearson Correlation test with significance level set at 0.01

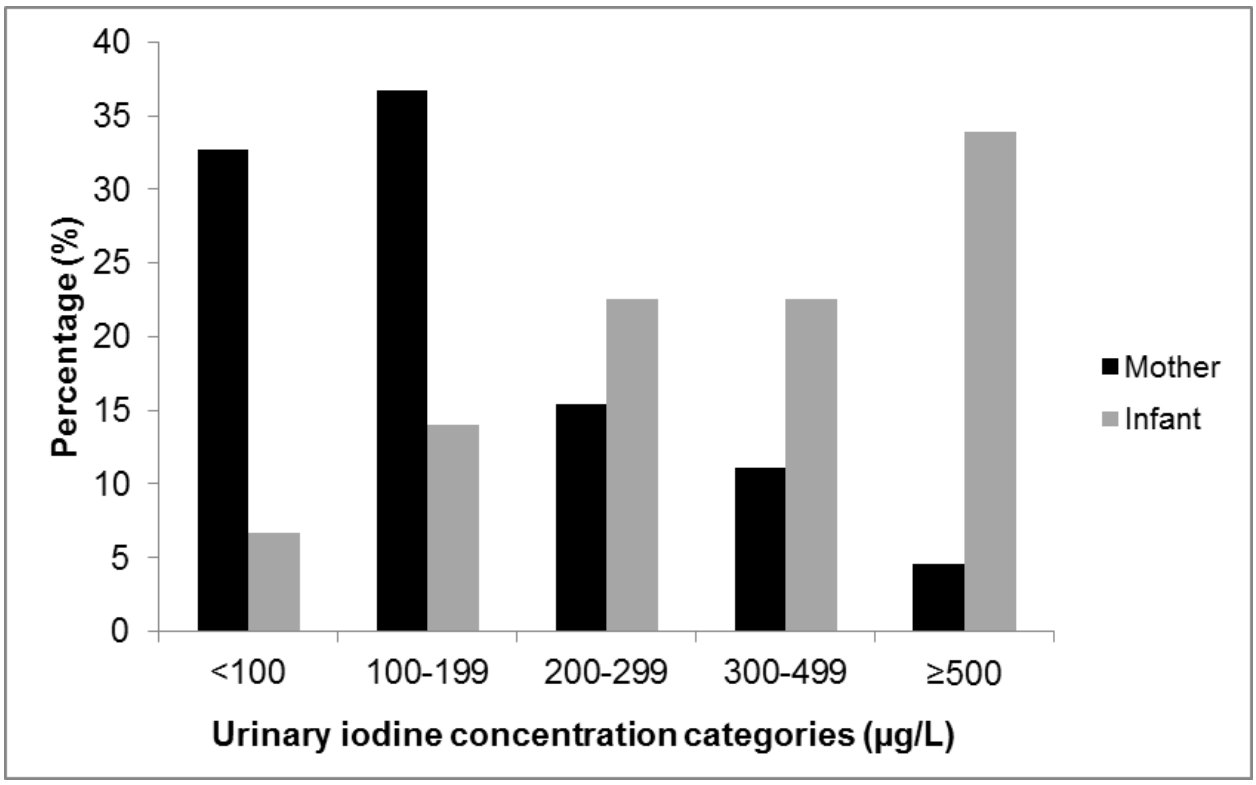

Figure 4.1: Frequency distribution of spot urinary iodine concentration in six-month-old infants (n=386) and their mothers (n=371).

A total of 216 household salt samples were collected, and 143 corresponding infant urine samples were obtained from these households. The median (25^{th}-75^{th} percentile) SIC was 45 (27-70) ppm, and ranged from 0.4 to 762 ppm. The majority (90%) of salt samples had iodine concentrations greater than 15 ppm, with 27% even greater than 65 ppm. Infants from households with SIC greater than 65 ppm had a significantly higher UIC compared to those from households with SIC ranging from 40-65 ppm (*P* =0.049). More than 50% of the caregivers reported that salt was never added to the infant's food, and there were no significant differences in UIC when compared with infants that had reported added salt in their food (Table 4.2).

Table 4.2: Comparison of infant UIC in relation to household salt concentration and use in peri-urban South African infants aged six months

Household salt characteristics	n	%	UIC (µg/L) Median	25th Percentile	75th Percentile	*P*
Household SIC (ppm)	143					
<15 ppm	16	11.2	265	211	455	0.04
15-39.9 ppm	47	32.9	367	238	548	
40-65 ppm	41	28.7	256	131	523[a]	
>65 ppm	39	27.3	454	235	722[b]	
Daily addition of salt to infants food	381					
Everyday	13	3.4	403	235	588	0.921
Most days	19	5.0	357	212	595	
Once a week	142	37.3	357	205	598	
Never	207	54.3	327	216	632	

Abbreviations: SIC =Salt iodine concentration

P value for Krustall Wallis test with significance level set at p=0.05

[a,b] median UIC of infants from households with SIC > 65 ppm were significantly higher compared to infants from households with SIC ranging from 40-65 ppm

Of the 386 infants included in the study, 72% were still breastfed, and the UIC tended to be higher in breastfed infants than non-breastfed infants (*P* =0.074) (Table 4.3). More than half (52%) of the infants were not exclusively breastfed beyond the age of two months. More than half (56%) of the infants had not yet been introduced to any milk feeds (other than breast milk). Of the infants already consuming milk feeds, 41% received formula milk, while 4% received cow's milk. Other liquids that were commonly introduced to infants before or at the age of six months were water (54%), rooibos tea (3%) and juice (3%). Almost all infants (95%) were already consuming solid foods and the most common solid foods that were first introduced included commercial infant cereal (60%); jarred infant foods (20%); maize meal porridge (3%); and others such as sorghum/oats porridge and mashed vegetables (3%). We found that infants who were reported to consume commercial infant cereals more than four days weekly (68%) had significantly higher UIC than those reported to seldom/never consume commercial infant cereals (*P* =0.023) (Table 4.3).

The mean ± SD scores for psychomotor development and parental rating in the studied infants were [37.1 ± 6.4], [20.7 ± 3.9], [16.5 ± 3.4] and [20.4 ± 3.2] for combined psychomotor score, eye-hand coordination sub-scale, Locomotor skills sub-scale and parent rating, respectively. No differences in psychomotor developmental scores across the different infant UIC categories were found, even after adjusting for Hb and LAZ (Table 4.4).

Table 4.3: Urinary iodine concentration (UIC) in relation to infant feeding practices of peri-urban South African infants six months of age

Infant feeding practices	n	%	UIC (µg/L) Median	25th Percentile	75th Percentile	*P*[c]
Currently breastfed						
Yes	279	72.3	355	229	602	0.074
No	107	27.7	298	189	591	
Duration of breastfeeding						
Age 0-2 months	53	13.9	378	211	646	0.114
Age 3-4 months	34	8.9	222	146	518	
Age 5-6 months	14	3.7	367	190	556	
still breastfeeding	279	72.3	355	229	602	
Liquids already introduced[a]						
Yes	364	94.3	347	212	596	0.866
No	22	5.7	321	246	535	
Milk feeds already introduced						
Yes	170	44.3	405	210	632	0.216
No	214	55.7	326	216	561	
Type of milk feeds first introduced						
Cow's milk	14	3.6	338	162	613	0.513
Formula milk	158	40.9	405	210	632	
Not started yet	214	55.4	326	216	560	
Currently formula feeding						
Yes	168	47.7	400	210	629	0.431
No	213	54.8	329	218	578	
Semi-solid/ solid foods already introduced[b]						
Yes	365	95.3	344	213	595	0.819
No	18	4.7	383	189	686	
Weekly consumption of formula milk						
≥ 4 days per week	146	46.9	405	211	632	0.447
Seldom/ never	235	54.7	330	215	576	
Weekly consumption of jarred infant foods						
≥ 4 days per week	88	23.1	302	210	546	0.353
Seldom/ never	293	76.9	358	218	606	
Weekly consumption of commercial cereals						
≥ 4 days per week	262	68.8	372	225	637	0.023
Seldom/ never	119	31.2	308	200	517	
Weekly consumption of maize-meal porridge						
≥ 4 days per week	33	8.7	367	209	562	0.734
Seldom/ never	348	91.3	342	216	607	

[a] Liquids first introduced were water; formula milk; other (rooibos tea, sweetened drink, sugar water, cow's milk).

[b] Foods first introduced were commercial infant cereal; jarred infant foods; maize meal porridge; other (sorghum/oats porridge, mashed vegetables).

[c] *P* value for Mann-Whitney U test with significance level set at p=0.05

Table 4.4: Distribution of psychomotor development scores (n=386) in relation to infant UIC categories

		Psychomotor development scores[a] (mean ± SD)							
		Combined psychomotor score	*P*	Eye-hand coordination sub-scale	*P*	Locomotor skills sub-scale	*P*	Parent rating	*P*
UIC categories (µg/L)	n								
<100	26	37.2 ± 8.5	0.974[b]	20.8 ± 4.2	0.928[b]	16.3 ± 4.9	0.987[b]	21.0 ± 2.5	0.770[c]
100-199	54	36.3 ± 5.3		20.1 ± 3.7		16.2 ± 2.4		20.3 ± 3.2	
200-299	88	37.4 ± 6.2		20.7 ± 3.8		16.7 ± 3.3		20.4 ± 3.2	
300-499	87	37.6 ± 5.9		21.1 ± 3.8		16.6 ± 3.2		20.1 ± 3.6	
≥500	131	36.9 ± 6.9		20.5 ± 4.2		16.5 ± 3.6		20.5 ± 3.1	

Maximum possible scores for psychomotor development are: Combined psychomotor score= 53; Eye-hand coordination sub-scale= 27; Locomotor skills subscale= 26; Parent rating= 31; *P* value calculated by one-way ANCOVA, adjusting for haemoglobin concentrations and length-for-age z-score.

[b] Haemoglobin concentration is a significant predictor, *P* <0.05 (Rothman, 2015).

[c] Length-for-age z-score is a significant covariate *P* <0.05 (Rothman, 2015)

Discussion

In this peri-urban population, complementary fed infants and their mothers had adequate iodine status. Infants' urinary iodine concentrations (UIC) were positively associated with breast milk iodine concentrations (BMIC) and there was a trend for higher UIC in breastfed than in non-breastfed infants. The first solid foods that were introduced to most infants were commercial infant cereals and infants consumed these cereals more frequently than maize flour porridge. Significantly higher UIC were observed in infants who consumed commercial infant cereals frequently as opposed to those who seldom/never consumed these cereals. This study did not find any associations between infants' iodine status and psychomotor development.

In this study population over 90% of the households had access to adequately iodized salt (>15 ppm) as recommended by the WHO (WHO *et al.* 2007), thereby indicating a successful salt iodization programme. This finding is consistent with an earlier study in South Africa that showed that 90% of lactating mothers of younger infants (aged two to four months), from the same province and similar socio-economic background but different location, also consumed adequately iodized salt (>15 ppm) (Osei *et al.* chapter 3). In South Africa, a revised legislation in 2006 mandated iodization of household salt to the levels of 35 to 65 ppm to ensure adequate access by all (Jooste & Zimmermann 2008).

The transition from a diet of breast milk and/or infant formula to a diet that includes solid foods and other beverages during infancy, can lead to paramount changes in intake of certain nutrients; hence the importance of ensuring the nutritional adequacy of complementary foods given to infants (Agostoni *et al.* 2008, Fein *et al.* 2008, Monte & Giugliani 2004). It is recommended that as infants are being introduced to complementary foods, supplementary breastfeeding should be continued until the age of two years, to cater for the infants' iodine requirements (Andersson *et al.* 2007). The results show that the majority of infants in this study population were still breastfed at six months and, furthermore, the median BMIC of 170 µg was higher than reported in other countries (Andersson *et al.* 2010, Azizi 2007, Bazrafshan *et al*. 2005). Although there were no significant differences in UIC between breastfed and non-breastfed infants, a trend for higher UIC was found in infants that were breastfed. Furthermore, an observed significant association between BMIC and infant UIC is an indication that breast milk greatly contributed to the adequate iodine status of infants. In addition to this, infants from households that had salt iodine concentrations (SIC) above 65 ppm had significantly higher UIC when compared to infants from households that had SIC ranging 40-65 ppm, leading to the assumption that iodized salt contributed to the adequate iodine status indirectly through breast milk (Hess *et al.* 2015).

Contrary to the findings of this study, Swiss breastfed infants who were given home-prepared complementary foods (containing little or no added salt) were reported to be at highest risk of

low iodine intake compared to breastfed infants who also received infant formula milk and or complementary foods (Andersson *et al.* 2010). However, in the Swiss study, the median BMIC (50.6 µg/kg) of lactating mothers of six-month-old infants and the median household SIC (19.8 ppm) was lower than what is reported in this study done with a South African population. Furthermore, although 84% of the Swiss women reported to use iodized salt, lactating mothers were iodine deficient (median UIC 67 µg/L) (Andersson *et al.* 2010).

Because of the potential detrimental effects of excessive sodium intake on the developing kidneys during infancy, the addition of salt to home-prepared complementary foods is not encouraged (Cribb *et al.* 2012), because iodized salt may not directly provide for an infant's iodine needs, except when complementary foods are fortified with iodine (Andersson *et al*. 2007). In this present study no significant difference in UIC based on the addition of salt to infants' food were observed, and 54% of caregivers reported to never add salt to infants' foods. Furthermore, the consumption of salt could not have been high amongst infants, as the majority (91%) of infants were reported to seldom/never consume home prepared infant cereals.

Results on infant feeding practices showed that the consumption of commercial infant cereals was more common in these infants as opposed the staple maize flour porridge. These findings were consistent with national data showing that commercial infant cereals (51.2%) was the most commonly introduced complementary food followed by homemade cereals (29.0%) (HSRC 2013). However, the findings were in contrast to earlier individual studies from different parts of the country reporting that maize flour porridge forms an integral part of the diet of most six to 12-month-old infants (Faber 2005, Mamabolo *et al.* 2004), whilst commercial infant cereals were mainly given to supplement the maize flour porridge (Mamabolo *et al.* 2004). This is also an indication of a shift in feeding practices from staple cereals to more commercially available cereals. Commercial cereals tend to be more expensive than staple cereals, therefore, not sustainable in poor communities (HSRC 2013). No significant differences in infants' UIC were found by frequency of maize flour porridge consumption. However, a significant difference in infant UIC by frequency of commercial infant cereal consumption was observed. A limitation of this study was that the iodine content of frequently consumed commercial infant cereals was not determined. Therefore, conclusions about the contribution of the commercial infant cereals to the adequate iodine status of these infants cannot be made.

Despite adequate iodine nutrition in both stunted and non-stunted infants, the findings of this study pointed out a significantly lower UIC in stunted infants compared to non-stunted infants. This implies that iodine nutrition may be associated with linear growth in these children. It could also be assumed that these infants experienced poorer feeding practices, which were further reflected in lower iodine status. The stunting rate of about 27% in this sub-study population was similar to what was reported nationally (26.1%) in children <5 years old in 2012 (HSRC 2013). If

not addressed at an early stage, stunting can have detrimental effects on an infant's psychomotor development (Abubakar *et al*. 2008, Grantham-McGregor *et al.* 1997). In mildly deficient South African school-going children, supplementation with iodized oil significantly increased ($p<0.05$) median Insulin-Like Growth Factor (IGF)-I concentration, but did not result in any significant changes in median height-for-age z-scores (HAZ) and WAZ scores or median IGF Binding Protein-3 (IGFBP-3) concentration (Zimmermann *et al.* 2007).

No significant differences in the mean psychomotor development scores (Eye-hand coordination, Locomotor sub-scale, combined psychomotor) and parental rating scores across the various UIC categories were observed, even after adjusting for Hb and length-for-age Z-scores (LAZ). Previously Rothman (2015) has shown Hb and LAZ to be associated with motor milestone development in these same infants (Rothman 2015). Iodine deficient infants are at risk for cognitive disability, especially cretinism manifested by delayed motor and mental development (Federal Commission for Nutrition 2013). Reports from a review by Zimmermann (2007) on the effects of iodine deficiency in childhood, showed that even mild to moderate iodine deficiency has resulted in delayed mental development However, only very few infants in this present study had low UIC (6.7%), which may explain why no association between UIC and motor milestone development were found.

Limitations of this study were that the success rate of obtaining complete sets of urine samples from infants and mothers was low, reducing the sample size for some analyses. Furthermore, the analysis of thyroid hormones (thyroxine; T_4 and triiodothyronine; T_3) and thyroglobulin (Tg) would have been useful to investigate whether high UIC in infants were associated with abnormal thyroid function (WHO 2007, Zimmermann *et al.* 2013). Unfortunately, blood samples were not collected for these analyses. No established cut-off values exist for excess iodine intakes during infancy, however median UIC >300 µg/L is considered excessive in children older than six years (Delange *et al.* 2002, WHO *et al.* 2007). Although BMIC was associated with adequate iodine status, the amount of breast milk consumed by individual infants was unknown.

The results of this research suggest that complementary fed infants living in a peri-urban setting in South Africa have adequate iodine status. The results further suggest that in countries with successful salt iodization programs, breast milk may significantly contribute to adequate iodine intake in weaning fed infants who continue to breast feed. Commercial infant cereals potentially also contribute to the sufficient iodine intake in these infants. However, iodine content of commercial infant cereals available in South Africa needs to be investigated.

Key messages

- **Six-month-old complementary fed infants living in a peri-urban setting in South Africa have adequate iodine status.**
- **In countries with successful salt iodization programs, breast milk significantly contributes to adequate iodine status in complementary fed infants who continue to breastfeed.**
- **Commercial infant cereals could potentially contribute to adequate iodine intakes in complementary fed infants, however, the iodine content of these cereals need to be investigated in South Africa.**

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Conflict of interest

The authors declare no conflict of interests except CMS who received a travel grant from Unilever.

Contributions

CMS, MF, NC and JB conceptualized and designed the study; JO, MR and JB executed the study and collected data; JO performed biochemical analyses; JB and JO performed statistical analyses. JO wrote the first draft of the manuscript and all authors read and edited the manuscript.

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CHAPTER 5

Efficacy of novel small-quantity lipid-based nutrient supplements in maintaining adequate iodine status during complementary feeding: A randomized controlled trial in South African infants

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Abstract

Iodine fortification of complementary foods is recommended to ensure sufficient intake by infants and young children. This study aimed to assess the efficacy of small-quantity lipid-based nutrient supplements (SQ-LNS) in maintaining adequate iodine status in complementary fed South African infants. In a randomized controlled trial, 750 infants aged six months were randomized to receive: a daily fortified SQ-LNS with essential fatty acids providing 45 µg of iodine (SQ-LNS A); a daily fortified SQ-LNS with docosahexaenoic acid, arachidonic acid and phytase, providing 45 µg of iodine (SQ-LNS B); or a control group not receiving SQ-LNS. Urinary iodine concentrations (UIC) were measured at baseline (n=386) and at 12 months (n=262).

The geometric mean (95% CI) UIC at baseline was 333.8 (310.5, 358.9) µg/L and decreased to 214.9 (189.2, 242.6) µg/L at 12 months. Infants who no longer received breast milk had significantly lower UIC (159.6 [65.9, 397.5] µg/L) and higher odds (OR=4.9 [2.5, 9.3]) for having a UIC <100 µg/L (38%), than infants who continued to be breastfed (373.2 [202.6, 522.9] µg/L) at 12 months. Infants receiving SQ-LNS (combined group) had higher UIC (*P*=0.025) and lower odds for having a UIC <100 µg/L (OR=0.289 [0.11, 0.75]) at 12 months than the control group; adjusting for maternal baseline UIC, age, sex and continued breastfeeding. In sub-group analysis, the effect of SQ-LNS for higher UIC at 12 months was only apparent in the infants who no longer received breast milk at 12 months (*P*=0.039). These effects were no longer significant after adjusting for infant baseline UIC, resulting in a smaller sample size (n=124).

In the studied infants, iodine intake decreased from six to 12 months of age, but only in those infants who were no longer breastfed. In these infants, the provision of 45 µg of iodine per day as SQ-LNS resulted in higher UIC at 12 months, but was not efficacious in counteracting an overall decline in iodine status.

This trial is registered at clinicaltrials.gov as NCT01845610.

Keywords: Iodine, small-quantity lipid-based nutrient supplements, complementary feeding

Introduction

The prevalence of micronutrient deficiencies in infants and young children is of high public health concern in most developing countries. Adequate nutrition during the first two years of life is crucial for optimal physical and mental development in these vulnerable groups (Bellamy, 2004, Rivera and Lutter, 2001). Iodine is required by the body for thyroid hormone production. Thus, adequate iodine intake during infancy and childhood is particularly crucial due to the essential role of thyroid hormones in growth and development (Pearce et al., 2013). Deficiency in iodine may provoke a broad spectrum of disorders including hypothyroidism, cretinism, endemic goitre, mental retardation and increased infant mortality (Zimmermann and Boelaert, 2015)

Recommendations by the International Council for the Control of Iodine Deficiency Disorders (ICCIDD) and WHO indicate that children younger than five years should have a daily iodine intake of 90 µg for optimal growth (WHO et al., 2007), whereas the US Institute of Medicine recommends 110–130 µg/daily for children aged between 0 and 12 months (Institute of Medicine et al., 2001). Between the ages of approximately four to six months, infants are introduced to complementary foods and the contribution of iodine-rich breast milk or fortified infant formula in their diet decreases. Furthermore, the native content of iodine in food and beverages is said to be generally low (Zimmermann et al., 2008) and although plants and animal foods produced in iodine rich soils may contain iodine, they may not provide significant amounts (Zimmermann et al., 2008, Swanson et al., 2012). Thus, the transition to complementary foods increases infants' risk for becoming iodine deficient (Agostoni et al., 2008, Untoro et al., 2010).

Although salt iodization remains the key strategy to control iodine deficiency and to ensure universal adequate iodine intake (WHO et al., 2007), the use of salt in infants' food is not recommended, especially during the first year of life (Agostoni et al., 2008, Heird et al., 2006). This is because of the potential harmful effects excessive sodium intake can have on an infant's developing kidneys and blood pressure later in life (Cribb et al., 2012); therefore, alternative approaches are needed to ensure that infants have adequate and safe iodine intake during complementary feeding. The WHO recommends that in regions where the coverage of salt iodization is between 20% and 90%, children aged seven to 24 months should be supplemented with iodine through fortified complementary foods whilst breastfeeding is being maintained (Andersson et al., 2007). Dunn proposed that a daily amount of 90 µg iodine be added to complementary foods suitable for infants and young children (Dunn, 2003). Iodine can be added to complementary foods through home fortification products such as micronutrient powders (MNP) and small-quantity lipid-based nutrient supplements (SQ-LNS) (Bhutta et al., 2013, WHO, 2011).

A recent cluster-randomized study carried out in Burkina Faso tested the efficacy of SQ-LNS as a vehicle to provide a daily portion of 90 µg iodine together with additional protein, essential fatty acids and other micronutrients to infants aged nine months (Hess et al., 2015). At nine months and 18 months, the children had UIC indicating adequate intake, and the provision of 90 µg iodine did not affect any of the measured iodine status indicators. Burkina Faso has a successful salt iodization programme and over 90% of the studied infants were breastfed. Thus the authors stipulated that they may have received sufficient iodine, either indirectly via breast milk or directly through the consumption of salt-containing foods, and suggested a reduction of SQ-LNS iodine content in similar settings (Hess et al., 2015).

Iodization of table salt to a concentration of 35-65 ppm at the point of production was revised in South Africa since 2006/2007 (Jooste and Zimmermann, 2008). The last national survey on iodine nutrition in the country revealed that 77% of households in South Africa had access to, and consumed adequately iodized salt (Jooste et al., 2007). More recently, adequate iodine intake were observed in a convenience sample of two to four-months-old South African breastfed infants, which were associated with breast milk iodine concentrations (BMIC). BMIC in turn were predicted by household salt iodine concentrations (SIC) (Osei et al. under review: chapter 3).

In the present study we tested the efficacy of SQ-LNS products, providing 45 µg of iodine per day together with essential fatty acids or long-chain polyunsaturated fatty acids, phytase and other micronutrients, in maintaining adequate iodine status in South African infants during complementary feeding.

Methods

Participants and study site

The present study was nested within the Tswaka trial, which was designed as a randomized, controlled trial to investigate the effects of two different small-quantity lipid-based nutrient supplements (SQ-LNS) on child growth. The trial was conducted from September 2013 to January 2015 in the Jouberton area of the greater Matlosana Municipality in the North-West Province, South Africa. This study site is 200 km from the nearest metropolitan area (Johannesburg) and has a relatively stable population migration. Infants included in the trial were six months of age, and were excluded if they had 1) not received any breast milk previously; 2) severe obvious congenital abnormalities; 3) severe anaemia (haemoglobin < 7 g/dL); 4) severe malnutrition (weight-for-length Z-score <-3.00); 5) other diseases referred for hospitalization by clinic staff; 6) plans to move out of the study area in the next seven months; 7) known allergies/intolerances to peanuts and/or soy, milk and/or lactose, fish; 8) received

special nutritional supplements as part of feeding programmes; 9) known to be HIV+; 10) not been born as a singleton. Infants with severe malnutrition or severe anaemia were referred for treatment.

Sample size

The sample size for the Tswaka trial was 750 (250 infants per group), which was based on an expected difference in growth achieved during the six months of active intervention and also accounting for an expected drop-out rate of 25%. For the iodine status assessments, all infants in the study had an equal chance of being included and only infants who successfully provided urine samples at six and/or 12 months were included in this study.

Delivery of the intervention

The trial statistician used NQuery version 7 to prepare randomization lists. Block randomisation of sizes three, six and nine were used to randomly allocate the infants to one of the following three groups for a six month period: (1) fortified fat-based paste (SQ-LNS A); (2) fortified fat-based paste with DHA, ARA and phytase (SQ-LNS B); and (3) control group. The control group received no supplement during the six-month trial period, but was provided with a six-month supply of the fortified fat-based paste with essential fatty acids (SQ-LNS A) after completion of the six-month trial period. Both SQ-LNS products contained 45 µg of iodine (**Table 5.1**). The three groups were colour coded to facilitate implementation and monitoring. The infants' details were recorded on the enrolment form and each study participant was issued with a colour-coded identification card which was used during all project-related home, clinic and research site visits. The study was blinded only to the people involved in the laboratory, anthropometric, child development and dietary intake assessments; as well as the people involved in data and dietary analysis due to the visible differences in study products.

Table 5.1: Nutrient composition of the small-quantity lipid-based nutrient supplement (SQ-LNS) products used in the study

	Fortified fat-based paste (SQ-LNS A)	Fortified fat-based paste containing DHA/ARA (SQ-LNS B)
Amount of food supplement (g)	20	20
Energy (kcal)	114	113
Energy density (kcal/g)	5.7	5.7
Protein (g)	3.0	3.7
% calories from protein	10%	13%
Fat (g)	8.0	8.8
% calories from fat	63%	70%
Essential fatty acids		
Linoleic acid (LA) (g)	1.5	1.8
α-linolenic acid (ALA) (mg)	265	348
n-6/n-3 ratio	5.7	5.0
Long-chain polyunsaturated fatty acids		
Docosahexaenoic acid (DHA) (mg)		75
Arachidonic acid (ARA) (mg)		75
Micronutrients		
Vitamin A (µg)	200	200
Vitamin D (µg)	2.5	2.5
Vitamin E (mg)	2.5	2.5
Vitamin K (µg)	7.5	7.5
Thiamin (mg)	0.25	0.25
Riboflavin (mg)	0.25	0.25
Niacin (mg)	3	3
Pantothenate (mg)	1.0	1.0
Vitamin B6 (mg)	0.25	0.25
Biotin (mg)	4.0	4.0
Folate (B9) (µg)	80	80
Vitamin B12 (µg)	0.45	0.45
Vitamin C (mg)	23.3	23.3
Calcium (mg)	250	396
Iodine (µg)	45	45
Iron (mg)	5.8	5.8
Zinc (mg)	6.2	6.2
Copper (mg)	0.28	0.28
Selenium (µg)	8.5	8.5
Magnesium (mg)	-	30
Manganese (mg)	-	0.6
Phosphorus (mg)	-	230
Potassium (mg)	-	300
Other		
L-Lysine (mg)	-	160
Phytase (FTU)	-	200

The SQ-LNS products were manufactured internationally by DSM and UNILEVER and were tested to be acceptable by the study participants (Rothman et al., 2015). Caregivers with infants in the supplement groups were shown how to mix the study SQ-LNS into the infants' food. The caregivers were encouraged to mix 20 g of the SQ-LNS with approximately 20 g of the infants' first complementary feed of the day. The importance of using the product explicitly for the research participant was also stressed. Every caregiver was provided with two weeks supply of the SQ-LNS packed in individual single-dose sachets. An assigned fieldworker visited the caregiver within seven days after enrolment to ensure that the SQ-LNS products were used correctly. The fieldworker then visited the caregiver weekly to collect data on daily adherence, and any illness that may have occurred as well as to provide a new supply of the SQ-LNS. All three groups were treated the same.

Data collection and measurements

During enrollment, infants' age was confirmed from the health card and a detailed questionnaire was used to collect and record information on family socio-economic characteristics, infant feeding practices and infant morbidity during the past seven days. At 12 months an exit questionnaire was used to record information on infant feeding practices, morbidity and feedback on the delivery and consumption of the SQ-LNS (Addendum 8). Haemoglobin (Hb) concentrations were determined on an aliquot of whole blood samples obtained via antecubital venepuncture of the arm. If this was not successful, a capillary blood sample was obtained via a finger prick. Hb measurements were determined by using the HemoCue system (HemoCue 201+; HemoCue® AB). Anaemia was defined as Hb <11 g/dL.

Weight and recumbent length were measured in duplicate according to WHO standardized techniques (WHO, 1995). Infants were weighed without clothing to the nearest 0.01 kg using a digital baby scale (Seca model 354, GmbH & Co. KG., Hamburg, Germany, maximum 20 kg). Recumbent length was measured to the nearest 0.1 cm (Seca 416 infantometer; Seca GmbH & Co. KG., Hamburg, Germany). Anthropometry data were entered using EpiData 3.1. Anthropometric indices; length-for-age Z-score, weight-for-age Z-score and BMI-for-age Z-scores (BAZ) were calculated using the SAS macros for the WHO Child growth standards (WHO, 2006). Stunting was defined as LAZ < -2 standard deviations (SD), Wasting was defined as BAZ < -2 SD, normal weight as BAZ ≥ -2 and ≤ 2 SD, overweight as BAZ >2 SD and obese as BAZ > 3 SD.

Spot urine samples were collected in the morning (08:00-12:00 noon) at six and 12 months from infants using sterile urine collection pads (SteriSets Uricol Set) and from volunteering mothers. Samples were aliquoted and stored at -80ºC until analysis.

Laboratory analyses

Iodine status was determined based on urinary iodine concentrations (UIC). UIC was determined in duplicate at the North-West University in Potchefstroom by using a modification of the Sandell-Kolthoff reaction with spectrophotometric detection (Jooste and Strydom, 2010). The laboratory successfully participates in the Program to Ensure the Quality of Urinary Iodine Procedures (EQUIP, U.S. Centres for Disease Control and Prevention, Atlanta GA, USA) (Caldwell et al., 2005). Iodine in spot urine samples were expressed as geometric mean concentrations (µg/L). A geometric mean UIC of 100 µg/L and more was considered to indicate adequate iodine intake in infants (WHO et al., 2007).

Data management and statistical methods

Statistical analyses were performed using IBM SPSS Statistics (version 21; IBM Co). Data were checked for normality using Q-Q plots and the Shapiro-Wilk test. Normally distributed data were expressed as mean ± SD values. Not normally distributed data were log-transformed for statistical analysis and presented as geometric mean with 95% confidence interval (CI). To compare baseline characteristics of infants included in the analysis (urine sample provided at six or at 12 months) with the remaining infants participating in the Tswaka trial, as well as between randomization groups, one-way ANOVA were used for continuous data and the Pearson Chi-square test for categorical data. Independent t-tests were used to determine differences in UIC at six and 12 months between infants who were breastfed and who no longer received breast milk. Odds ratios for having UIC <100 µg/L at six and 12 months by breastfeeding status was further determined using Chi-Square tests.

Differences in UIC at 12 months by groups were assessed using one-way ANCOVA, adjusting for baseline maternal UIC, age, sex and continued breastfeeding at 12 months. In an additional model, baseline infant UIC was added as a covariate. Moreover, we examined the odds for having UIC <100 µg/L at 12 months by randomization group, using binary logistic regression analysis, including baseline maternal UIC, age, sex and continued breastfeeding at 12 months. In an additional model, baseline infant UIC was added as a covariate. Maternal baseline UIC was added as a covariate because it significantly correlated with baseline infant UIC (r = 0.162, P = 0.017). P-values <0.05 were considered significant.

Ethical considerations

The randomized control trial was conducted in accordance with the guidelines laid down in the Declaration of Helsinki involving human subjects and was registered as a clinical trial at Clinicaltrials.gov registry (NCT01845610). The trial was approved by the Health Research Ethics Committee (HREC) (NWU-00001-11-A1) of the North-West University and the Ethics Committee of the South African Medical Research Council (SAMRC) (EC-01-03/2012). The trial was also reviewed by the North-West Provincial Department of Health and Social Development and registered with the Directorate for Policy, Planning and Research.

All parents, legal guardians or caregivers of infants received information letters and completed informed consent forms. The study and its implications were explained to mothers/guardians/caregivers in a one-on-one setting and only infants whose parent/legal guardian signed informed consent were included in the study. In case of illiterate parents/legal guardians the fieldworker read the information out to them, and they used their thumb print as a signature for consent. Parents or legal guardians had the right to withdraw their child from the study at any time for any reason, without being obliged to give reasons and without penalty or loss of benefits they were entitled to.

Results

Of the 750 infants that were enrolled in the Tswaka trial, 386 and 262 successfully provided urine samples at six and 12 months, respectively. However, only 124 (24%) provided urine samples at both time points (**Figure 5.1**). The mean age of infants who provided urine at baseline (six months) was 6.2 ± 0.2 months, and of those infants, 27% were stunted (LAZ <-2 SD), whilst 8% were overweight or obese (BAZ >2 SD) (Table 5.2). The baseline prevalence of anemia was 35.8%. Of the infants who provided a urine sample at six and 12 months, 72% and 53% were still breastfeeding, respectively. Furthermore, at six and 12 months, 68.8% and 23.5% of infants were reported to consume commercial infant cereals frequently (≥4 times per week), respectively. There was no significant difference in baseline characteristics between the randomization groups (Table 5.2). There was also no significant difference between the infants who provided a urine sample at six months, at 12 months and the remaining infants participating in the Tswaka trial who did not provide a urine sample (Supplementary table 5.1).

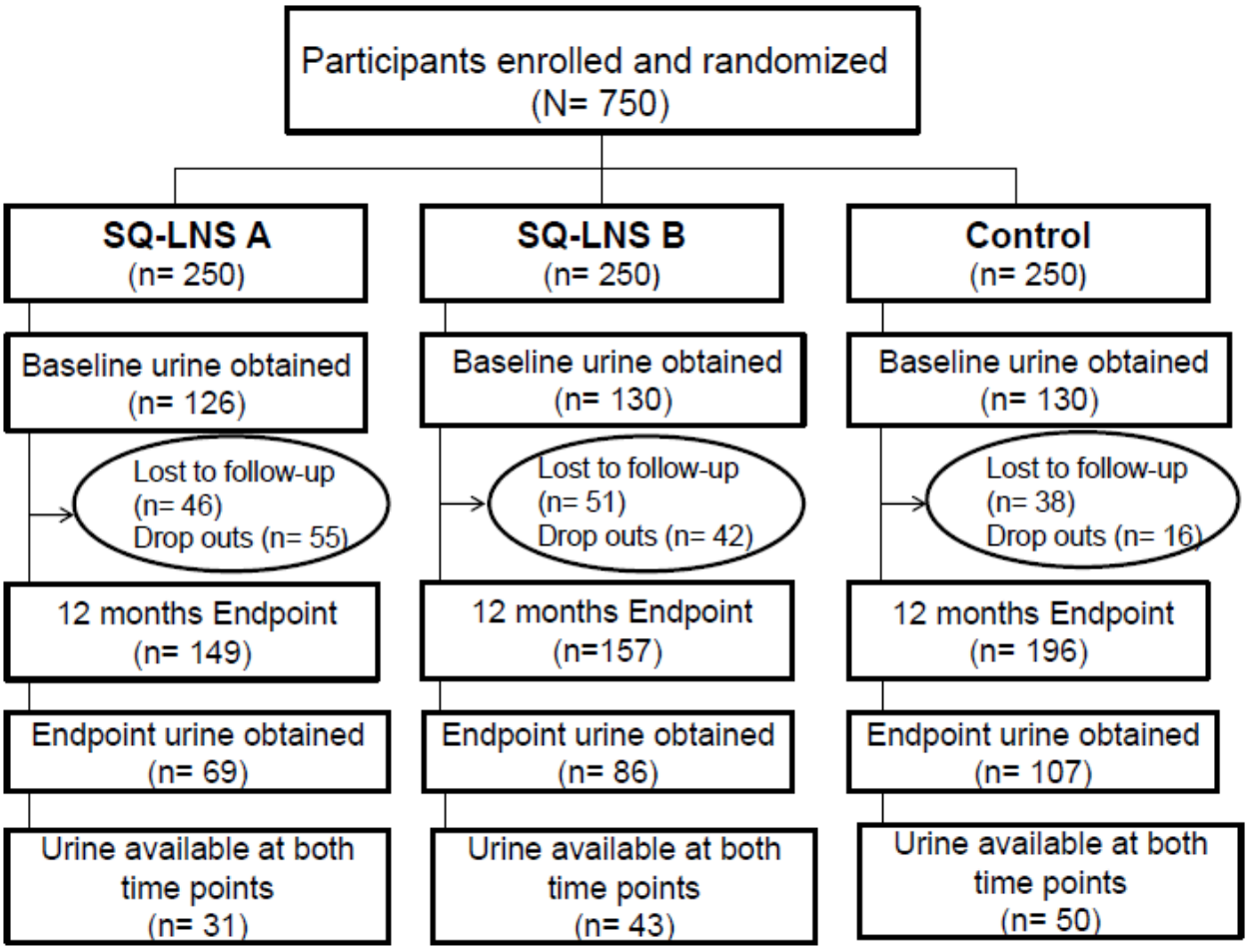

Figure 5.1: Flow diagram of participant randomization and progress throughout the study. SQ-LNS, small-quantity lipid-based nutrient supplement

The geometric mean (95% CI) UIC at six months was 333.8 (310.5, 358.9) µg/L in the infants who provided a urine sample. Only 6.7% of these infants had a UIC <100 µg/L. The geometric mean UIC at six months tended to be lower (P = 0.053) in infants who no longer received breast milk (294.8 [252.6, 344.2] µg/L; n = 107) than in infants who continued to be breastfed at six months (350.1 [322.9, 379.6] µg/L; n = 279). Furthermore, the odds for having a UIC <100 µg/L at six months was significantly higher in the infants who no longer received breast milk (OR = 2.8 [1.3, 6.3]) (Figure 5.2).

The geometric mean UIC at 12 months was 214.9 (189.2, 242.6) µg/L in the infants who provided a urine sample, and was significantly lower than what was observed at six months (P = 0.003). Furthermore, infants who no longer received breast milk had significantly lower UIC at 12 months (159.6 [65.9, 397.5] µg/L) than infants who continued to be breastfed at 12 months (373.2 [202.6, 522.9] µg/L) (P <0.001). They also had significantly higher odds for having a UIC

<100 µg/L at 12 months (OR = 4.9 [2.5, 9.3]) (Figure 5.2). Of the infants who no longer received breast milk at 12 months (53%), the infants who received commercial infant cereals frequently (≥4 times per week) had significantly higher UIC (350.1 [81.7-594.0] µg/L) than the ones who seldom or never received commercial infant cereals (138.8 [35.3-368.5]) (*P* = 0.030), while there was no difference in the breastfed infants (*P* = 0.198).

Table 5.2: Baseline characteristics of infants who provided urine both at ages six and 12 months, and of infants who provided urine at 12 months by randomization group[1]

	Infants who provided urine both at ages 6 and 12 months				Infants who provided urine at age 12 months			
	SQ-LNS A (*n*=31)	SQ-LNS B (*n*=43)	Control (*n*=50)	*P*[3]	SQ-LNS A (*n*=69)	SQ-LNS B (*n*=86)	Control (*n*=107)	*P*[3]
Age (m)	6.2 ± 0.2[2]	6.2 ± 0.2	6.2 ± 0.3	0.551	6.2 ± 0.3	6.2 ± 0.2	6.2 ± 0.3	0.850
Male [*n* (%)]	11 (35.5)	23 (53.5)	26 (52.0)	0.250	25 (36.2)	49 (56.9)	56 (52.3)	0.595
Baseline anthropometric indexes [*n* (%)]								
Stunting (LAZ <-2 SD to -3 SD)	5 (16.1)	6 (14.0)	9 (18.0)	0.869	20 (28.9)	23 (26.7)	26 (24.3)	0.222
Severe stunting (LAZ <-3 SD)	1 (3.2)	3 (7.0)	2 (4.0)	0.712	7 (10.1)	6 (6.9)	4 (3.7)	0.364
Wasting (BAZ <-2 SD to -3 SD)	1 (3.2)	1 (2.3)	0 (0)	0.940	1 (1.4)	2 (2.3)	1 (0.9)	0.282
Overweight (BAZ >2 SD)	0 (0)	4 (9.3)	5 (10.0)	0.197	2 (2.9)	8 (9.3)	12 (11.2)	0.547
Obese (BAZ >3 SD)	0 (0)	4 (9.3)	0 (0)	0.020	0 (0)	4 (4.6)	1 (0.9)	0.350
Hb (g/dl)	11.4 ± 1.2	11.6 ± 1.3	11.4 ± 1.3	0.642	11.3 ± 1.3	11.4 ± 1.4	11.3 ± 1.4	0.265
Anaemia (Hb <11 g/dl) [n (%)	8 (25.8)	10 (23.3)	18 (36.0)	0.362	17 (24.6)	27 (31.4)	42 (39.2)	0.287
Breastfeeding [*n* (%)]	21 (67.7)	29 (67.4)	36 (72.0)	0.871	47 (68.1)	60 (69.7)	69 (64.5)	0.354

[1] BAZ, BMI-for-age z score; HAZ, height-for-age z score; SQ-LNS, small quantity lipid nutrient supplement; WAZ, weight-for-age z score;
[2] Mean ± SD (all such values)
P[3] Value for differences between randomization groups (including only infants who provided a urine sample at baseline and endpoint) determined using one-way ANOVA or Pearson Chi-square test.

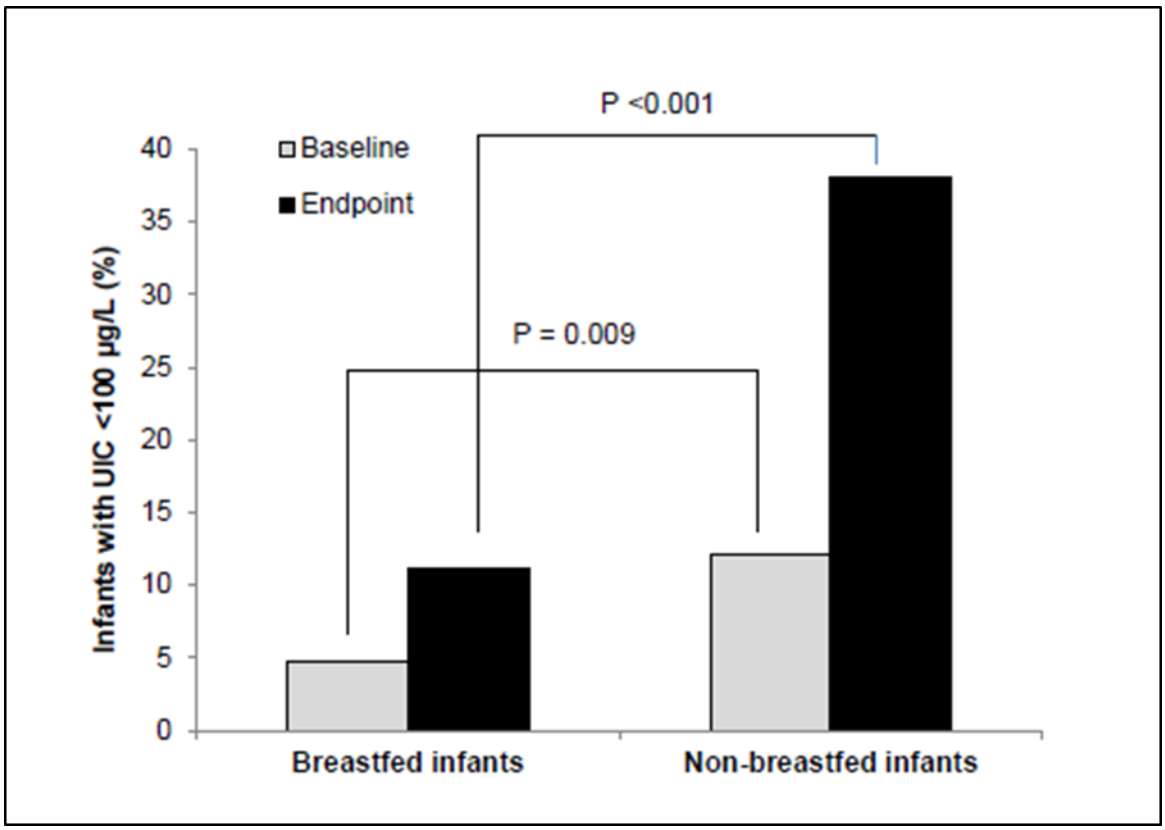

Figure 5.2: Proportion of breastfed and non-breastfed infants with urinary iodine concentrations (UIC) <100 µg/L at baseline (six months) and endpoint (12 months). *n* = 279 and 107 in the breastfed and non-breastfed group, respectively, at baseline. *n* = 134 and 121 in the breastfed and non-breastfed group, respectively, at endpoint. Differences in the proportion of infants with a UIC <100 µg/L between breastfed and non-breastfed infants at baseline and at endpoint were determined using Chi-Square tests.

The geometric mean UIC at six and 12 months by randomization groups are presented in **Table 5.3**. Using ANCOVA models adjusting for baseline maternal UIC, age, sex and continued breastfeeding at 12 months, there tended to be an effect of the two different SQ-LNS for higher UIC at 12 months. When merging the SQ-LNS A and SQ-LNS B infants into a combined SQ-LNS group, there was a significant effect of SQ-LNS for higher UIC at 12 months (*P*=0.025). However, when adjusting the ANCOVA model additionally for baseline UIC – resulting in a markedly smaller sample size – the effect was no longer significant. In all models, continued breastfeeding until 12 months was a significant predictor of endpoint UIC (P <0.05).

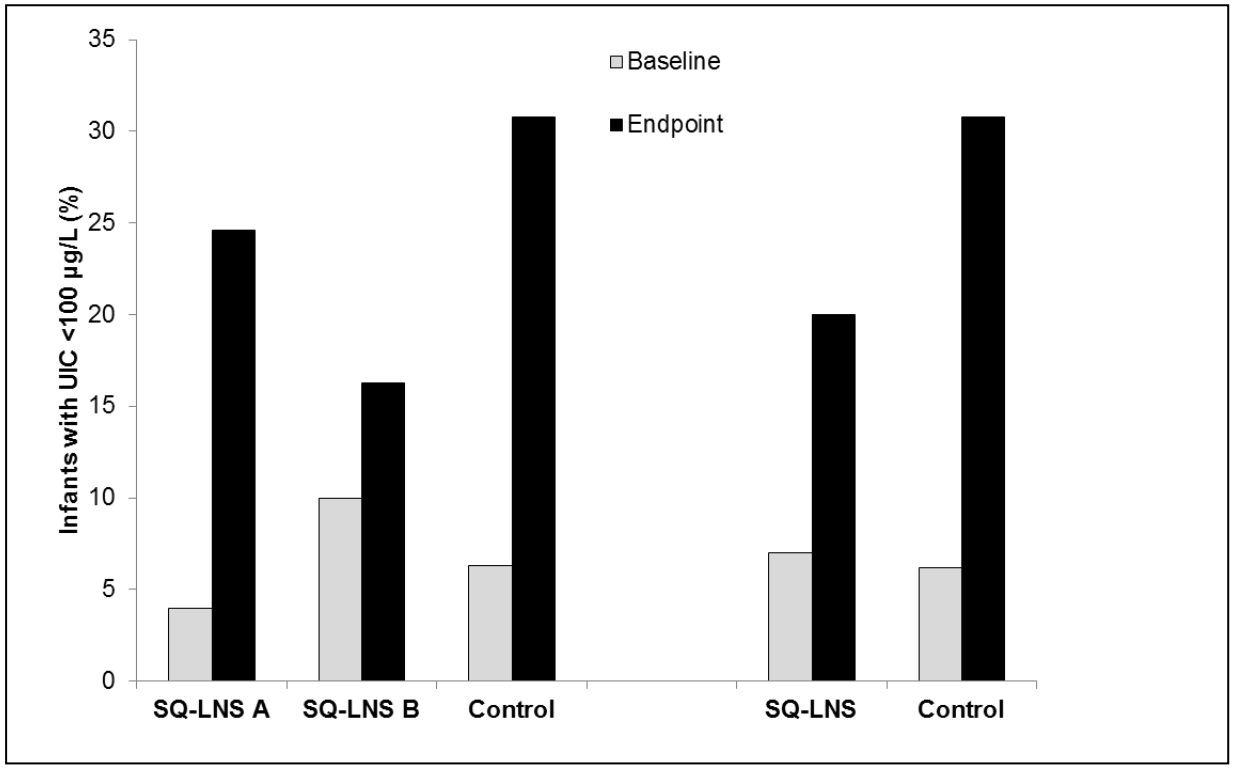

Figure 5.3: Proportion of infants with urinary iodine concentrations (UIC) <100 µg/L at baseline (six months) and endpoint (12 months) in the two small-quantity lipid-based nutrient supplement (SQ-LNS) groups and the control group; and in the pooled small-quantity lipid-based nutrient supplement group and the control group. n = 126, 130 and 130 for the SQ-LNS A, SQ-LNS B and control group, respectively, at baseline. n = 69, 86 and 107 for the SQ-LNS A, SQ-LNS B and control group, respectively, at endpoint. Differences in the endpoint proportion of infants with a UIC <100 µg/L between the SQ-LNS B and control group, as well as between the pooled SQ-LNS and the control group were determined using binary logistic regression analysis including maternal UIC, age, sex, and continued breastfeeding until 12 months as covariates. When adding baseline UIC <100 µg/L as covariate the differences were no longer significant.

Using binary logistic regression analysis including baseline maternal UIC, age, sex and continued breastfeeding at 12 months as covariates, SQ-LNS B significantly decreased the odds for infants having UIC <100 µg/L at 12 months (OR = 0.132 [0.04, 0.46]) compared to the control group (**Figure 5.3**). When merging the SQ-LNS A and SQ-LNS B infants into one SQ-LNS group, infants who received SQ-LNS had significantly lower odds for having a UIC <100 µg/L at 12 months (OR = 0.289 [0.11, 0.75]) compared to infants in the control group. However, when adding baseline infant UIC as covariate these effects were no longer significant.

In a sub-group analysis, we determined the effect of SQ-LNS (separate groups and combined) on UIC in infants who no longer received breast milk and who continued to be breastfed at 12 months separately (**Table 5.4**). A significant effect of SQ-LNS (combined group) for higher UIC at 12 months was found in the infants who no longer received breast milk (P = 0.039), but not in the infants who continued to be breastfed (*P* = 0.290). When further dividing the non-breastfed infants into sub-groups, to those who consumed commercial infant cereals frequently or seldom/never, a trend for a significant effect of SQ-LNS (combined group) for higher UIC at 12 months was only apparent in the non-breast fed infants who seldom/never consumed commercial infant cereals (*P* = 0.075), but not in the ones who consumed commercial infant cereals frequently (*P* = 0.253).

Table 5.3: Urinary iodine concentrations (UIC) at baseline (age six months) and endpoint (age 12 months) by randomization group (geometric means and 95% confidence intervals)[a]

	SQ-LNS A	SQ-LNS B	Control	*P*[b]	*P*[c]	SQ-LNS	Control	*P*[b]	*P*[c]
n baseline UIC	126	130	130			256	130		
n endpoint UIC	69	86	107			155	107		
n baseline and endpoint UIC	31	43	50						
Baseline	349.3 (307.1, 397.3)[d]	323.3 (283.7, 368.5)	331.1 (292.5, 372.0)			335.9 (306.5, 368.0)	331.1 (292.5, 372.0)		
Endpoint	232.9 (186.0, 295.1)	233.5 (193.2, 282.3)	190.9 (154.3, 236.2)	0.080[e]	0.724[e]	233.2 (202.0, 269.3)	190.9 (154.3, 236.2)	0.025[e]	0.424[e]

[a]SQ-LNS, small quantity lipid nutrient supplement.
[b]*P* value for difference in endpoint UIC between the groups determined by using ANCOVA adjusted for baseline maternal UIC, age, sex and continued breastfeeding at 12 months. UIC were log-transformed to perform ANCOVA
[c]*P* value for difference in endpoint UIC between the groups determined by using ANCOVA adjusted for baseline infant and maternal UIC, age, sex, continued breastfeeding at 12 months. UIC were log-transformed to perform ANCOVA.
[d]Values are geometric means and 95% CI, all such values.
[e]Continued breastfeeding until 12 months was a significant predictor of endpoint UIC, $P<0.05$.

Table 5.4: Urinary iodine concentrations (UIC) at endpoint (12 months) by randomization group (geometric means and 95% confidence intervals) for infants who no longer received breast milk and who continued to being breastfed at 12 months separately[a]

	SQ-LNS A	SQ-LNS B	Control	*P*[b]	SQ-LNS	Control	*P*[b]
Breastfed at 12 months	n = 34	n = 47	n = 53		n = 81	n = 53	
Endpoint UIC	341.3 (268.0, 434.5)[c]	341.6 (255.4, 387.4)	263.7 (203.1, 342.2)	0.557	325.5 (278.9, 379.9)	263.7 (203.1, 342.2)	0.290
Non-breastfed at 12 months	n = 33	n = 38	n = 50		n = 71	n = 50	
Endpoint UIC	169.8 (119.0, 242.3)	160.2 (117.0, 219.3)	140.1 (99.2, 197.7)	0.123	164.6 (130.8, 207.1)	140.1 (99.2, 197.7)	0.039

[a]SQ-LNS, small quantity lipid nutrient supplement.
[b]*P* value for difference in endpoint UIC between the groups determined by using ANCOVA adjusted for baseline maternal UIC, age, sex and continued breastfeeding at 12 months. UIC were log-transformed to perform ANCOVA
[c]Values are geometric means and 95% CI, all such values.

Discussion

Iodine status of the complementary fed infants included in this study declined significantly from six to 12 months of age (334 µg/L to 215 µg/L, respectively). Based on the geometric mean UIC, however, the infants were considered iodine sufficient at both time points. The daily provision of 45 µg of iodine through SQ-LNS resulted in higher UIC and decreased the odds for having a UIC <100 µg/L at 12 months, but was not efficacious in counteracting an overall decline in iodine status. Sub-group analyses revealed that iodine status only declined in infants who no longer received iodine-rich breast milk at 12 months, and that it was these same non-breastfed infants who benefited from the SQ-LNS.

This study clearly showed that breast milk was a prominent source of iodine for these infants. Breastfeeding reduced the odds of being iodine deficient (UIC <100 µg/L) at six months, and continued breastfeeding maintained adequate iodine intake at 12 months (350.1 µg/L and 373.2 µg/L at six and 12 months respectively), whilst greatly reducing the odds for having a UIC indicating inadequate iodine intake. This is in agreement with the statement made by Alexy *et al*., that milk, in the form of either breast milk, formula, or milk porridge, has the highest impact on the iodine intake of infants during complementary feeding (Alexy et al., 2009). Furthermore, assessment of breast milk iodine concentrations (BMIC) in this study population at six months revealed a median BMIC of 170 µg/L (Osei et al., chapter 4), which is relatively high when compared to previously reported BMIC in other iodine sufficient countries (Azizi, 2007, Bazrafshan et al., 2005, Andersson et al., 2010). Median BMIC in this study population were, however, comparable to the median BMIC that was measured in 100 lactating mothers of younger infants (two to four months) living in a different town within the same province in South Africa, but with similar socio-economic status and living conditions (Osei et al. under review, chapter 3).

In South Africa early cessation of breastfeeding is highly practiced (Doherty et al., 2012, Siziba et al., 2015). Results from a recent study carried out in a peri-urban community revealed that certain social circumstances drove mothers to use formula milk as compared to breastfeeding (Ijumba et al., 2014). However, compared to formula milk, breast milk is a more affordable, reliable and potentially better source of iodine for infants, especially in this particular population with high BMIC. Moreover, although findings on feeding practices in this population reported that over 68% of the infants frequently consumed potentially fortified commercial infant cereals at six months (Osei et al. chapter 4); the consumption of these infant cereals drastically decreased within the six month intervention period to 24% at 12 months. Thus, even though we showed that commercial infant cereals significantly contribute towards adequate iodine status in infants at 6 months (Osei et al. chapter 4), and in non-breastfed infants at 12 months, the marked decline in consumption of commercial infants cereals from six to 12 months confirms

previous concerns that commercial infant cereals may not be a sustainable source of micronutrients in low-socioeconomic populations (HSRC, 2013).

Home fortification of staple foods (e.g. maize porridge) through the use of micronutrient powders or lipid-based nutrient supplements, are potentially feasible interventions to be introduced in South Africa. However, thoughtful behaviour change communication programmes to support their adoption would be required (Pelto et al. 2013), and of course, mothers of nutritionally vulnerable children should have easy access to these products.

Assessment of household salt iodine concentration (SIC) showed that over 90% of households in this study had access to adequately iodized salt (Osei et al. chapter 4), as mandated in South Africa (Jooste and Zimmermann, 2008); hence, providing sufficient iodine indirectly through maternal breast milk for infants who continued to breastfeed. Iodine concentrations in breast milk are reported to be higher in areas where iodized salt is consumed (Azizi and Smyth, 2009). It was recently also shown that household SIC were significantly associated with BMIC (β=0.329; P =0.005) in South African lactating mothers (Osei et al. chapter 3).

Overall, infants receiving SQ-LNS (combined group) had significantly higher UIC at 12 months compared to infants in the control group. Sub-group analyses further revealed that it was only the infants who no longer received breast milk at 12 months who benefitted from the SQ-LNS. This is not surprising considering that the high UIC of breastfed infants observed at six months was maintained at 12 months, implying that breastfed infants received sufficient amounts of iodine from breast milk. Therefore, the daily addition of 45 µg iodine in the form of SQ-LNS did not have any additional benefits for these infants. Hence, iodine fortified SQ-LNS may not be of additional benefit in populations with successful salt iodization programmes, high iodine concentrations in breast milk and a high rate of continued breastfeeding. This is in line with the conclusions drawn by Hess and colleagues, who recently reported no effects on urinary iodine, thyroxine or thyroglobulin concentrations in iodine sufficient Burkinabe infants who were breastfed and consumed 90 µg of iodine daily in SQ-LNS over a nine month period (Hess et al., 2015).

It has to be acknowledged that the beneficial effects of SQ-LNS on infant UIC at 12 months were no longer significant after adjusting for baseline UIC. This can be explained by the marked reduction in sample size from 262 to 124 when adding baseline UIC as covariate into the ANCOVA and binary regression models. The small sample of infants with corresponding UIC data at both time points is therefore a major limitation of this study. However, adjusting the ANCOVA models for individual baseline UIC may not be appropriate, since UIC determined in spot urine reflects recent iodine intake, and should not be used as an individual marker of iodine status.

In conclusion, this study indicates that continued breastfeeding and the consumption of commercial infant cereals during the weaning period effectively maintains adequate iodine intake in infants from a country with a successful salt iodization programme, and that iodine fortified SQ-LNS may not have any additional benefits in this population. Non-breastfed infants, however, are at risk of developing iodine deficiency during weaning period. Even though the provision of a daily dose of 45 µg iodine in the form of SQ-LNS improved their status, this fortification level was not efficacious in counteracting a decline in iodine status from six to 12 months of age and therefore a risk for deficiency at a later age. Thus, a higher fortification level providing a daily dose of 90 µg as recommended by Dunn (2003) and WHO (2007) may be more efficacious in reducing the risk of inadequate iodine intake in infants who do not receive iodine from breast milk or in iodine deficient populations. More studies are needed investigating the potential of iodine fortified SQ-LNS as a strategy to reduce the risk for inadequate iodine intake in weaning infants who are breastfed and are residing in areas where universal salt iodization programmes are not well established.

Public health nutrition interventions should therefore continue to promote supplementary breastfeeding up to the age of two years, whilst infants consume complementary foods, in regions with high coverage of universal salt iodization, as recommended by the WHO (Andersson et al., 2007). Furthermore, in these same regions, whilst encouraging breastfeeding, it may also be necessary to increase the iodine content in home fortification products such as SQ-LNS to the recommended iodine fortification level of 90 µg, so as to fill the nutrition gap for non-breastfed infants, especially in countries where breastfeeding practices are known to be sub-optimal and access to iodine-fortified commercial infant cereals is limited.

Key messages

- **Breast milk plays an important role in maintaining adequate iodine intake during weaning period; therefore, supplementary breastfeeding should be encouraged.**
- **SQ-LNS fortified with iodine at 45 µg could potentially improve iodine status of infants, however, it is not efficacious in maintaining adequate intake and therefore an increase to 90 µg is recommended.**

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Conflict of interest

The authors declare no conflict of interests except CMS who received a travel grant from Unilever.

Contributions

CMS, MF and JB conceptualized and designed the study; TM, MR, JO and JB executed the study and collected data; JO performed biochemical analyses; JB and JO performed statistical analyses. JO wrote the first draft of the manuscript and all authors read and edited the manuscript.

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Supplementary material

Supplementary Table 5.1: Baseline characteristics of infants who provided urine at baseline, at endpoint, or who did not provide urine[a]

	Provided urine at baseline (*n*=386)	Provided urine at endpoint (n=262)	Provided no urine sample (*n*=226)	*P*
Age (months)	6.2 ± 0.2[b]	6.2 ± 0.3	6.2 ± 0.3	0.858
Male [*n* (%)]	197 (51)	130 (49.6)	120 (53.1)	0.323
Baseline anthropometric indexes [*n* (%)]				
Stunting (LAZ <-2 SD to -3 SD)	104 (26.9)	69 (26.3)	68 (30.1)	0.315
Severe stunting (LAZ <-3 SD)	26 (6.7)	17 (6.5)	23 (10.2)	0.099
Wasting (BAZ <-2 SD to -3 SD)	9 (2.3)	4 (1.5)	9 (4.0)	0.083
Overweight (BAZ >2 SD)	25 (6.5)	22 (8.4)	13 (5.8)	0.282
Obese (BAZ >3 SD)	7 (1.8)	5 (1.9)	5 (2.2)	0.349
Hb (g/dl)	11.3 ± 1.4	11.4 ± 1.3	11.4 ± 1.4	0.274
Anaemia (Hb <11 g/dl) [n (%)	138 (35.8)	86 (32.8)	85 (37.6)	0.355
Breastfeeding [*n* (%)]	279 (72.3)	176 (67.2)	156 (69.3)[c]	0.415

[a] BAZ, BMI-for-age z score; HAZ, height-for-age z score; SQ-LNS, small quantity lipid nutrient supplement; WAZ, weight-for-age z score;

[b] Mean ± SD (all such values)

[c] infants that provided no urine, *n*=225

P Value for differences between randomization groups determined using one-way ANOVA or Pearson Chi-square test.

CHAPTER 6: GENERAL SUMMARY AND RECOMMENATIONS

6.1. Introduction

Adequate iodine intake during the period of infancy is crucial as the consequences of iodine deficiency can lead to thyroid malfunction which may cause irreversible brain damage, impaired growth and increased mortality (Zimmermann *et al.*, 2008). Excessive iodine intake, in turn, may also be detrimental to infants' immature thyroid glands (Connelly *et al.*, 2012). Universal salt iodization is the key strategy to control iodine deficiency, and in areas where lactating mothers consume adequately iodized salt, iodine can be transferred from the mother to the infant via breast milk (WHO et al., 2007). Complementary fed infants are recommended to consume iodine fortified complementary foods whilst breastfeeding is continued (Andersson *et al.*, 2007). It is not recommended to add iodized salt to home prepared complementary foods because of the potential harmful effects of excessive sodium intake on infants developing kidneys and blood pressure later in life (Cribb *et al.*, 2012)

Although South African school going children and women of child-bearing age have adequate iodine status (Jooste *et al.*, 2007), to date, the iodine status of infants and lactating mothers in the country has not been explored. Against this background, this PhD research aimed at assessing and improving the iodine nutrition of lactating mothers and their infants during the periods of breastfeeding and complementary feeding in the North-West Province, South Africa. The specific objectives of the research were the following:

1. to assess breast milk iodine concentration (BMIC), iodine status and thyroid function of lactating women and their breastfed infants aged two to four months old;

2. to determine the iodine status of six months old infants in relation to their feeding practices and psychomotor milestone development; and

3. to investigate whether the provision of novel small-quantity lipid-based nutrient supplements (SQ-LNS) fortified with iodine to infants from six to 12 months will improve/maintain iodine status compared to a control group.

This chapter will give a summary and highlight the main findings of the three manuscripts provided in chapters 3 to 5 of this thesis. Based on these main findings, the public health implications of the study and recommendations for future research will be given.

6.2. Breast milk iodine concentrations, iodine status and thyroid function of South African breastfed infants aged two to four months and their lactating mothers

The findings of this research suggested that in a convenience sample of 100 lactating mother and infant pairs, breastfed infants received adequate iodine from breast milk. Based on a median BMIC of 179 µg/L and a breast-milk consumption of 0.78 L at three months (Institute of Medicine *et al.*, 2001), infants consumed 140 µg iodine/day, which was well above the recommended daily iodine intake of 90 µg and 110 µg for infants younger than six months of age, by WHO and the IOM, respectively (Institute of Medicine *et al.*, 2001; WHO et al., 2007). The median thyroglobulin (Tg) concentrations of 77.1 µg/L measured in infants was six times higher than reported in iodine sufficient school aged children (Zimmermann *et al.*, 2013). However, infant reference values are lacking for Tg in dried blood spots and therefore it was not possible to estimate the prevalence of elevated Tg. None of the infants had subclinical hypothyroidism, and no associations of infant UIC with Tg, thyroid stimulating hormone (TSH) and thyroxine (T4) concentrations were found.

Lactating mothers had adequate iodine intake and normal TSH concentrations. It was found that maternal UIC was a predictor for BMIC, which in turn was a predictor for UIC in infants.

The median iodine concentration in the household salt was 44 ppm, and therefore above the upper level of 40 ppm recommended by WHO (WHO et al., 2007) but was within the South African mandatory fortification levels of 35-65 ppm at production. Moreover, 21% of households consumed salt iodized above the upper level of 65 ppm. Household salt iodine concentrations predicted both BMIC and maternal UIC. Adequately iodized salt (>15 ppm) was consumed by 90% of women.

The vast amount of data collected in this study provided a complete picture of the iodine status in the studied infants and their mothers despite the small sample size. No data was collected on the use of any iodine containing disinfectants applied for maternal wound disinfection or continuous umbilical care of the infants, and it was acknowledged to be a limitation of this research.

The study concluded that the South African salt iodization programme provided sufficient iodine to lactating mothers and their infants. However, salt iodine levels appeared to be poorly monitored and therefore on-going monitoring and surveillance of salt fortification at production is required to ensure sustenance of optimal iodine status in vulnerable population groups.

Breast milk samples collected for this study were used to optimize a new inductively coupled plasma mass spectrometry (ICP-MS) method and further evaluate the effect of analytical method and timing of sample collection on BMIC. These data have already been published

(Dold *et al.*, 2016) and is part of the PhD thesis of Miss Susanne Dold of the Human Nutrition Laboratory in Zurich, Switzerland (see Addendum 9).

6.3. Iodine status and associations with feeding practices and psychomotor milestone development in six-months-old South African infants

The study showed that six-months-old complementary fed infants (n=386) and their mothers (n=371) had adequate iodine status with median UIC of 345 µg/L and 142 µg/L in infants and mothers, respectively. More than half (72%) of the infants were still breastfed and the median iodine concentrations in breast milk was 170 µg/L. Infant UIC was positively associated with BMIC and there was a trend for higher UIC in breastfed infants as compared to non-breastfed infants. The consumption of commercial infant cereals was more frequent (68%) compared to the consumption of home-made maize flour porridge (9%). Infants who consumed commercial infant cereals frequently had higher UIC compared to those who seldom/never consumed these cereals. However, no associations between infants' iodine status and psychomotor development were found.

Limitations to this study included low success rate of obtaining complete sets of urine samples from infants and mothers, thus reducing the sample size for some analyses. Blood samples were not collected hence thyroid function could not be assessed. Although BMIC were associated with adequate iodine status, the amount of breast milk consumed by individual infants was unknown, which was another limitation. Because the iodine content in frequently consumed commercial infant cereals was not measured, a conclusion about the impact it had on the adequate iodine status observed in infants could not be reached.

This study concluded that in a peri-urban setting in South Africa, supplementary breast milk and the consumption of commercial infant cereals may have significantly contributed to the adequate iodine intake in weaning infants. However, iodine content of commercial infant cereals available in South Africa needs to be investigated.

6.4. Efficacy of novel small-quantity lipid-based nutrient supplements (SQ-LNS) in maintaining adequate iodine status during complementary feeding in South African infants

Iodine status of the complementary fed infants included in this study declined significantly from six to 12 months of age (333.8 µg/L to 214.9 µg/L, respectively). However, the infants had adequate iodine status at both time points. The daily provision of 45 µg of iodine through SQ-LNS resulted in higher UIC and decreased the odds for being deficient at 12 months, but was

not efficacious in counteracting an overall decline in iodine status. Sub-group analyses revealed that iodine status only declined in infants who were no longer breastfed at 12 months (UIC of 373 µg/L in breastfed versus 160 µg/L in non-breastfed infants at 12 months), and that it was the same infants who benefited from the SQ-LNS. In addition, the consumption of commercial infant cereals also contributed to improved iodine status and prevented a decline in non-breastfed infants. However, only few infants received commercial infant cereals at 12 months (26%).

The small sample of infants with corresponding UIC data at both time points (n=124) was a major limitation of this study.

From this study it was concluded that in areas with successful salt iodization programmes, supplementary breastfeeding and the consumption of commercial infant cereals maintained adequate iodine intake of weaning infants, and iodine fortified SQ-LNS may not have any additional benefits in these infants. In contrast, non-breast fed infants were at risk of developing iodine deficiency and even though the provision of a daily dose of 45 µg iodine in the form of SQ-LNS improved their status, this fortification level was not efficacious in counteracting a decline in iodine status from six to 12 months of age. Therefore, a higher fortification level providing a daily dose of 90 µg as recommended by Dunn (2003) may be more efficacious in reducing the risk of inadequate iodine intake in non-breastfed infants who do not have access to iodine-fortified cereals.

6.5. Public health perspective

Findings from this research confirmed the success South Africa has made with regards to universal access to adequately iodized salt. Over 90% of households in this research had access to adequately iodized salt (>15ppm), which was an improvement when compared to the 77% and 80% that were previously reported nationally, and in the North-West Province, respectively, in 2005 (Jooste *et al.*, 2007). Analysis of household salt in the two surveyed towns in this research pointed out that the population was also at risk of consuming over-iodized salt. About 59% and 28% of households in Potchefstroom and Jouberton respectively, had salt iodine concentrations (SIC) above 40 ppm, which is the upper fortification level set by the WHO (WHO et al., 2007). Furthermore, 21% and 27% of the same households, respectively, had SIC above 65 ppm which is the upper mandatory fortification level at production in South Africa (Jooste & Zimmermann, 2008). Hence, there is a dire need for on-going monitoring of salt fortification in the country to ensure compliance with the set recommendations.

The successful salt iodization programme in the country contributed to the adequate iodine status that was observed in the studied lactating mothers and infants. In younger infants (aged

two to four months), who were mostly breastfed in this research, iodine in breast milk contributed to their iodine intake. In weaned infants, however, there was an observed risk for inadequate iodine intakes, especially at 12 months of age. This finding is in line with current literature that points out that the transition from a diet of breast milk and/or infant formula to a diet that includes solid foods and other beverages, is associated with major changes in both macronutrient and micronutrient intake by infants, which puts them at risk of insufficient intakes (Agostoni *et al.*, 2008; Fein *et al.*, 2008).

In weaned infants the results showed that if breastfeeding was continued at six months and further until 12 months, infants were at a lower risk of being iodine deficient. However, in the infants who were no longer breastfed at 12 months, the iodine status drastically declined and this put them at higher risk of being deficient. Additionally, the research findings also pointed out the potential contribution of frequently consumed commercial infant cereals towards adequate iodine status. Six month old weaning infants who frequently consumed commercially available infant cereals had significantly higher UIC than those who did not. Although we observed a decline in the iodine status from six to 12 months in non-breastfed infants, of interest was that in non-breasted infants who frequently consumed commercial infant cereals UIC did not decline at 12 months, indicating that iodine fortified commercial infant cereals could potentially contribute to the iodine status of non-breastfed infants. Despite the limitation of not knowing the exact iodine content in frequently consumed infant cereals, it can be assumed that they were potentially fortified with iodine.

Moreover, this study showed that iodine fortified SQ-LNS improved the iodine status of non-breastfed infants, but that it was not efficacious in counteracting the decline in iodine status. These results lead us to query the adequacy of 45 µg of iodine provided by the SQ-LNS products used in this present study, and perhaps a higher dose of 90 µg as recommended by the WHO may have been more efficacious.

Although commercial infant cereals could potentially be a good source of iodine as shown in this research, in poorer communities, the frequent consumption of these cereals may not be sustainable. In this study, the reduction in the percentage of infants that consumed commercial cereals within the six month intervention period confirms this. Therefore, in circumstances where mothers cannot afford to buy commercially available infant cereals, public health nutrition programmes should consider the introduction of iodine fortified SQ-LNS for fortification of home prepared staple cereals (porridges).

Results from this research confirm that during the weaning period, supplementary breastfeeding will ensure that infants have adequate iodine status. Furthermore, it also shows that iodine fortified commercial infant cereals can potentially be a good source of iodine for infants. These

findings are in line with the consensus reached by the WHO Secretariat that states that in regions where universal salt iodization has been effective for at least two years, with adequately iodized salt being consumed by more than 90% of the population, it can be reasonably expected that the iodine requirements of women of child-bearing age and pregnant and lactating women are covered by their diet. However in these same regions, iodized salt may not provide enough iodine to meet a child's needs during complementary feeding, especially if the lactating mother is only marginally iodine sufficient unless complementary foods are fortified with iodine (Andersson *et al.*, 2007). The recommendations further state that in circumstances where iodine fortified complementary foods are not available, additional iodine should be given to infants until they are old enough to eat the normal family food (Andersson *et al.*, 2007). In this research additional iodine that was provided to infants was in the form of SQ-LNS, which proved to be beneficial in improving iodine status of vulnerable non-breastfed infants, but the dose of 45 µg/day was not efficacious in counteracting a decline in iodine status.

Public health nutrition interventions should therefore continue to promote exclusive breastfeeding for the first six months of life, and complementary fed infants should be supplemented with breast milk up to the age of two years in regions with high coverage of universal salt iodization, as recommended by the WHO (Andersson *et al.*, 2007; WHO, 2010). Furthermore, in the same regions, whilst encouraging breastfeeding, it may also be necessary to encourage the use of commercially available iodine fortified infants cereals. In areas where the use of these cereals is not feasible, the introduction of home fortification products such as SQ-LNS fortified with iodine may be necessary. The iodine content of these products and commercial infant cereals should be monitored to ensure the recommended iodine fortification level of 90 µg is met, in an attempt to fill the nutrition gap for non-breastfed infants, especially where breastfeeding practices are known to be sub-optimal.

6.6. Recommendations for future research

More research is still needed to establish the cut-off for iodine excess during infancy. Furthermore, investigations are required to establish and define the cut-offs and reference values for thyroglobulin (Tg) in dried blood spots (DBS) during infancy.

In South Africa the universal salt iodization programme needs to focus on the monitoring of the salt fortification levels of table salt to ensure that households do not consume over-iodized salt.

Future studies that aim to assess and improve iodine status of infants and lactating women should attend to the following:

- Measure the iodine content of frequently consumed commercial infant cereals. Alexy *et al*., (2009) investigated the fortification practice of commercial complementary foods in Germany, which is exemplary for other European countries. Based on a market survey in autumn 2008, the results showed that 51% of the commercial complementary foods were fortified with iodine at varying degrees. The highest variation of iodine content was found in fortified milk-cereal porridges (Alexy *et al.*, 2009). However, more research is needed in other countries.
- Assess the potential of iodine fortified SQ-LNS at a dose of 90 µg per day, as a strategy to reduce the risk of inadequate iodine intake in complementary fed infants who are breastfed and are residing in areas where universal salt iodization programmes are not well established.
- Assess the potential of iodine fortified SQ-LNS at a dose of 90 µg per day in areas where supplementary breastfeeding rates are low.

6.7. References

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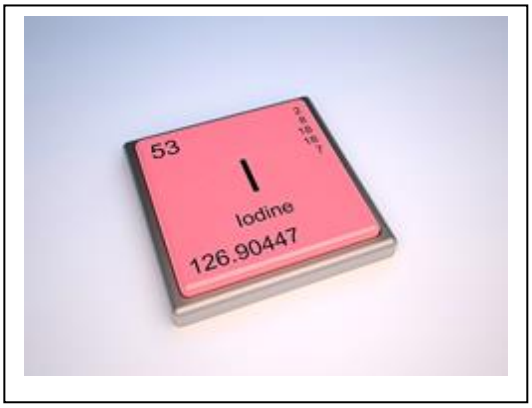
ADDENDA



ADDENDUM 1: Instructions for authors (JCRPE)

JCRPE Journal of Clinical Research in Paediatric Endocrinology

INSTRUCTIONS TO AUTHORS

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Keywords are included according to MeSH (Medical Subject Headings) National Library of Medicine.

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Frederick P. Rivara, MD, MPH in Arch Pediatr Adolesc Med. 2002;156:11-13

ADDENDUM 2: Instructions for authors (Maternal & Child Nutrition)

Maternal & Child Nutrition

Edited By: Victoria Hall Moran and Rafael Pérez-Escamilla

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Online ISSN: 1740-8709

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Baby Friendly Health Initiative (2012) 10 Steps to Successful Breastfeeding. Available at: http://www.babyfriendly.org.au/about-bfhi/ten-steps-tosuccessful-breastfeeding/ (Accessed 23 November 2014).

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ADDENDUM 3: Informed consent-iodine cross-sectional study

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INFORMATION SHEET

Study title: Assessment of breast milk iodine and n-3 fatty acid concentrations, iodine, iron and n-3 fatty acid status and thyroid function of lactating women and their infants
Go kgethisa masi a letsele gore a na le iodine, n-3 fatty acids go go kanakang le modiro wa kgeleswa ya thyroid.

Project leader/ Moeteledi pele: Prof Marius Smuts
Co-project leader/ Motlatsa-moeteledi pele: Dr. Jeannine Baumgartner

Dear Parent / Legal Guardian/
Motsadi/motlhokomedi

Who are we?
Re bo mang?
We are from the North-West University, Potchefstroom. We are testing the breast milk of mothers who are mainly breast feeding their babies, for iodine and n-3 fatty acid concentrations. We are also testing the iodine, iron and n-3 fatty acid status and thyroid function of lactating mothers and their babies. We invite you to participate in this study.
Re tswa ko Yunibesiting ya bokone-bophirima, Potchefstroom. Re tlhatlhoba mashi a letsele a bo mme ba ba amusang masea a bone gore a na le iodine the n3-fatty acids go le kanakang. Gape re tlhatlhoba modiro wa kgeleswa ya thyroid mo maseeng le bo mme ba ba antshang masea. Re go mema go tsaya karolo.

Why do we want to do the research?
Lebaka la tlhatlhobe e, ke lefe?
Micronutrients such as iron and iodine, as well as n-3 fatty acids are needed for proper brain development and functioning. The micronutrient and n-3 fatty acid status of breast fed babies depends on the micronutrient and n-3 fatty acid concentrations in the breast milk, which they get from their mothers. Currently there is no available data on breast milk iodine and n-3 fatty acid concentrations of lactating mothers in South Africa. There is also no existing data on the iodine and n-3 fatty acid status and thyroid function of lactating South African women and their breast fed babies. This information is needed to optimise the nutritional status of lactating mothers and their developing infants.
Dikotla tse di tshwanang le iodine, iron le n2-fatty acids di tlhokagala mo mebeleng go thusa book go dira le go gola. Masea a ikanyetse go bo mme go ba neya dikotla tse go tswa mo masing a letsele. A go di tokomane tse di tlhalosang maemo a dikotla tse mo bommemg ba Afrika borwa le masea a bona, ke ka moo re batlang go itse gore re kgone go fa bomme le masea a bone dikotla tse di lekanetseng.

What do we expect from the participants in the study?
Re solofela eng go tswa go bomme ba ba tsayang karolo?

If you agree to participate in this study, we will ask you and your baby to visit our metabolic unit at the North-West University (Pukke) for a day where you will be asked to do the following:

Ga o dumela go tsaya karolo, re tlo kopa o tle kwa tliniking ya rona ko Yunibesiting ya bokone-bophirima letsatsi le le nngwe fela go re go dire tse di latelang:

We will ask you some questions on food sources that provide iodine as well as on infant feeding practices.
Re tlo go go botsa ka mefuta ya dijo tse o dijang le tse o dineelang lesea la gago.

Your weight and height will be taken. The weight, length and head circumference of your baby will also be taken.
Re tlo go tsaya bo kete le bo telle bag ago le lesea la gago

Urine (40ml for mother; 5ml for baby) and blood (4 ml for mother; 2 ml for baby) samples will be taken from both you and your baby.
Re tlo kopa go tsaya moroto le madi go wean le lesea la gago.

Urine from your baby will be collected using a special urine collection nappy.
Moroto wa lesea la gago o tla tsewa go tswa mo leiring le tle tlo mo apesang lona.

You will be asked to provide a 15 ml breast milk sample. This can be expressed manually or by using an electric breast pump.
Re tlo go kopa go re neya masi a letsele la gago.

All the information collected is confidential and will not be given to anybody else; only the researchers will have access to it.
Polelo yotlhe e o re neyang yona e tlo ba sephiri magareng a ron ale wean fela.

Must I participate?
Ke tshwanetse go tsaya karolo?
Participation in this study is completely voluntary (your own choice). Whether you do, or do not, give your permission will not influence you or your child's access to health care in any way.
Go tsaya karolo go tswa go wena, ga se kgapeletso. Go se tseye karolo go ka se ame tokelo ya gago le ngwana wa gago mo dipetleleng le ditliniki.

May I change my mind?
A nka fetola mogopolo?
You may change your mind at any time without having to give a reason. The study is completely voluntary and it will not be kept against you in any way should you decide to withdraw from the study.
O ka fetola mogopolo nako nngwe le nngwe le ga o sa re fe lebaka. Go tsaya karolo go tswa go wena gape o na le tokelo ya go tswa.

What happens to the collected data?
Go diriwang ka tsotlhe tse di buiwang le tse di tsewang go nna le ngwana wa me?
All the data collected during the study will be made anonymous, handled with strict confidentiality and used only for scientific purposes. We will keep all biological samples (blood, urine) frozen at the study site for 5 years. After these 5 years all samples will be destroyed and discarded. The obtained data will be stored safely and reported only in an anonymous form. Only the responsible investigators and the members of the ethical committee have access to the original data under strict confidentiality.
Tsotlhe tse di diriwang le tse di buiwang magareng a rona le wena di tlo itsiwe ke rona fela. Re tlile go boloka di rekhoto tsotlhe go fitlhela me nwgaga e le metlhano, morage ga moo di tlo go latlhiwa.

Do I get a reimbursement for participating in the study?

A ke a lebogiwa ga ke tseya karolo?
You will receive a hamper with products for your baby as a token of appreciation and as a compensation for the time you are spending at the metabolic unit (half day).
Ga o tsaya karolo o tlo go fiwa hamper ya dimpho tsa ngwana.

Who can you contact if there are any queries?
Nka ikgolaganya le mang ga ken a le dipotso
For more information on the study you may contact Prof Marius Smuts at 018-299 2086 or 082 451 0486 **OR** Dr. Jeannine Baumgartner at 018-299 4011 or 0764364439 during office hours.
Ga o na le dipotso o ka letsetsa Prof Marius Smuts go 018-299 2086 kgotsa 082 451 0486 kgotsa Dr. Jeannine Baumgartner go 018-299 4011 or 0764364439

The study has been approved by the Ethics Committee of the North-West University. If you have any queries or problems regarding the study, you can contact Prof Amanda Lourens the chairperson of the NWU Ethics Committee at (018) 299 2606.
Ga o na le ditletlebo mapi le se se dirwang, o ka letsetsa Prof Amanda Lourens (Modula setilo wa NWU Ethics Committee) go (018) 299 2606.

If you are happy for you and your child to take part in the study, please read and sign the consent form.
Ga o batla go tsaya karolo wena le lesea la gago, buisa tsebe e e latelang ga o fetsa, o e tshwae.

NORTH-WEST UNIVERSITY
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT
POTCHEFSTROOM CAMPUS

PARTICIPANT CONSENT FORM

Study title: Assessment of breast milk iodine and n-3 fatty acid concentrations, iodine, iron and n-3 fatty acid status and thyroid function of lactating women and their infants.
Go kgethisa masi a letsele gore a na le iodine, n-3 fatty acids tse di kanakang le modiro wa kgeleswa ya thyroid mo basading ba ba amusing masea le mo maseeng a bone.

	YES/Eya	**NO/Nnya**
I voluntarily agree to take part in the study. Ke dumela go tsaya karolo		
I have been informed about and understand the purpose of the study. Ke bolelletswe tsotlhe e bile ke tlhaloganya mosola wa teng.		
I understand that I can withdraw my consent at any time and it will not affect my routine health care service received at any health care center. Ke tlhaloganya gore kena le tokelo ya go tlogela go tsaya karolo ga ke batla.		
I have been informed that all information will be treated as private and confidential. Ke bolelletswe gore tsotlhe di tla ba sephiri sa me le ba batlisisi ba Yunibesiti ya Bokone-Bophirima		
I understand that the following will be expected from me: (i) I and my child will be assessed for weight, height and circumferences at the beginning of the study. Nna le lesea la me re tlile go sekesekiwa bokete, bo llele le bo phara ba tlhogo kwa thsimologong.		
(ii) Blood and urine samples will be collected from me and my baby. Go tlo tseiwa madi le moroto go nna le lesea la me.		
(iii) I am required to express a sample of my breast milk. Ke tlhoka go ga molola bonnye ba masi a letsele la me.		
I have been given an opportunity to ask any questions regarding the study Kefilwe sebaka sa go botsa dipotso ga ke sa tlhaloganye.		

Would you be willing to be approached for other studies? Yes ☐ No ☐
A oka rata go ka lalediwa ka di patlisiso tse dingwe gape?

If I have any further queries or problems regarding the study, I can contact Prof Amanda Lourens the chairperson of the NWU Ethics Committee at (018) 2992606.
Ga ke na le dipotso tse dingwe ke filwe tetla ya go letsetsa Prof Amanda Lourens go (018) 2992606.

Name of mother/Lebitso la mme: .....

Name of baby/Lebitso la ngwana: .....

Participant code/Nomoro ya karolo: .....

Phone number/Nomoro ya mogala: .....

I hereby give consent that I and my baby may participate in the study.

Ke dumela gore nna le lesea la me re tseye karolo.

Signature/Letshwao: ..... Date/Letatsi: .....

Signed at/Tshailwe ko: .....

Witness/Paki: .....

I hereby give consent that my weight, height and circumferences and that of my baby can be measured.

Ke dumela gore nna le lesea la me re tseye karolo, go kadiwa boima le bo telele.

Signature/Letshwao: ..... Date/Letsatsi: .....

Signed at/Tshwailwe ko: .....

Witness/Paki: .....

I hereby give consent that my blood sample (4 ml) can be taken.

Ke dumela gore madi (4 ml) a tseiwe go nna.

Signature/Letshwao: ..... Date/Letsatsi: ……..

Signed at/Tshwailwe ko: .....

Witness/Paki: .....

I hereby give consent that the blood (2 ml) of my baby can be taken.

Ke dumela gore madi (2 ml) a tseiwe go lesea la me.

Signature/Letshwao: ..... Date/Letsatsi: ……..

Signed at/Tshwailwe ko: .....

Witness/Paki: .....

I hereby give consent that my breast milk (15 ml) and urine (40 ml) samples, and urine (5 ml) samples of my baby can be taken. Ke dumela gore moroto (40 ml) le masi a letsele (15 ml) a tseiwe go nna le lesea la me.

Signature/Letshwao: ..... Date/Letsatsi: ……..

Signed at/Tshwailwe ko: ..... Witness/Paki: .....

Fieldworker who informed the mother/Motlhalosi: ……..

Researcher/Motlhotlhomisi: .....

Thank you for your participation!
Re lebogela go tseya karolo ga gago!

ADDENDUM 4: Demographic questionnaire-iodine cross-sectional study

NORTH-WEST UNIVERSITY
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NOORDWES-UNIVERSITEIT
POTCHEFSTROOMKAMPUS

Cross-sectional study on assessment of breast milk iodine concentrations and iodine status and thyroid function of lactating women and their infants

Participant number:

C	**S**			

Date of Interview (DD/MM/YYYY): ...../...../.....

Fieldworker's name: .....

Section A: Socio-demographic and economic data

Baby

1) Gender: M ☐ F ☐
2) Age (in weeks): .....
3) Date of birth (DD/MM/YYYY): ...../...../.....
4) Birth weight (grams):

   .....

Mother

5) Age (in years): .....
6) Date of birth (DD/MM/YYYY): ...../...../.....
7) Ethnicity:
   - ☐ Black
   - ☐ Coloured
   - ☐ Indian or Asian
   - ☐ White

8) Marital status:

☐ Married

☐ Single

☐ Living together

☐ Divorced/ separated

☐ Widowed

9) Educational level:

☐ Primary (grade 1-7)

☐ Secondary (grade 8-12)

☐ Tertiary (college/ university)

☐ None

☐ Other: .....

10) How many children do you have? .....

11) Are you currently employed? Yes ☐ No ☐

12) Do you smoke at present? Yes ☐ No ☐

13) Did you smoke before pregnancy? Yes ☐ No ☐

Section B: Information on breast feeding, infant feeding practices and on diet

1) Are you currently breastfeeding your baby? Yes ☐ No ☐

2) How long do you plan to breast feed your baby? .....

3) Do you exclusively breastfeed your baby (incl. bottled breast milk)? Yes ☐ No ☐

4) If yes, how many times per day (24h) do you breastfeed? .....

5) If no, how many times per day (24h) do you breastfeed? .....

6) If no, please describe everything that the baby ate and/or drank yesterday during the day and night:

Breakfast	Midmorning	Lunch	Mid afternoon	Supper	After supper

7) Is this diet recall typical of what your baby eats on a daily basis? Yes ☐ No ☐

8) What breast milk substitute do you use?

Type	Yes / No	Brand / Ingredients (for home-made substitute)
Commercial **infant** cereal		
Homemade porridge/cereal		
Commercial cereal/porridge (not specifically made for infants)		
Cow's milk		
Tea		
Juice		
Water		
other		

9) What is the reason for using a breast milk substitute? .....

Diet of mother

Did you take any supplements during pregnancy? Yes ☐ No ☐

If yes, what type/brand of supplements? .....

Do you take any supplements at the moment? Yes ☐ No ☐

If yes, what type/brand of supplements? .....

10) Where do you buy your salt (choose an option)?

☐ Supermarket

☐ Spaza shop

☐ Other: .....

11) Do you add table salt to your food before eating (or tasting)?

Never	Rarely	Sometimes	Often	Always
1	**2**	**3**	**4**	**5**

12) In the food you eat at home, is table salt added during cooking?

Never	Rarely	Sometimes	Often	Always
1	**2**	**3**	**4**	**5**

13) Do you consume seafood (apart from fish)?

Never	Rarely	Sometimes	Often	Always
1	**2**	**3**	**4**	**5**

If yes, what types of seafood do you consume?

.....

.....

14) Do you consume fish in your household?

Never	Rarely	Sometimes	Often	Always
1	**2**	**3**	**4**	**5**

If yes, what type of fish do you consume?

☐ Tinned fish specify: .....

☐ Fresh fish: salt water fish specify: ..... ☐ Fresh water fish specify: ..... ☐ Dried fish specify: ..... ☐ Other specify: .....

15) Do you consume cow's milk?

Never	Rarely	Sometimes	Often	Always
1	**2**	**3**	**4**	**5**

16) Do you eat eggs?

Never	Rarely	Sometimes	Often	Always
1	**2**	**3**	**4**	**5**

17) Do you eat meat? (Beef, poultry, pork)

Never	Rarely	Sometimes	Often	Always
1	**2**	**3**	**4**	**5**

18) What type of meat do you consume the most (indicate with 1) and second most (indicate with 2) etc.?

☐ Chicken

☐ Liver (from any animal)

☐ Beef

☐ Lamb

☐ Goat

☐ Pork

☐ Sausages (incl. polony, viennas and boerewors)

19) What type and brand of margarine/oil do you use for food preparation and on bread?
Food preparation: .....

Bread: .....

22) How often do you eat away from home (at fast food outlets or take-aways)?

Never	Rarely	Sometimes	Often	Always
1	**2**	**3**	**4**	**5**

Thank you for your participation!!

ADDENDUM 5: Tswaka baseline questionnaire

BASELINE QUESTIONNAIRE

Baby's code:				

Date of the interview (dd/mm/yyyy):			/			/	2	0	1	

Fieldworker's code:	

SOCIO-ECONOMIC INFORMATION

1. With whom is interview?	Mother of the baby ***(go to question 3)***	1	
	Caregiver of the baby	2	

2. Caregiver's relationship to the child?	Father	1	
	Aunt	2	
	Uncle	3	
	Grandmother	4	
	Grandfather	5	
	Not related caregiver	6	

3.a How old are you? (in years)		

3.b What is your birth date?			/			/	**1**	**9**		

4. Are you married?	Yes, married	1	
	Common-law husband/wife	2	
	Living together	3	
	No, unmarried	4	
	Separated / divorced	5	
	Widowed/widow	6	

5. Did you ever attend school?	Yes	1	
	No ***(go to question 7)***	2	

6. What was the highest standard that you passed at school?	Sub A / Grade 1	01		
	Sub B / Grade 2	02		
	Standard 1 / Grade 3	03		
	Standard 2 / Grade 4	04		
	Standard 3 / Grade 5	05		
	Standard 4 / Grade 6	06		
	Standard 5 / Grade 7	07		
	Standard 6 / Grade 8 / Form I	08		
	Standard 7 / Grade 9 / Form II	09		
	Standard 8 / Grade 10 / Form III / NTC I	10		
	Standard 9 / Grade 11 / Form IV / NTC II	11		
	Standard 10 / Grade 12 / Form V / NTC III	12		
	Higher qualifications	13		

7. How many people live in the house?	a. Total number of people		
	b. Babies and small children		
	c. Primary school children		
	d. High school children		
	e. Adults		
	f. Elderly people		

8.a How many people in the household are employed and earns a salary or wage (weekly or monthly income)		

8.b How many children in the household gets a child grant		

8.c How many people in the household gets either an old age pension or disability t		

9. Where does the household usually get its drinking water from? *(Mark only one)*	Own tap – inside the house	1	
	Own tap – outside the house	2	
	Neighbour's tap	3	
	Public tap	4	
	Borehole	5	
	Other, specify ……..	6	

10. What type of toilet does the household have?	Flush toilet	1	
	Pit toilet	2	
	None	3	
	Other; specify ……..	4	

11. Do you have electricity available inside your home?	Yes	1	
	No ***(go to question 13)***	2	

12. During the last 4 weeks, were there times that you did not	Yes	1	

use electricity because you had not money to pay it?	No	2	

INFANT FEEDING

13. Are you currently breastfeeding your baby/Is the baby currently breastfed?	Yes ***(go to question 15)***	1	
	No	2	
	Don't know ***(go to question 15)***	3	

14. How old was the baby when breastfeeding was stopped? *(in months)*	

15. What was the <u>first</u> drink other than breast milk that your baby was ever given to drink?
……

16. How old was your baby when you gave this drink for the first time? *(in months)*	

17. At the moment, does your baby get any <u>milk feeds</u> other than breast milk?	Yes	1	
	No ***(go to question 20)***	2	
	Don't know ***(go to question 20)***	3	

18. What type of milk, other than breast milk is your baby getting?	Cow's milk (full strength)	1	
	Cow's milk (diluted)	2	
	Klim / Nespray	3	
	Infant formula Give name:	4	
	Other, specify:	5	

19. How old was your baby when he/she was given this <u>milk feed</u> for the first time? *(in months)*	

20. At the moment, does your baby get any semi-solid or solid food (with a spoon)?	Yes	1	
	No ***(go to question 24)***	2	
	Don't know ***(go to question 24)***	3	

21. What was the <u>first</u> semi-solid or solid food (with a spoon) that your baby ate?
……

22. How old was your baby when he/she ate semi-solid or solid food (with a spoon) for the first time? *(in months)*	

23a. How many times did your baby eat solid, semi-solid or soft foods (with a spoon) other than liquids yesterday during the day?	

23b. How many times did your baby eat solid, semi-solid or soft foods (with a spoon) other than liquids yesterday during the night?	

24. Did your baby drink anything from a bottle with a teat yesterday during the day and/or the night?	Yes	1	
	No	2	
	Don't know	3	

25. Does your baby get any dietary supplements (e.g. vitamin syrup, vitamin tablets)?	Yes, specify:	1	
	No	2	
	Don't know	3	

MORBIDITY

M1. During the past week (7 days), did your baby have diarrhoea (at least three watery stools per day)	1	Yes *	
	2	no ***(go to question M2)***	
	3	don't know ***(go to question M2)***	

M1a. For how many days did he/she have diarrhoea?	

When did your baby's diarrhoea start?	Date: _____/_____/ 20____
When did your baby's diarrhoea stop?	Date: _____/_____ / 20____ Or is it carrying on?_____

**** If marked "yes": Fieldworkers please collate information for study nurse to complete AE form***

M2. During the past week (7 days), did your baby vomit?	1	Yes *	
	2	no ***(go to question M3)***	
	3	don't know ***(go to question M3)***	

M2a. For how many days did he/she vomit?	

When did your baby's vomiting start?	Date: _____/_____/ 20____
When did your baby's vomiting stop?	Date: _____/_____ / 20____ Or it is carrying on? _____

**** If marked "yes": Fieldworkers please collate information for study nurse to complete AE form***

M3. During the past week (7 days), did your baby have a continuous cough (at least for one whole day)?	1	Yes *	
	2	no ***(go to question M4)***	
	3	don't know ***(go to question M4)***	

M3a. For how many days did he/she cough continuously?	

M3b. Was the cough wet or dry?	1	Wet	
	2	Dry	

When did your baby's coughing start?	Date: _____/_____/ 20____

When did your baby's coughing stop?	Date: _____/_____ / 20____ Or is it carrying on? _____

**** If marked "yes": Fieldworkers please collate information for study nurse to complete AE form***

M4. During the past week (7 days), did your baby have wheezing or difficult breathing?	1	Yes *	
	2	no ***(go to question M5)***	
	3	don't know ***(go to question M5)***	

M4a. For how many days did he/she have wheezing or difficult breath?	

When did your baby's wheezing / difficulty in breathing start?	Date: _____/_____/ 20____
When did your baby's wheezing / difficulty in breathing stop?	Date: _____/_____ / 20____ Or is it carrying on? _____

**** If marked "yes": Fieldworkers please collate information for study nurse to complete AE form***

M5. During the past week (7 days), did your baby have a hot body?	1	Yes *	
	2	no ***(go to question M6)***	
	3	don't know ***(go to question M6)***	

M5a. For how many days did he/she have a hot body?	

When did you notice that your baby first have a hot body?	Date: _____/_____/ 20____
When did you notice that your baby's body is no longer hot?	Date: _____/_____ / 20____ Or is it carrying on? _____

**** If marked "yes" : Fieldworkers please collate information for study nurse to complete AE form***

M6. During the past week (7 days), did your baby have any sores on the skin or rash?	1	Yes *	
	2	no ***(go to question M7)***	
	3	don't know ***(go to question M7)***	

M6a. For how many days did he/she have the sores or rash?	

When did your baby first have a sore or rash?	Date: _____/_____/ 20____
When did your baby's sore or rash stop?	Date: _____/_____ / 20____ Or is it carrying on? _____

**** If marked "yes": Fieldworkers please collate information for study nurse to complete AE form***

M7. During the past week (7 days), did your baby have a continuous runny nose/ blocked nose?	1	Yes *	
	2	no ***(go to question M8)***	
	3	don't know ***(go to question M8)***	

M7a. For how many days did he/she have a runny nose/blocked nose?	

When did your baby's runny nose / blocked nose start?	Date: _____/_____/ 20____
When did your baby's runny nose / blocked nose stop?	Date: _____/_____ / 20____ Or is it carrying on? _____

**** If marked "yes": Fieldworkers please collate information for study nurse to complete AE form***

M8. During the past week (7 days), did your baby have any other illness?	1	Yes *	
	2	no ***(go to question M9)***	
	3	don't know ***(go to question M9)***	

M8a. Which illness did your baby have? ....

When did your baby's illness start?	Date: _____/_____/ 20____
When did your baby's illness stop?	Date: _____/_____ / 20____ Or is it carrying on?_____

**** If marked "yes": Fieldworkers please collate information for study nurse to complete AE form***

M9. Did your baby visit any health service during the last week (7 days) because he/she was ill?	1	Yes	
	2	no ***(end of questionnaire)***	
	3	don't know ***(end of questionnaire)***	

M10.	Which health services did your baby go to during the last week?			
	a. Clinic	yes = 1	no = 2	
	b. Private physician / doctor	yes = 1	no = 2	
	c. Hospital	yes = 1	no = 2	
	d. Traditional healer	yes = 1	no = 2	
	e. Other (specify): …….....	yes = 1	no = 2	

M11. Did your baby receive any medicine prescribed by a nurse or doctor for his/her illness during the last week (7 days)	1	yes	
	2	no ***(go to question M12)***	

M11 What type of medicine? ....

M11b. For how many days did he/she receive medicine?	

M12. Was your baby officially diagnosed with any illness during the last week (7 days)?	1	yes	
	2	no ***(go to question M14)***	

M13. With what illness was your baby diagnosed? ....

M14. Was your baby hospitalised during the last week (7 days)	1	Yes *	
	2	no ***(end of questionnaire)***	

M15. How long was your baby in hospital? (days)	

When did your baby's stay in hospital start?	Date: _____/_____/ 20____
When did your baby's stay in hospital stop?	Date: _____/_____ / 20____ Or is it carrying on?_____

**** If marked "yes": Fieldworkers please collate information for study nurse to complete AE form***

M16. Why was your baby hospitalised? .....

ADDENDUM 6: Tswaka food frequency questionnaire

FOOD FREQUENCY

Baby's code: | | | | |

Ask for each food item, one at a time, how often the child usually eats the specific food item. The last week (or last seven days) should be taken as guideline, therefore the frequency that the child ate the food item during the last week. Make a cross on the option that describes the mother's answer the best). The options are as follows: Every day

Most days: not every day, but at least 4 times per week
Once a week: less than 4 times per week, but at least once per week
Never

Food item	***Frequency of intake during the last week***			
	Baseline	**Month 2**	**Month 4**	**Month 6 (end)**
Date (dd/mm/yyyy)**:**				
Fieldworker's code:				
Breastmilk	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Formula milk *If formula milk was used, please give name of the formula milk:*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never* .....
Cow's milk / amasi / maas Milk powder e.g. Klim, Nespray	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Yoghurt / danone	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Baby foods in a jar e.g. Purity	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Infant cereals or infant porridge e.g. Nestum, Cerelac, Cream of Maize, Baby Mabele	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Porridge made with maize meal (soft, stiff or crumbly)	*1___Every day* *2___Most days* *3___Once a week*	*1___Every day* *2___Most days* *3___Once a week*	*1___Every day* *2___Most days* *3___Once a week*	*1___Every day* *2___Most days* *3___Once a week*

Food item	*Frequency of intake during the last week*			
	Baseline	**Month 2**	**Month 4**	**Month 6 (end)**
	4___Never	*4___Never*	*4___Never*	*4___Never*
Cooked porridge, other than maize meal porridge e.g. oats, mabele	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Instant porridge, e.g. instant Maize, Mabele	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Bread	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Rice	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Potatoes	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Vegetables, any type (NOT potatoes) *If vegetables were eaten, please name the type of vegetables eaten mostly:*	*1___Every day* *2___Most days* *3___Once a week* *4___Never* 1 ..... 2 ..... 3	*1___Every day* *2___Most days* *3___Once a week* *4___Never* 1 ..... 2 ..... 3	*1___Every day* *2___Most days* *3___Once a week* *4___Never* 1 ..... 2 ..... 3	*1___Every day* *2___Most days* *3___Once a week* *4___Never* 1. ..... 2. ..... 3. .....
Fruit juice (includes juice squeezed from the fruit)	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Fresh fruit (any type) *If fruit were eaten, please name the type of fruit eaten mostly:*	*1___Every day* *2___Most days* *3___Once a week* *4___Never* 1 ..... 2 ..... 3	*1___Every day* *2___Most days* *3___Once a week* *4___Never* 1 ..... 2 ..... 3	*1___Every day* *2___Most days* *3___Once a week* *4___Never* 1 ..... 2 ..... 3	*1___Every day* *2___Most days* *3___Once a week* *4___Never* 1. ..... 2. ..... 3. .....
Eggs	*1___Every day* *2___Most days* *3___Once a week*	*1___Every day* *2___Most days* *3___Once a week*	*1___Every day* *2___Most days* *3___Once a week*	*1___Every day* *2___Most days* *3___Once a week*

Food item	*Frequency of intake during the last week*			
	Baseline	**Month 2**	**Month 4**	**Month 6 (end)**
	4___Never	*4___Never*	*4___Never*	*4___Never*
Red meat (beef, pork, mutton) / stew / sausage / mince meat	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*
Chicken / poultry	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*
Liver (e.g. chicken liver, beef liver, sheep liver etc)	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*
Fish (fresh or canned)	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*
Sweets / Chocolates	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*
Chips / Cheese curls / Niknaks	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*
Fizzy cold drink e.g. Coke	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*
Juice concentrate, mix with water e.g. Oros	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*
Rooibos	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*
Tea, normal	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*
Sugar (any type), eaten as such, in drinks (e.g. tea) or added to food	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*	*1___Every day 2___Most days 3___Once a week 4___Never*

Food item	*Frequency of intake during the last week*			
	Baseline	**Month 2**	**Month 4**	**Month 6 (end)**
Salt (added to food)	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
How often did you use oil when preparing the baby's food?	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
How often did you use margarine when preparing the baby's food? [any type of margarine]	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
How often did you use peanut butter when preparing the baby's food?	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*	*1___Every day* *2___Most days* *3___Once a week* *4___Never*

ADDENDUM 7: Tswaka informed consent form

INFORMATION SHEET

STUDY: Randomized controlled trial in South Africa comparing the impact of complementary food products on child growth

Principal investigator: Prof Marius Smuts
Co-principal investigator: Prof Mieke Faber

Dear Parent / Legal Guardian

Who are we?
We are from the North-West University (PUKKE), Potchefstroom and the Medical Research Council, Cape Town. We are studying the effect of newly-developed nutritional supplements on the growth and development of babies. The supplements are in the form of a paste and can be mixed with the food babies normally eat. We invite you and your baby to participate in this important study**.**

Why are we doing this?
Iron and fatty acids in foods are important for the growth and development of babies. For optimal growth and development of babies, the different types of fatty acids in food need to be present in the right amounts. The aim of this study is to test two types of nutritional supplements in order to see if these supplements can improve the growth and development of babies. This will be done by measuring nutritional status, growth and development of babies after they have eaten the supplement mixed with their usual food every day for six months.

What do we expect from participants during the study?
Seven hundred and fifty (750) babies and their mothers (or primary caregiver) will be recruited through the local clinics. All babies will be 6 months old at the start of the study and will be followed up until they are 12 months old. Only mothers who planned to stay in the Jouberton area for at least the next 7 months can take part in the study. Only apparently healthy babies with no known allergy to soy, peanut, milk/lactose and fish, who are currently breastfeeding or have previously breastfed, will be included in the study. The products contain allergens from soy, milk, and fish, and may also contain traces of peanuts. Twins cannot participate in the study.

Participants will be divided into three groups. The first group will receive a fortified fat-based paste that contains essential fatty acids with other added fatty acids as well as a substance that may improve the absorption of iron. The second group will receive a paste that contains essential fatty acids. Both products contain soy. The third group will not receive any

supplement during the 6-month study period, but they will receive a 6-month supply of the fat-based paste when the baby is 12 months old. Each child has an equal chance to be in any of the three groups. The amount of nutrients (e.g. iron and fatty acids) used in the two supplements is safe and no side effects such as nausea or diarrhoea is expected. The supplement will be provided free of charge to all study participants. Mothers will be asked to mix a certain amount of the supplement with the child's usual food daily for 6 months.

If you agree to participate in the study, we will ask the following from you:

When your baby is 6 and 12 months old, you will be asked to go with your baby to the research site which is at the Baptist Church. During these two visits, we will ask the following from you:
- You will be asked questions about your household (at the start of the project), the foods that your baby eats and drinks, any illnesses that your baby had during the previous weeks, and development that your baby has achieved.
- You will be asked to recall the foods and drinks that your baby consumed the day before.
- Your baby's weight, length, head circumference and upper arm circumference will be measured.
- Your weight and height will be measured (at the start of the project).
- You will be maybe asked to express a small amount of breast milk (approximately ¼ cup). We will measure the fatty acids and iodine in the breast milk. After the fatty acids and iodine have been measured, the remaining breast milk will be discarded according to standard procedures.
- A nursing sister will take a 4 mL (less than one teaspoon) blood sample from the vein in your baby's arm to measure the levels of nutrients in blood. We will also use this blood to measure your baby's response to the measles immunization routinely given at the clinic to babies when they are 9 months old. If the nursing sister cannot get blood from your baby's arm, she will take blood from the vein in top of your baby's hand or by a finger prick. If your baby is showing too much resistance during this process, blood will not be taken from him/her. The procedure is completely safe.
- You will be maybe asked to provide a small amount of urine (at the start and end of the project). We will measure the iodine in the urine.
- We will collect a small amount of urine from your baby using a special nappy. We will measure the iodine in the urine.

You and your baby will be asked to further visit the research site when the baby is 8 and 10 months old. During these two visits you will be asked questions about the foods that your baby eats and drinks, and your baby's weight and length will be measured.

You will also be asked to record daily how much product your baby consumed and to report illness.

A field worker will visit you at home once a week. During these visits the fieldworker will ask you questions on the usage of the supplement, illnesses that your baby may experience and developmental milestones that your baby has achieved.

Payment, Expenses and Costs
You will not have to pay for any costs that are directly related to the research study, for example blood tests. Your taxi-fare from your home in Jouberton to the research site at the Baptist Church will be refunded on the day that you visit the research site.

Who will have access to my child's information?
All information collected about your baby will be treated as confidential (will not be given to or discussed with anybody) and only the researchers and the ethics committees at the Medical research Council and North-West University will have access to it. No abnormal finding is expected, but should anything abnormal be found we will refer the baby to the local clinic or a medical doctor for the necessary treatment. You will be kept informed in this

regard and are welcome to discuss any concerns that you may have with us.

What will the benefit be for my child who participates?
Your child may not benefit from the study, but children may in future benefit from the results. Your child will be monitored for the 6-month period and, should anything abnormal be found, be referred to the local clinic or a medical doctor for the necessary treatment. You will also gain information on your child's nutritional status and development.

What will the risks be for my child who participates?
The nutritional supplement is safe and should not harm your child or make your child sick. Your child may experience some discomfort when the blood samples are taken or when the weight and length are taken. This discomfort will be minimised as the staff taking these measurements will be experienced. If your child does not want to cooperate, the procedures will be stopped.

Must I participate?
Participation in this study is completely voluntary (your own choice). Whether you do, or do not, give your permission will not influence your baby's access to health care in any way.

May I change my mind?
Certainly, you may do this at any time without having to give a reason. The study is completely voluntary and it will not be kept against you in any way should you decide to withdraw from the study.

Who can you contact if there are any queries?
For more information on the study you may contact Prof Marius Smuts at 018-299 4670 or 082 451 0486 **OR** Prof Mieke Faber at 021-938 0404 or 0824602946 during office hours.

The study has been approved by the Ethics Committee of North-West University (NWU), the Ethics Committee of the Medical Research Council, as well as the Department of Health. If you have any queries or problems regarding the study, you can contact either Prof Amanda Lourens the chairperson of the NWU Ethics Committee at (018) 2992606 or Prof Danie du Toit who is the chairperson of the MRC Ethics Committee at (021) 9380341 or you can send an e-mail to adri.labuschagne@mrc.ac.za.

If you are happy for you and your child to take part in the study, please read and sign the consent form.

Thank you!

ADDENDUM 8: Tswaka Exit questionnaire

EXIT QUESTIONNAIRE

Baby's code: | | | | |

Date of the interview (dd/mm/yyyy): | | | / | | | / | 2 | 0 | 1 | |

Fieldworker code: | | |

INFANT FEEDING

1. Are you currently breastfeeding your baby / Is the baby currently breastfed?	Yes ***(go to question 3)***	1	
	No	2	
	Don't know ***(go to question 3)***	3	

2. How old was the baby when breastfeeding was stopped? *(in months)*		

3. At the moment, does your baby get any <u>milk feeds</u> other than breast milk?	Yes	1	
	No ***(go to question 5)***	2	
	Don't know ***(go to question 5)***	3	

4. What type of milk, other than breast milk is your baby getting?	Cow's milk (full strength)	1	
	Cow's milk (diluted)	2	
	Klim / Nespray	3	
	Infant formula Give name:	4	
	Other, specify:	5	

5.a. How many times did your baby eat solid, semi-solid or soft foods (with a spoon) other than liquids yesterday during the day?	

5.b. How many times did your baby eat solid, semi-solid or soft foods (with a spoon) other than liquids yesterday during the night?	

6. Did your baby drink anything from a bottle with a teat yesterday during the day and/or night?	Yes	1	
	No	2	
	Don't know	3	

7. Does your baby get any dietary supplements (e.g. vitamin syrup, vitamin tablets)?	Yes, specify:	1	
	No	2	

	Don't know	3	

INFORMATION ON THE PASTE

8. In general, how do feel about the paste?

.....

.....

.....

Why do you feel this way?

.....

.....

.....

.....

9. During the past week (7 days), how often did you give the paste to your baby?	Every day	1	
	4-6 days per week	2	
	1-3 days per week	3	
	Never	4	

10. Was the paste acceptable for you?	Yes	1	
	No	2	
	Don't know / No answer	3	

11. Was the paste acceptable for your baby?	Yes	1	
	No	2	
	Don't know / No answer	3	

12. With what foods did you <u>usually</u> mix the paste with?

.....

.....

.....

13. With what foods did you mix the paste with <u>yesterday</u>?

.....

.....

.....

14. Please use the five faces, and tick the number under the face that best describes how you feel about each of the following statements

	 Disagree	 Tend to disagree	 Un-decided	 Tend to agree	 Agree	
a. The baby liked the paste	1	2	3	4	5	
b. You liked giving the paste to the baby	1	2	3	4	5	
c. It was easy to mix the paste with the baby's food	1	2	3	4	5	
d. You had problems feeding the baby the paste	1	2	3	4	5	
e. You are interested in using the paste in future if for sale	1	2	3	4	5	
f. You will buy the paste if available	1	2	3	4	5	
g. You sometimes gave the paste with foods other than porridge	1	2	3	4	5	
h. You sometimes gave the daily dose of the paste in small portions throughout the day	1	2	3	4	5	

MORBIDITY

M1. During the past week (7 days), did your baby have diarrhoea (at least three watery stools per day)	1	Yes *	
	2	no ***(go to question M2)***	
	3	don't know ***(go to question M2)***	

M1a. For how many days did he/she have diarrhoea?	

When did your baby's diarrhoea start?	Date: _____/_____/ 20____
When did your baby's diarrhoea stop?	Date: _____/_____ / 20____ Or is it carrying on?_____

**** If marked "yes": Fieldworkers please collate information for study nurse to complete AE form***

M2. During the past week (7 days), did your baby vomit?	1	Yes *	
	2	no ***(go to question M3)***	
	3	don't know ***(go to question M3)***	

M2a. For how many days did he/she vomit?	
When did your baby's vomiting start?	Date: _____/_____/ 20____

When did your baby's vomiting stop?	Date: _____/_____ / 20____ Or it is carrying on? _____

**** If marked "yes": Fieldworkers please collate information for study nurse to complete AE form***

M3. During the past week (7 days), did your baby have a continuous cough (at least for one whole day)?	1	Yes *	
	2	no ***(go to question M4)***	
	3	don't know ***(go to question M4)***	

M3a. For how many days did he/she cough continuously?	

M3b. Was the cough wet or dry?	1	Wet	
	2	Dry	

When did your baby's coughing start?	Date: _____/_____/ 20____
When did your baby's coughing stop?	Date: _____/_____ / 20____ Or is it carrying on? _____

**** If marked "yes": Fieldworkers please collate information for study nurse to complete AE form***

M4. During the past week (7 days), did your baby have wheezing or difficult breathing?	1	Yes *	
	2	no ***(go to question M5)***	
	3	don't know ***(go to question M5)***	

M4a. For how many days did he/she have wheezing or difficult breath?	

When did your baby's wheezing / difficulty in breathing start?	Date: _____/_____/ 20____
When did your baby's wheezing / difficulty in breathing stop?	Date: _____/_____ / 20____ Or is it carrying on? _____

**** If marked "yes": Fieldworkers please collate information for study nurse to complete AE form***

M5. During the past week (7 days), did your baby have a hot body?	1	Yes *	
	2	no ***(go to question M6)***	
	3	don't know ***(go to question M6)***	

M5a. For how many days did he/she have a hot body?	

When did you notice that your baby first have a hot body?	Date: _____/_____/ 20____

When did you notice that your baby's body is no longer hot?	Date: _____/_____ / 20____ Or is it carrying on? _____

**** If marked "yes" : Fieldworkers please collate information for study nurse to complete AE form***

M6. During the past week (7 days), did your baby have any sores on the skin or rash?	1	Yes *	
	2	no ***(go to question M7)***	
	3	don't know ***(go to question M7)***	

M6a. For how many days did he/she have the sores or rash?	

When did your baby first have a sore or rash?	Date: _____/_____/ 20____
When did your baby's sore or rash stop?	Date: _____/_____ / 20____ Or is it carrying on? _____

**** If marked "yes": Fieldworkers please collate information for study nurse to complete AE form***

M7. During the past week (7 days), did your baby have a continuous runny nose/ blocked nose?	1	Yes *	
	2	no ***(go to question M8)***	
	3	don't know ***(go to question M8)***	

M7a. For how many days did he/she have a runny nose/blocked nose?	

When did your baby's runny nose / blocked nose start?	Date: _____/_____/ 20____
When did your baby's runny nose / blocked nose stop?	Date: _____/_____ / 20____ Or is it carrying on? _____

**** If marked "yes": Fieldworkers please collate information for study nurse to complete AE form***

M8. During the past week (7 days), did your baby have any other illness?	1	Yes *	
	2	no ***(go to question M9)***	
	3	don't know ***(go to question M9)***	

M8a. Which illness did your baby have?

.....

.....

When did your baby's illness start?	Date: _____/_____/ 20____
When did your baby's illness stop?	Date: _____/_____ / 20____

	Or is it carrying on?_____

**** If marked "yes": Fieldworkers please collate information for study nurse to complete AE form***

M9. Did your baby visit any health service during the last week (7 days) because he/she was ill?	1	Yes	
	2	no ***(end of questionnaire)***	
	3	don't know ***(end of questionnaire)***	

M10.	Which health services did your baby go to during the last week?			
	a. Clinic	yes = 1	no = 2	
	b. Private physician / doctor	yes = 1	no = 2	
	c. Hospital	yes = 1	no = 2	
	d. Traditional healer	yes = 1	no = 2	
	e. Other (specify): …….....	yes = 1	no = 2	

M11. Did your baby receive any medicine prescribed by a nurse or doctor for his/her illness during the last week (7 days)	1	yes	
	2	no ***(go to question M12)***	

M11 What type of medicine?
.....
.....

M11b. For how many days did he/she receive medicine?

M12. Was your baby officially diagnosed with any illness during the last week (7 days)?	1	yes	
	2	no ***(go to question M14)***	

M13. With what illness was your baby diagnosed?
.....
.....

M14. Was your baby hospitalised during the last week (7 days)	1	Yes *	
	2	no ***(end of questionnaire)***	

M15. How long was your baby in hospital? (days)

When did your baby's stay in hospital start?	Date: _____/_____/ 20____
When did your baby's stay in hospital stop?	Date: _____/_____ / 20____ Or is it carrying on?_____

**** If marked "yes": Fieldworkers please collate information for study nurse to complete AE form***

M16. Why was your baby hospitalised?
.....

ADDENDUM 9: Publication (Dold *et al.,* 2016)

Optimization of a New Mass Spectrometry Method for Measurement of Breast Milk Iodine Concentrations and an Assessment of the Effect of Analytic Method and Timing of Within-Feed Sample Collection on Breast Milk Iodine Concentrations

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Optimization of a New Mass Spectrometry Method for Measurement of Breast Milk Iodine Concentrations and an Assessment of the Effect of Analytic Method and Timing of Within-Feed Sample Collection on Breast Milk Iodine Concentrations

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Background: Breast milk iodine concentration (BMIC) may be an indicator of iodine status during lactation, but there are few data comparing different analytical methods or timing of sampling. The aims of this study were: (i) to optimize a new inductively coupled plasma mass spectrometry (ICP-MS) method; and (ii) to evaluate the effect of analytical method and timing of within-feed sample collection on BMIC.
Methods: The colorimetric Sandell–Kolthoff method was evaluated with (a) or without (b) alkaline ashing, and ICP-MS was evaluated using a new ^{129}I isotope ratio approach including Tellurium (Te) for mass bias correction (c) or external standard curve (d). From iodine-sufficient lactating women ($n = 97$), three samples were collected within one breast-feeding session (fore-, mid-, and hind-feed samples) and BMIC was analyzed using (c) and (d).
Results: Iodine recovery from NIST SRM1549a whole milk powder for methods (a)–(d) was 67%, 24%, 105%, and 102%, respectively. Intra- and inter-assay coefficients of variation for ICP-MS comparing (c) and (d) were 1.3% versus 5.6% ($p = 0.04$) and 1.1% versus 2.4% ($p = 0.33$). The limit of detection (LOD) was lower for (c) (0.26 μg/kg) than it was for (d) (2.54 μg/kg; $p = 0.02$). Using (c), the median [95% confidence interval (CI) obtained by bootstrap] BMIC (μg/kg) in foremilk (179 [CI 161–206]) and in mid-feed milk (184 [CI 160–220]) were not significantly different ($p = 0.017$), but were higher than in hindmilk (175 [CI 153–216]; $p < 0.001$). In foremilk using (d), BMIC was 199 ([CI 182–257]; $p < 0.001$ vs. (c)). The variation in BMIC comparing (c) and (d) (13%) was greater than variation within feeding (5%; $p < 0.001$).
Conclusions: Because of poor recoveries, (a) and (b) should not be used to measure BMIC. Compared with (d), (c) has the advantages of higher precision and a lower LOD. In iodine-sufficient women, BMIC shows low variation within a breast-feeding session, so timing of sampling is not a major determinant of BMIC.

Introduction

Iodine deficiency during infancy may impair growth and development (1–4). Infants have low thyroidal iodine stores and are dependent on iodine in breast milk or infant formula to meet their requirements (2,5). However, there is no scientific consensus on optimal dietary iodine intake during infancy or on optimal breast milk iodine concentration (BMIC) (1,2,6–8). BMIC is influenced by maternal iodine status (1,9–11), recent maternal iodine intake (12), duration of lactation (13–17), and maternal fluid intake (17). Sampling procedures may also be important. One study found differences in BMIC between fore- and hind-milk samples (17) while another did not (18). Broad ranges of BMIC have been reported in iodine-sufficient countries (9–11,19), but comparing BMIC across studies is difficult because of differences in study design, analytical methods, and the lack of external quality control (9,17,20–24).

Breast milk is a complex sample matrix (20,21,25), and inductively coupled plasma mass spectrometry (ICP-MS) is considered the standard method for iodine determination in such matrices, providing high levels of accuracy and precision and low detection limits (20–23,26). However, obtaining accurate measurements using ICP-MS requires optimization of sample

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preparation and measurement parameters (20–23,26). Isotope dilution analysis (IDA) for ICP-MS uses the determination of isotope intensity ratios for quantification and is a promising approach for improving iodine measurements in breast milk. This is because, compared with conventional standard curve applications, IDA allows for correction for losses, matrix effects, and instrumental drifts (20,21,25–28).

The European Committee for Standardization and the AOAC International have adopted ICP-MS-based methods for the determination of iodine content in foods, infant formula, and nutritional products (22,26,29,30), but no reference analytical method has been recommended for BMIC. Thus, the aim of this study was to evaluate differences in BMIC by analytical method and timing of sample collection within a feeding session. Specifically, (i) two methods for measurement of BMIC were compared: the spectrophotometric Sandell–Kolthoff method and ICP-MS analysis; (ii) an ICP-MS method based on IDA with ^{129}I and Tellurium (Te) for mass bias correction was optimized to quantify BMIC, and then this ICP-MS ^{129}I isotope ratio method was compared with an ICP-MS procedure using external iodine calibrators for quantification; and (iii) breast milk samples were collected from an iodine-sufficient population and BMIC was compared between fore-, mid-feed, and hind-milk samples.

It was hypothesized that:

H1: The spectrophotometric method will not obtain reliable BMIC results.

H2: The new ICP-MS method based on IDA with ^{129}I and Te for mass bias correction will improve precision of BMIC analysis compared with conventional ICP-MS approaches.

H3: Differences in BMIC by sampling time point within a breast-feeding session will be negligible in iodine-sufficient women.

Subjects and Methods

Subjects

Breast milk samples were obtained as part of a cross-sectional study conducted in the Potchefstroom municipal area of the North-West Province, South Africa. Briefly, a convenience sample of lactating women ($n = 100$) was recruited at local health centers during a routine infant polio vaccination visit. Inclusion criteria were: (i) apparently healthy lactating women; (ii) no history of thyroid disease; (iii) currently breast-feeding a singleton infant; and (iv) not consuming iodine-containing dietary supplements. Trained study assistants explained the study protocol to the women in the local language (Setswana or Afrikaans). Written informed consent was obtained from the participating women. The Health Research Ethics Committee of the Faculty of Health Sciences of the North-West University, South Africa, approved the study protocol (NWU-00016-13-S1). Permission was also granted from the Provincial and District Health Departments, South Africa. South Africa has a long-standing salt iodization program with mandatory table salt iodization at 35–65 ppm (31). The median urinary iodine concentration (UIC) of the participating women was 118 μg/L (interquartile range 67–179 μg/L), indicating adequate iodine intake.

Study design

The subjects provided three consecutive breast milk samples from one feeding session by manual expression, following a sample collection scheme adapted from Neville *et al.* (32). After cleaning the breast with a wet cloth, mothers expressed a 5 mL milk sample before breast-feeding to obtain a foremilk sample. The baby was then put to the breast to suckle for 2–2.5 min after letdown, judged as the time when the baby began to swallow actively. Another 5 mL of breast milk was then collected to obtain a mid-feed sample. The baby then suckled on the breast until fully satisfied, and a third 5 mL sample (the hindmilk sample) was collected when the feeding was finished. When sample volume allowed, a pooled milk sample was generated from equal parts of the fore-, mid-, and hind-feed samples. All breast milk samples were collected between 8:00 am and 12:00 am. The breast milk samples were frozen within an hour after collection, shipped frozen to the Human Nutrition Laboratory of the ETH Zurich, and stored at −25°C until analysis.

BMIC analysis: Sandell–Kolthoff methods

The milk iodine concentration was analyzed using the colorimetric Sandell–Kolthoff procedure (33) with and without prior alkaline ashing. Alkaline ashing was applied to remove interfering substances using modified procedures from Jones *et al.* (34) and Aumont and Tressol (35).

Reagents. Analytical grade reagents were used for all analyses: potassium hydroxide pellets (Merck, Darmstadt, Germany), ammonium persulfate (Riedel de Haën; Sigma-Aldrich, St. Louis, MO), sodium hydroxide pellets (Sigma-Aldrich), arsenic trioxide (Fluka; Sigma-Aldrich), sulfuric acid 95–97% (Sigma-Aldrich), sodium chloride (Sigma-Aldrich), ammonium cerium(IV) sulfate dihydrate (Sigma-Aldrich), and potassium iodate (Fluka; Sigma-Aldrich). Water (18 MΩ cm) was generated by an ultrapure water purification system (Barnstead E-Pure; Thermo Scientific, Waltham, MA).

Ashing. For the ashing procedure, 0.5 mL of milk was pipetted into a ceramic crucible, and 0.5 mL of 4 M potassium hydroxide solution was added. The samples were dried in a heat cabinet at 105°C for 20 h. Then, the samples were placed in a muffle furnace and heated at 150°C for 30 min. The temperature was raised to 600°C and maintained for 1 h. After cooling, the ash was dissolved in 1 mL of ultrapure water by putting the ceramic crucibles into an ultrasonic bath for 10 min. The solution was filtered into microtubes using 3 mL syringes and microfilters (Chromafil MV, A-45/25; Macherey-Nagel, Dueren, Germany). The calibration blanks were prepared using ultrapure water.

Sandell–Kolthoff procedure. The iodine content in the untreated milk samples and in the dissolved ash samples was measured using the modification by Pino *et al.* of the Sandell–Kolthoff reaction with spectrophotometric detection (33). For each sample, 250 μL was pipetted in duplicate into Pyrex tubes. The samples were digested by adding 1 mL of 1 M ammonium persulfate solution to all tubes and by heating them on a heating block for 60 min at 95°C. After cooling, 50 μL of each sample was pipetted onto a flat-bottom 96-well PS microplate (Greiner Bio-One, Kremsmuenster, Austria), and 100 μL of arsenious acid solution (0.05 M) was added. The plate was placed on a microplate shaker for 15 min. Then, 50 μL of ceric ammonium solution (0.019 M) was

added to all wells, and the plate was shaken for another 28 min before the absorbance was measured at 405 nm using a microplate reader (PowerWave HT; BioTek, Winooski, VT).

The authors' laboratory routinely uses the described modification by Pino *et al*. of the Sandell–Kolthoff method for UIC analysis. The laboratory is certified by the Program to Ensure the Quality of Urinary Iodine Procedures (EQUIP; Centers for Disease Control and Prevention, Atlanta, GA), and participates successfully in its quarterly external validation.

Calculations. Iodine calibrators for a concentration range between 0 and 600 μg/L were prepared for each Sandell–Kolthoff run from a 1 g/L iodine stock solution using potassium iodate and ultrapure water. The calibration curve was established by linear regression of the iodine concentrations of the standards versus the logarithm of the absorbance at 405 nm. The iodine concentrations in the milk samples were calculated using the calibration curve obtained for each run and the dilution factors for each milk sample.

ICP-MS methods

The BMIC was analyzed using a two-step procedure. First, iodine was extracted from the breast milk samples using tetramethylammonium hydroxide (TMAH) with a procedure modified from Fecher *et al*. (22) and Andrey *et al*. (36). Then, the iodine content in the TMAH extracts was measured using multicollector (MC) ICP-MS. The quantification was done using two different approaches based on: (i) ICP-MS IDA using ^{129}I, and (ii) ICP-MS standard curve analysis using external potassium iodide calibrators in an aqueous 0.25% TMAH solution. Instrumentation, operation, reagents, and sample preparation procedure were identical for both ICP-MS methods under study.

Instrumentation and operation. A Finnigan NEPTUNE high-resolution double focusing MC-ICP-MS (Thermo Scientific) equipped with an ASX-520 Autosampler (Cetac, Omaha, NE), a Perimax pump (Spetec, Erding, Germany) for sample introduction, a Scott Style combined cyclonic-double-pass high-stability quartz Spray Chamber for NEPTUNE (Thermo Scientific), and a MicroFlow PFA-ST self-aspirating or pumped nebulizer, 50 μL/min uptake version (Epond, Vevey, Switzerland), were used for the analysis. The MC-ICP-MS was operated with the conditions listed in Table 1. Signals were measured in volts (V). The low-resolution mode was used in order to obtain higher ICP-MS signals. The instrument was tuned before each run using iodide standards in 0.25% TMAH.

Reagents. Tama pure-AA TMAH 25% (Tama, Kawasaki, Japan) and argon with a purity grade of 99.998% (Carbagas, Guemligen, Switzerland) were used. Water (18 MΩ cm) was generated by an ultrapure water purification system (Barnstead E-Pure; Thermo Scientific).

Sample preparation. To assure homogeneity of the frozen breast milk samples, the samples were defrosted on a Speci-Mix mixer for 1 h. Any coagulates were dissolved by placing the defrosted samples in a 40°C oven for 15–30 min. After cooling, the samples were thoroughly homogenized by using a vortex mixer immediately before pipetting. For the sample preparation procedure, 1.5 mL of each milk sample was pipetted into a graduated disposable 50 mL PP-tube with a screw cap (Sarstedt, Nuembrecht, Germany), and the exact weight was noted. Further, 0.5 mL of 25% TMAH was added to each tube. After mixing, the screw caps of the tubes were closed, and the samples were put in the heat cabinet for 3 h at 90 ± 3°C for iodine extraction. The samples were shaken carefully by hand from time to time during the extraction. After cooling to room temperature, the tubes were filled to 50 mL with ultrapure water, resulting in a TMAH concentration of 0.25%. The tubes were closed, mixed, and left for sedimentation at room temperature overnight. The next day, 10 mL aliquots of the extract were taken from the middle of the tubes, and they were filled into graduated disposable 15 mL PP-tubes with screw caps (Semadeni, Ostermundigen, Switzerland). Sediments from the bottom or parts of the fat fraction floating on the top of the extract were avoided. The calibration blanks were prepared using ultrapure water.

Table 1. MC-ICP-MS Operating Conditions for the Analysis

Settings	
Operation power	1.3 kW
Auxiliary gas flow rate	0.4 L/min
Cooling gas flow rate	16.2 L/min
Sample gas flow rate	0.9 L/min
Sample uptake	50 μL/min
Vacuum conditions	
Plasma interface	10e-004 mbar
Electrostatic analyzer (ESA)	10e-008 mbar
Ion getter press	10e-009 mbar
Data acquisition	
Monitored ions (m/z)	124 (Te), 125 (Te), 126 (Te), 127 (I) 128 (Te), 129 (I), 130 (Te), 131 (Xe)
Mode	Low resolution ($m/\Delta m \approx 2000$)
Measurement cycles per sample	20
Time per measurement cycle	4 s
Take-up time	105 s
Wash time	10 s

MC-ICP-MS, multicollector inductively coupled plasma mass spectrometry.

ICP-MS ^{129}I isotope ratio method

Standards. The NIST SRM 4949C ^{129}I radioactivity standard (Standard Reference Material 4949C; National Institute of Standards and Technology, Gaithersburg, MD) was used for IDA. The specific activity of the solution was certified at 3451 Bq/g (RSD 0.6%), which is equal to a concentration of 528 μg/g ^{129}I. A ^{129}I stock solution was prepared with a concentration of 174 μg/g ^{129}I, corresponding to a specific activity of 1136 Bq/g, by diluting the NIST SRM 4949C ^{129}I radioactivity standard in a 0.01 M NaOH solution. The stock solution was used to prepare a ^{129}I spike solution in a 0.01% TMAH solution with a concentration of 1.3 μg/g ^{129}I, corresponding to a specific activity of 9 Bq/g. Then, 50 μL of the ^{129}I spike solution was added to each 10 mL extracted sample. The concentration of ^{127}I in NIST SRM 4949C ^{129}I radioactivity standard was not certified by the

producer. By doing standard additions of ^{127}I, an isotopic fraction of 14% ^{127}I and 86% ^{129}I was obtained, corresponding to a ^{127}I/^{129}I ratio $R_{\text{iso true}}$ of 0.16, in agreement with Platzner (37). For each ICP-MS run, 50 μL ^{129}I spike solution was added to 10 mL 0.25% TMAH solution and the ^{127}I/^{129}I intensity ratio R_{iso} was measured at the beginning, middle, and end of each run, and was used for the IDA calculations.

Te was used for mass bias correction, and the spike solution (10 mg/L) was prepared from a 1000 mg/L standard solution for ICP (AppliChem, Darmstadt, Germany). Te isotope ratios in the standard solution were not specified by the producer and were assumed to be equivalent to the relative abundances of naturally occurring isotopes (38). A total of 50 μL of the 10 mg/L Te spike solution was added to each 10 mL sample.

Calculations. The ^{127}I/^{129}I intensity ratio R_{meas} was measured for all samples. Solvent blanks (0.25% TMAH) were analyzed before and after each sample, and were used for respective blank correction of the acquired sample ICP-MS intensities. Microsoft Excel 2010 (Microsoft, Redmond, WA) was used for all calculations. R_{meas} results from the ^{127}I in the milk samples and the ^{129}I and ^{127}I added with the ^{129}I spike solution. It can be described as:

$$R\left(\frac{^{127}I}{^{129}I}\right)_{meas} = \frac{n_n + n_s{}^{*127}h_s}{n_s{}^{*129}h_s} \quad (1)$$

with n_n representing the number of moles of ^{127}I in the milk samples and n_s representing the number of moles of ^{129}I respectively ^{127}I added with the spike solution. The isotopic abundance in the spike solution is described by h_s. By substituting the number of moles for the respective m/M terms and rearranging equation 1, the unknown mass of ^{127}I in the milk samples can be calculated as:

$$m_{127_I} = \left(R\left(\frac{^{127}I}{^{129}I}\right)_{meas} - R\left(\frac{^{127}I}{^{129}I}\right)_{iso}\right) * \frac{m_{129_I}}{M_{129_I}} * M_{127_I} \quad (2)$$

with $m_{129\text{I}}$ representing the mass of added ^{129}I, M representing the molar masses of ^{127}I respectively ^{129}I, and R_{iso} representing the ^{127}I/^{129}I intensity ratio measured for the 50 μL ^{129}I spike solution that was added to 10 mL 0.25% TMAH solution.

The Russell's law model was applied for mass bias correction. A calibrator with known isotope ratio (Te) is measured. From the difference between the known isotope ratios and the measured isotope ratios, a correction factor $f(t)$ is calculated, and this correction factor $f(t)$ is then applied to correct the measured isotope ratios of the measurand (iodine). For the present instrumental settings, Russell's law was found to be suitable to correct for mass bias of the ^{126}Te/^{128}Te intensity ratio, and it was used further to correct the measured ^{127}I/^{129}I intensity ratios (39). Using MC-ICP-MS, the ^{126}Te/^{128}Te intensity ratio was measured simultaneously to the ^{127}I/^{129}I ratio. It was thus not susceptible to variation in the magnitude of bias. The mass bias correction factors f were calculated as:

$$f = \frac{\ln\left(R\left(\frac{^{126}Te}{^{128}Te}\right)_{meas} / R\left(\frac{^{126}Te}{^{128}Te}\right)_{true}\right)}{\ln\frac{m_{126_{Te}}}{m_{128_{Te}}}} \quad (3)$$

with m_{Te} representing the mass of ^{126}Te respectively of ^{128}Te added with the Te spike solution, $R_{\text{Te meas}}$ being the experimentally measured ^{126}Te/^{128}Te intensity ratio, and $R_{\text{Te true}}$ being the true ^{126}Te/^{128}Te intensity ratio obtained from the relative abundances of naturally occurring isotopes (38). Then, the correction factors were applied to calculate the mass bias corrected ^{127}I/^{129}I intensity ratios R_{corr} from the measured ^{127}I/^{129}I intensity ratios R_{meas}:

$$R\left(\frac{^{127}I}{^{129}I}\right)_{corr} = \frac{R\left(\frac{^{127}I}{^{129}I}\right)_{meas}}{\left(\frac{m_{127_I}}{m_{129_I}}\right)^f} \quad (4)$$

Then, the mass bias corrected ^{127}I/^{129}I intensity ratios R_{corr} were applied to calculate the unknown mass of ^{127}I in the milk samples corresponding to:

$$m_{127_I} = \left(R\left(\frac{^{127}I}{^{129}I}\right)_{corr} - R\left(\frac{^{127}I}{^{129}I}\right)_{iso}\right) * \frac{m_{129_I}}{M_{129_I}} * M_{127_I} \quad (5)$$

The iodine concentration in the milk samples was calculated using the dilution factors applied to each sample.

ICP-MS standard curve method

Standards. A 1000 μg/g iodide stock solution was prepared from analytical grade potassium iodide (Riedel de Haën; Sigma-Aldrich). A 1 μg/g iodine spike solution was prepared from the stock solution, and it was used to prepare iodine standards of 0, 5, 10, 20, and 40 ng/g in 0.25% TMAH solutions. Te was used for continuous monitoring of the ICP-MS signal, and it was prepared from a 1000 mg/L standard solution for ICP (AppliChem). Then, 50 μL of the 10 mg/L Te spike solution was added to each 10 mL iodine standard. All standards were prepared gravimetrically, and the concentrations were verified using ICP-MS IDA. To account for possible variations and drifts during an ICP-MS run consisting of 40 milk samples, the set of iodine standards were measured at the beginning, middle, and end of each run. The same standard solutions were used for all ICP-MS measurements reported.

Calculations. The calibration curve was established by linear regression of the iodine concentrations of the three measurements of the set of iodine standards for each ICP-MS run versus the measured intensity at m/z 127. The standard curve was linear and the correlation coefficient was consistently >0.978. If required, the samples were diluted so that the measured BMIC fell within the calibration range of the calibration curve. Solvent blanks (0.25% TMAH) were analyzed before and after each sample and were used for respective blank correction of the acquired sample ICP-MS intensities. Microsoft Excel 2010 was used for all calculations. The BMIC in the samples were calculated using the calibration curve obtained for each ICP-MS run and the dilution factors obtained for each milk sample.

Validation of analytical methods for BMIC analysis

Accuracy and precision of the four methods described above were determined by analyzing the iodine content in NIST SRM1549a whole milk powder (Standard Reference

Material 1549a; National Institute of Standards and Technology). The reference milk powder was dissolved in ultrapure water according to instructions, and the iodine content was analyzed using (i) the Sandell–Kolthoff method after alkaline ashing, (ii) the Sandell–Kolthoff method without alkline ashing, (iii) the ICP-MS ^{129}I isotope ratio method, and (iv) the ICP-MS standard curve method. The intra-assay variability was determined by analyzing the NIST SRM1549a sample four times or more in a single assay. The inter-assay variability was determined by analyzing the reference sample two times or more in at least two separate assays on two different days. The NIST SRM1549a reference material was used as quality control sample and analyzed with each run for all methods and all samples.

The limit of detection (LOD) of the four methods was calculated as the mean concentration ± 3 standard deviations (*SD*) of the iodine concentration of a calibration blank measured at least seven times in different assays.

Statistical analysis

Microsoft Excel 2010 and IBM SPSS Statistics for Windows v22 (IBM Corp., Armonk, NY) were used for data processing and analysis. Normally distributed data were expressed as mean ± *SD*; non-normally distributed data were expressed as median ± confidence interval (CI) obtained by 1000 bootstrapped samples. Differences between the ICP-MS isotope ratio and the ICP-MS standard curve method were tested using Wilcoxon signed-rank test, and the correlation was tested using linear regression. Differences between the three sampling time points were tested using Friedman's analysis of variance (ANOVA) followed by Wilcoxon signed-rank tests with Bonferroni correction of the significance level. A p-value of <0.05 was considered significant.

Results

Fore-, mid-, and hind-feed milk samples were obtained from 97, 95, and 94 women, respectively, and the samples were analyzed using the ICP-MS isotope ratio and the ICP-MS standard curve method. A pooled breast milk sample was generated from 66 women.

Evaluation of analytical methods for BMIC analysis: Sandell–Kolthoff methods

The mean ± *SD* iodine content obtained in the NIST SRM1549a reference sample was 2236 ± 313 μg/kg ($n=12$) with prior alkaline ashing, and 790 ± 253 μg/kg ($n=11$) without prior ashing, both below the certified acceptable range (3040–3640 μg/kg), equaling to recoveries of 67% and 24%, respectively (Table 2). The intra-assay coefficient of variation (CV) for the iodine content of the NIST SRM1549a whole milk sample was 3.6% ($n=4$) with and 8.5% ($n=4$) without prior ashing. The inter-assay variability was 9.3% ($n=4$) with and 28.7% ($n=4$) without prior ashing. The LOD for the method was 10.22 μg/kg ($n=7$) with and 10.18 μg/kg ($n=10$) without prior ashing. Because of incomplete recovery using the Sandell–Kolthoff method, with or without prior alkaline ashing, this method was not used in further experiments.

Evaluation of analytical methods for BMIC analysis: ICP-MS methods

The mean ± *SD* iodine content for the NIST SRM1549a reference sample was 3502 ± 89 μg/kg ($n=16$) for the ^{129}I isotope ratio method and 3396 ± 370 μg/kg ($n=16$) for the standard curve method, both well within the certified acceptable range (3040–3640 μg/kg) and equaling to recoveries of 105% and 102%, respectively (Table 2).

The ^{129}I isotope ratio method quantified the iodine content with higher precision than the standard curve method; the intra-assay variability ($n=14$) was 1.3% for the ^{129}I isotope ratio method and 5.6% for the standard curve method ($p=0.04$). The inter-assay variability ($n=16$) was comparable for the two methods: 1.1% for the ^{129}I isotope ratio method and 2.6% for the standard curve method ($p=0.33$). The LOD ($n=10$) was lower for the ^{129}I isotope ratio method (0.26 μg/kg) than it was for the standard curve method (2.54 μg/kg; $p=0.02$).

Influence of choice of analytical method and of within-feed sampling time on BMIC

The BMIC measured by the two different ICP-MS methods in the fore-, mid-, and hind-feed milk samples are shown

Table 2. Precision, Accuracy, and LOD of the Sandell–Kolthoff Method with Prior Alkaline Ashing, the Sandell–Kolthoff Method Without Ashing, the ICP-MS ^{129}I Isotope Ratio Method, and the ICP-MS Standard Curve Method

	Sandell–Kolthoff		*ICP-MS*	
	With ashing	*Without ashing*	*^{129}I isotope ratio*	*Standard curve*
Iodine content of NIST SRM1549a whole milk sample[a]				
Mean ± *SD* (μg/kg)	2236 ± 313	790 ± 253	3502 ± 89	3396 ± 370
Range (μg/kg)	1934–3061	413–1150	3321–3655	2617–4377
Total assay variability (%)	14.0	32.1	2.6	10.9
Inter-assay variability (%)	9.3	28.7	1.1	2.4
Intra-assay variability (%)	3.6	8.5	1.3	5.6
LOD				
LOD (μg/kg)	10.22	10.18	0.26	2.54

Precision and accuracy of the methods was determined by analyzing NIST SRM1549a whole milk powder sample. The LOD was determined by analyzing calibration blanks.

[a]Certified iodine content 3340 μg/kg (range 3040–3640).

LOD, limit of detection; *SD*, standard deviation.

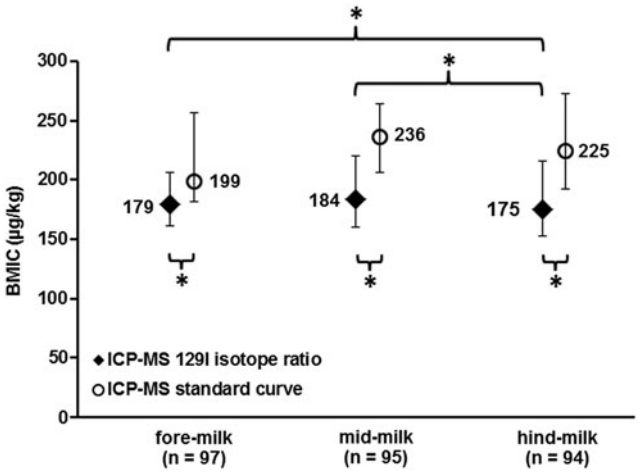

FIG. 1. Breast milk iodine concentration (BMIC, µg/kg) of fore-, mid-, and hind-milk samples obtained by the inductively coupled plasma mass spectrometry (ICP-MS) ^{129}I isotope ratio method and the ICP-MS standard curve method. Data are medians, and error bars are 95% confidence intervals obtained by 1000 bootstrapped samples. *Significant at $p < 0.05$.

in Figure 1. The median BMIC obtained by the ICP-MS ^{129}I isotope ratio method was 179 µg/kg [CI 161–206] in the foremilk, 184 µg/kg [CI 160–220] in the mid-feed milk, and 175 µg/kg [CI 153–216] in the hindmilk ($p < 0.001$). The measured iodine content in the hindmilk was lower than that in the foremilk ($p < 0.001$) and in mid-feed milk ($p < 0.001$). The iodine content in the foremilk did not differ significantly from the mid-feed milk ($p = 0.017$). The pooled milk samples ($n = 66$) yielded a median BMIC of 183 µg/kg [CI 158–257], higher than the iodine content in the hindmilk ($p < 0.001$) and in the foremilk ($p < 0.01$), but not different from the mid-feed samples ($p = 0.297$). In a sub-analysis, the three sampling time points for BMIC concentrations <150 µg/kg ($n = 28$) were compared. The significant difference between the hind- and the fore- ($p < 0.001$) and the hind- and the mid-feed samples ($p < 0.001$) remained. The median BMIC obtained by the ICP-MS standard curve method in the fore-, mid-, and hind-milk samples were 199 µg/kg [CI 182–257], 236 µg/kg [CI 206–264], and 225 µg/kg [CI 192–273], respectively ($p = 0.47$). The pooled milk samples ($n = 25$) yielded a median BMIC of 245 µg/kg [CI 189–364].

The ^{129}I isotope ratio method and the standard curve method strongly correlated ($R^2 = 0.855$, $p < 0.001$; $\beta = 18.3$ µg/kg, $p = 0.009$), but the median BMIC measured by the ICP-MS standard curve method in the fore-, mid-, and hind-milk samples, and in the pooled samples, were significantly higher than the median BMIC obtained by the ICP-MS ^{129}I isotope ratio method ($p < 0.001$) for all time points and pooled samples.

The mean relative standard deviation (RSD) for BMIC ($n = 286$) between the ICP-MS ^{129}I isotope ratio method and the ICP-MS standard curve method was 13% (Fig. 2A). The mean RSD for BMIC between the three within-feed sampling time points ($n = 94$) was 5% (Fig. 2B). The variation in BMIC for the two different ICP-MS methods was significantly higher than the variation in BMIC between the three different within-feed sampling time points ($p < 0.001$).

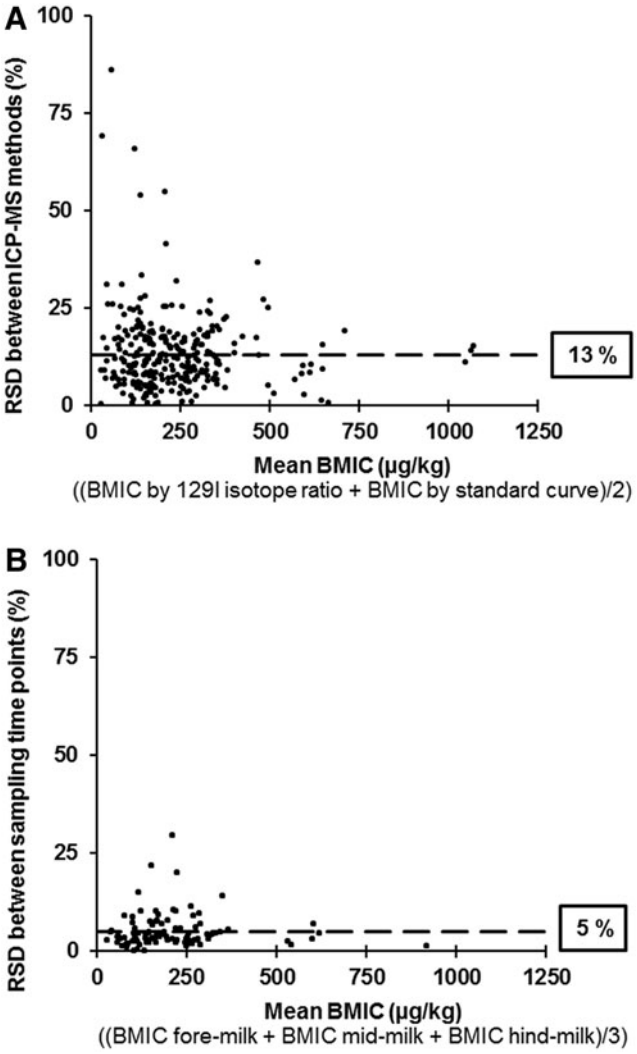

FIG. 2. The relative standard deviation (RSD, %) for BMIC. (**A**) RSD between the ICP-MS ^{129}I isotope ratio method and the ICP-MS standard curve method ($n = 286$). (**B**) RSD between the three different within-feed sampling time points ($n = 94$) using the ICP-MS ^{129}I isotope ratio method. The dashed line shows the mean RSD of the BMIC obtained by the two methods and for the three sampling time points.

Discussion

The Sandell–Kolthoff method is a standard method for measurement of UIC and is used for this analysis in more than 100 laboratories worldwide (40). It has also been applied and reported for iodine analysis of breast milk samples (12,17,41). However, with the Sandell-Kolthoff method, we were unable to retrieve iodine concentrations within the acceptable range for the whole milk reference sample; the iodine recovered, with and without prior alkaline ashing, was only 67% and 24% of the certified iodine content. Possible reasons may be losses during the ashing procedure, incomplete dissolution of the ash, inadequate mineralization, and interferences with the catalytic effect of iodide on the Sandell–Kolthoff reaction (23,42). Because volatile iodine species may be formed at low pH, alkaline ashing to prevent

losses and an ultrasonic bath to allow complete dissolution of the ash were used, but without success. The sensitivity and repeatability of the analysis were also low. Taken together, these data do not support the use of the Sandell–Kolthoff method, with or without prior alkaline ashing, for BMIC analysis. Studies reporting BMIC using the Sandell-Kolthoff method without reporting information on external quality control should be interpreted cautiously.

ICP-MS is the gold standard for determination of low-iodine concentrations in complex matrices (21,22,26,29,30) and the preferred method for BMIC. The data demonstrate the importance of the choice of ICP-MS quantification procedure for BMIC. Although both ICP-MS methods measured satisfactory iodine concentrations in the NIST reference sample, the BMIC differed by 13% between the two methods. The ICP-MS standard curve method measured on average 18 μg/kg higher BMIC than the ICP-MS ^{129}I isotope ratio method. While the standard curve method quantification was done using external iodine calibrators in aqueous TMAH solution, the ^{129}I isotope ratio method is based on IDA, which allowed for correction of analyte losses, matrix effects, and instrumental drifts (27). The simultaneous isotope measurement provided by MC-ICP-MS further allowed for continuous and simultaneous corrections of the acquired ratios. Due to processes in the ICP-MS instrument that favor the transmission of heavier isotopes, measured isotope ratios can defer from the true values by up to 25% (37). To achieve accurate isotope ratios, it is therefore essential to correct the ratios acquired with ICP-MS for this instrumental mass discrimination (37). In the present ^{129}I isotope ratio method, Te and Russell's law model was used for continuous and simultaneous mass bias correction (39). The ^{129}I isotope ratio method showed a higher precision and a lower LOD compared with the standard curve method.

In this study, samples were extracted at elevated temperatures using TMAH, a procedure previously validated for several foods including milk and infant formula (22,25,29). Other ICP-MS methods proposed for BMIC determination include digestion with ammonia (25) and quantification procedures using different internal standards, such as rhodium (Rh) (25), antimony (Sb) (21), or Te (21). The authors prefer to use TMAH for sample preparation, as it has been validated and documented to perform complete iodine extractions from breast milk (21). The high recovery rates of iodine in the NIST SRM1549a reference sample (105% for the ^{129}I isotope ratio method and 102% for the standard curve method) confirm complete iodine extraction using TMAH. Further digestion procedures such as ashing or filtration are likely superfluous and would rather increase the risk for iodine losses. Huynh *et al.* (21) recently proposed a method for BMIC determination using external iodine calibrators in aqueous TMAH solution for quantification and Sb as internal standard, and report a precision and accuracy similar to the present ^{129}I isotope ratio method. However, the current findings show that when using the same instrumental setup, quantification using IDA with ^{129}I and Te for mass bias correction provides more precise results than an external standard curve approach. Dyke *et al.* (28) tested several internal standards for milk iodine determination by ICP-MS and found that the iodine isotope ^{129}I is the preferred standard. The half-life of ^{129}I is 15.7 million years, and the radiation hazard during analysis is negligible (27,28,43). In many countries, including Switzerland, use of ^{129}I for ICP-MS IDA does not require radiological controls.

The present data show only small differences in BMIC between the within-feed sampling time points. Although the iodine content was statistically lower in the hindfeed samples compared with the fore- and mid-feed milk samples, the differences could only be detected by the more precise ICP-MS ^{129}I isotope ratio method. The lower BMIC in the hind-milk samples may be explained by the physiological change in breast milk composition during feeding: as the fat content of breast milk increases toward the end of the feed, the iodine-containing water phase may decrease (32). However, these small differences are unlikely to be physiologically relevant. The present findings are consistent with a small study in 13 borderline iodine deficient lactating women (BMIC 83 μg/L and median urinary iodine concentration 72 μg/L) that found a small (4 μg/L) but statistically significant difference between the iodine content of fore- and hind-milk samples (17). Another study of three lactating women did not observe a difference in BMIC between fore- and hind-milk; the average BMIC of participating women was 142 μg/kg (18). Neither of the two studies measured BMIC using ICP-MS. The present results suggest that BMIC from studies using the same analytical method and using breast milk from fore-, mid-, or pooled milk samples can be compared. However, data are limited, and because maternal iodine status and the prevailing BMIC concentration may play a role, the findings need confirmation in other populations with varying iodine status. The results also indicate that the ICP-MS ^{129}I isotope ratio method with Te for mass bias correction may be preferable to detect small differences in BMIC, which may not be discriminated by conventional ICP-MS methods that do not offer simultaneous and continuous correction of ICP-MS signals.

For iodine status monitoring in population-based studies, the World Health Organization recommends reference methods along with reference ranges for the determination of iodine in urine and salt (44), but not for BMIC. BMIC is a promising indicator for monitoring iodine nutrition in lactating women and breastfed infants. However, to establish reference ranges for BMIC, standardized procedures for sample collection and timing of collection, as well as the optimal analytical method, need to be defined. If future studies in populations with varying iodine status confirm the findings that the BMIC of foremilk samples does not differ significantly from mid-feed samples or pooled samples, collection of foremilk samples may be preferable in population-based studies; foremilk samples are easy to collect and do not interfere with the mother–infant interaction. The present findings also suggest that for BMIC analysis, the method described in this paper, based on extraction with TMAH followed by ICP-MS IDA using ^{129}I and Te for mass bias correction, may have advantages over other available methods.

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Author Disclosure Statement

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