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A Danish Regional Investigation

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IODINE STATUS IN PREGNANT & BREASTFEEDING WOMEN

A DANISH REGIONAL INVESTIGATION

BY
STINE LINDING ANDERSEN

DISSERTATION SUBMITTED 2014

AALBORG UNIVERSITY
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CV

Stine Linding Andersen obtained her medical degree at Aarhus University in 2011. She had her 6-month internal medicine training in the Department of Endocrinology, Aalborg University Hospital, and her 6-month general practice training in Aalborg. She was enrolled as a PhD student at Aalborg University on March 1, 2012. Her research focuses on iodine status and thyroid disease in and around pregnancy. The four publications combined in her PhD thesis address iodine status in pregnant and breastfeeding women. Within this field, she has contributed with invited lectures at Nordic Iodine meetings and at ICCIDD (International Council for the Control of Iodine Deficiency Disorders) European meetings. Simultaneously with her work on iodine, her research has focused on maternal thyroid dysfunction in and around pregnancy. Using Danish nationwide registers, she has examined the association between maternal thyroid dysfunction in pregnancy, pregnancy outcomes and child development, and she has studied the risk of birth defects associated with the drugs used for the treatment of hyperthyroidism in pregnancy. She is the first author of more than 10 publications within this field and the winner of three national and international awards. Since July 1, 2014 her employment has been split between the research fellowship in the Department of Endocrinology and employment at the Department of Clinical Biochemistry, Aalborg University Hospital, and she is planning to do her medical specialist training within this field.

ENGLISH SUMMARY

Iodine is required for the synthesis of thyroid hormones which are crucial regulators of early brain development. Denmark was previously iodine deficient with regional differences. The Danish mandatory iodine fortification of salt was introduced in the year 2000 and has improved iodine status in the general Danish population. The consequences of maternal iodine deficiency for the fetus and the breastfed infant may be severe and of major concern. Severe maternal iodine deficiency can cause brain damage and cretinism with stunted physical and mental growth, but also mild to moderate iodine deficiency has been associated with low child IQ.

Maternal thyroid hormones are essential for fetal brain development in the early pregnancy, but the fetal thyroid gland is increasingly able to synthesize thyroid hormones from the beginning of the second trimester which requires the transport of maternal iodide across the placenta. Several studies have shown that the transport of iodide in the human placenta is mediated by the sodium-iodide symporter (NIS), and studies in rats and *in vitro* have suggested that the transport of iodide in the placenta is autoregulated similar to in the thyroid gland. Our clinical data from a study performed before the introduction of the mandatory iodine fortification of salt in Denmark, at a time with a higher frequency of maternal smoking in pregnancy, support the hypothesis that placental iodide transport is autoregulated.

Before the introduction of the mandatory iodine fortification of salt in Denmark, Danish pregnant were iodine deficient with signs of thyroidal stress and also Danish breastfeeding women and their breastfed infants were iodine deficient.

The recommended method to evaluate iodine status in a population is to collect spot urine samples and to estimate the median urinary iodine concentration (UIC). In our new regional investigation performed more than 10 years after the introduction of the mandatory iodine fortification of salt in Denmark, Danish pregnant women living in an area with previously moderate iodine deficiency still had median UIC below the recommended range. However, even if the use of iodine-containing supplements had considerably increased since the previous investigation, 15% of the pregnant women did not use iodine-containing supplements, and this subgroup of pregnant women was particularly at risk of being iodine deficient. Maternal use of iodine-containing supplements was less frequent during breastfeeding, and iodine status in Danish breastfeeding women was inadequate both when evaluated by maternal UIC and breast milk iodine concentration. The use of iodine-containing supplements in pregnant and breastfeeding women should be officially recommended in Denmark.

The evaluation of urinary iodine status from spot urine samples in pregnant and breastfeeding women can be challenging. Our methodological considerations suggest that location and time of spot urine sampling, time of most recent iodine supplement intake prior to spot urine sampling, and urinary creatinine concentration optimally should be considered and reported.

DANSK RESUME

Jod indgår i dannelsen af stofskiftehormoner (thyroidea hormoner), som er essentielle udviklingsfaktorer, særligt hvad angår hjernens udvikling. I Danmark var der tidligere jodmangel med regionale forskelle betinget af geografisk varierende indhold af jod i drikkevandet. Således var der mild jodmangel i øst Danmark og moderat jodmangel i vest Danmark. En obligatorisk jodberigelse af husholdningssalt og salt anvendt til industriel fremstilling af brød blev indført i år 2000 og har generelt øget jodindtaget i den danske befolkning.

Jodmangel hos gravide og ammende kan have alvorlige konsekvenser for fosteret og det nyfødte barn. Svær jodmangel kan føre til irreversibel hjerneskade og kretinisme med hæmmet fysisk og psykisk udvikling hos barnet, men også lettere grader af jodmangel er fundet associeret med lav IQ hos barnet.

I den tidlige graviditet er fosterets hjerneudvikling helt afhængig af thyroidea hormoner fra moderen, men fra andet trimester af graviditeten bliver fosteret i stigende grad selv i stand til at producere thyroidea hormoner, og der er behov for, at jod transporteres via moderkagen (placenta) til fosteret. Flere studier har vist, at jodid transporten i den humane placenta ligesom i skjoldbruskkirtlen (glandula thyroidea) og i den lakterende mamma medieres af natrium-jodid symporteren (NIS), og studier i rotter og *in vitro* har antydet, at transporten af jodid i placenta er autoreguleret, ligesom det er tilfældet i glandula thyroidea. Vore kliniske data fra et dansk studie, der blev gennemført før indførelsen af den danske jodberigelse af salt, på et tidspunkt hvor hyppigheden af rygning hos danske gravide var større end i dag, støtter hypotesen om, at jodid transporten over placenta er autoreguleret.

Jodindtaget hos danske gravide og ammende blev senest undersøgt før indførelsen af den obligatoriske jodberigelse af salt i Danmark. På det tidspunkt havde danske gravide og ammende jodmangel. Den anbefalede metode til at vurdere jodindtaget i en befolkning er at indsamle spot urin prøver og beregne den mediane urinjods-koncentration. I vor nye regionale undersøgelse mere end 10 år efter indførelsen af den obligatoriske jodberigelse af salt i Danmark, var jodindtaget hos danske gravide fortsat for lavt vurderet ved den mediane urinjodskoncentration. Der var sket en markant stigning i antallet af gravide, som indtog jodholdigt kosttilskud i forhold til en tidligere undersøgelse, men der var fortsat 15% af de gravide, som ikke tog jodholdigt kosttilskud, og denne gruppe af gravide var særligt i risiko for at have jodmangel. Indtag af jodholdigt kosttilskud under amning var mindre hyppigt, og jodindholdet i mødrenes urin samt i ammemælk var under anbefalet niveau. Jodholdigt kosttilskud bør officielt anbefales til gravide og ammende i Danmark.

Evaluering af jodstatus hos gravide og ammende ud fra urinjodskoncentrationen være vanskelig. Vore metodemæssige overvejelser indikerer, at sted og tidspunkt for urinprøvetagningen, tidspunkt for seneste indtag af jodholdigt kosttilskud forud for urinprøvetagningen samt urin kreatinin koncentrationen optimalt set bør vurderes og rapporteres.

ACKNOWLEDGEMENTS

The work included in the thesis was carried out during my research fellowship in the Department of Endocrinology, Aalborg University Hospital. I would like to thank Aalborg University and the North Denmark Region for providing facilities and funding, and my main supervisor Prof. Peter Laurberg for introducing me to the field of iodine and thyroid research, for providing me the opportunity to do research within this field, and for his great interest in my work and excellent supervision including numerous inspiring conversations about study ideas and results. I would also like to thank my co-supervisors, Prof. Jørn Olsen and PhD Chunsen Wu, at the Section of Epidemiology, Department of Public Health, Aarhus University for introducing me to the field of epidemiology and register-based research. I would like to thank Susanne B. Nøhr and co-workers who collected the data included before the introduction of the mandatory iodine fortification of salt in Denmark. The new data collection took place in the Department of Obstetrics and Gynecology, Aalborg University Hospital, and I would like to thank Margrethe Møller for her willingness to participate in the project and the secretaries in the Department of Obstetrics and Gynecology for their help in recruiting the pregnant women arriving for obstetric ultrasound. A special thank to medical student Louise Kolding Sørensen who did an excellent job during the time of study inclusion. I would like to thank all the pregnant women who participated in the study, the women who also participated during breastfeeding and the families included. The study received a grant from Musikforlæggerne Agnes og Knut Mørks Fond and from Speciallæge Heinrich Kopps Legat. I would like to thank all my colleagues in the research unit at Department of Endocrinology, Aalborg University Hospital, including secretary Maggie Bloch who helped with important logistics prior to study inclusion, biomedical laboratory technologist Ingelise Leegaard who performed all the urinary iodine analyses and helped with the collection of samples from places which were hard to reach by bike, and PhD student Anne Krejbjerg who gave me great company during our daily work and during congress participation. Finally, I would like to thank my family and friends for their support and great interest in my research.

LIST OF ABBREVIATIONS

DanThyr	The Danish investigation on iodine intake and thyroid disease
DIT	Diiodotyrosine
D2	Type 2 iodothyronine deiodinase
D3	Type 3 iodothyronine deiodinase
GFR	Glomerular filtration rate
hCG	Human chorionic gonadotropin
ICCIDD	International Council for the Control of Iodine Deficiency Disorders
MIC	Breast milk iodine concentration
MIT	Monoiodotyrosine
NIS	Sodium-iodide symporter
PII	Plasma inorganic iodide
PPTD	Postpartum thyroid dysfunction
T3	Triiodothyronine
T4	Tetraiodothyronine
TBG	Thyroxine-binding globulin
Tg	Thyroglobulin
Tg-Ab	Thyroglobulin antibodies
TPO	Thyroid peroxidase
TPO-Ab	Thyroid peroxidase antibodies
TSH	Thyroid stimulating hormone
UIC	Urinary iodine concentration
UNICEF	United Nations Children's Fund
WHO	World Health Organization

LIST OF PUBLICATIONS

1.	**Thyroglobulin in smoking mothers and their newborns at delivery suggests autoregulation of placental iodide transport overcoming thiocyanate inhibition** Stine Linding Andersen, Susanne B. Nøhr, Chun Sen Wu, Jørn Olsen, Klaus M. Pedersen & Peter Laurberg. *European Journal of Endocrinology, 168 (5), 723-731, 2013.*
2.	**Iodine deficiency in Danish pregnant women** Stine Linding Andersen, Louise Kolding Sørensen, Anne Krejbjerg, Margrethe Møller & Peter Laurberg. *Danish Medical Journal, 60 (7), A4657, 2013 including a Danish summary in Ugeskrift for Læger, 175 (36), 2030, 2013.*
3.	**Challenges in the evaluation of urinary iodine status in pregnancy: The importance of iodine supplement intake and time of sampling** Stine Linding Andersen, Louise Kolding Sørensen, Anne Krejbjerg, Margrethe Møller & Peter Laurberg. *European Thyroid Journal, 3 (3), 179-188, 2014.*
4.	**Iodine concentrations in milk and in urine during breastfeeding are differently affected by maternal fluid intake** Stine Linding Andersen, Margrethe Møller & Peter Laurberg. *Thyroid, 24 (4), 764-772, 2014.*

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CHAPTER 1. INTRODUCTION

Iodine is required for the synthesis of thyroid hormones which are crucial regulators of early brain development [1,2]. The World Health Organization (WHO) describes iodine deficiency as the single most common preventable cause of brain damage [3], and adequate maternal iodine intake during pregnancy and breastfeeding is of major concern [4]. Historically, large parts of the world were iodine deficient, but preventive efforts have been made and today programs of universal salt iodization have been implemented in many countries [5]. Iodine is found naturally in foods and drinking water, artificially in foods fortified with iodine, in iodine-containing dietary supplements, radiographic contrasts agents, and in medicaments [6].

1.1. IODINE STATUS IN DENMARK

The main dietary sources of iodine in Denmark are milk, fish and drinking water [7]. Denmark was previously iodine deficient with regional differences caused by different levels of iodine in drinking water; moderate iodine deficiency in West Denmark and mild iodine deficiency in East Denmark [8,9]. A Danish voluntary iodine fortification (8 parts per million (ppm)) of household salt and salt used by the food industry was initiated in June 1998, but a voluntary program was not sufficient when evaluated after two years, and from July 2000 a mandatory iodine fortification (13 ppm) of household salt and salt used for commercial production of bread has been introduced in Denmark [10].

1.1.1. IODINE STATUS IN PREGNANT AND BREASTFEEDING WOMEN

No official recommendations for intake of iodine-containing supplements during pregnancy and breastfeeding exist in Denmark [11,12]. The iodine status in Danish pregnant and breastfeeding women has been examined before the introduction of the Danish iodine fortification of salt [13-19]. At that time, Danish pregnant and breastfeeding women were iodine deficient [13-19]. The Danish iodine fortification of salt has increased iodine intake in the general Danish population [9,20], but the significance of iodine fortification for Danish pregnant and breastfeeding women specifically has not been evaluated.

1.2. IODIDE TRANSPORT FROM MOTHER TO CHILD

Iodide is transported into the thyroid gland by the sodium-iodide symporter (NIS) [21]. NIS is also identified in a number of extrathyroidal tissues including the gastrointestinal tract, the kidney, the lactating mammary gland, and the placenta [22], and a functional role of NIS in extrathyroidal iodide transport has been proposed [23-26]. In early pregnancy, the developing fetus is dependent on

maternal thyroid hormones [27,28], but the fetal thyroid gland is increasingly capable of synthesizing thyroid hormones from 12-16 weeks of pregnancy with a need for transport of iodide from the mother to the fetus across the placenta [29,30].

1.2.1. PLACENTAL IODIDE TRANSPORT

In the thyroid gland, NIS-mediated transport of iodide is autoregulated to keep the level of iodine sufficient for thyroid hormone synthesis [31]. On the other hand, there is no apparent autoregulation of NIS-mediated transport of iodide into breast milk in the lactating mammary gland [19]. In the placenta, autoregulation of iodide transport has been demonstrated in rats and *in vitro* [32,33], but details on the regulation of placental iodide transport are still to be elucidated.
An indicator of fetal iodine deficiency is cord serum thyroglobulin (Tg) [34,35]. One way to evaluate the regulation of NIS-mediated placental iodide transport *in vivo* is to study the impact of a known NIS inhibitor such as thiocyanate from tobacco smoking [36]. The frequency of maternal smoking during pregnancy in Denmark has considerably declined during the last decades [37], but at the time of the previous investigation of iodine intake in Danish pregnant and breastfeeding women, maternal smoking was more frequent than today [19].

1.3. CHALLENGES IN EVALUATION OF IODINE STATUS

The recommended method to assess iodine status in a population is to collect spot urine samples for measurement of urinary iodine concentration (UIC) and calculation of the population median UIC [3]. But several factors may influence UIC and challenge the interpretation of the results.

1.3.1. IN PREGNANCY

Traditionally, the median UIC of schoolchildren has been the recommended method to assess urinary iodine status in a population, including pregnant women [38]. However, disparity between results of schoolchildren examination and that of pregnant women has been reported [39], and it can be speculated if such disparity could be partly explained by differences in urine sampling conditions. In the majority of studies evaluating iodine status in pregnancy, the pregnant women are recruited during a routine hospital visit. It can be speculated if results of such iodine status evaluation are representative for urine samples which were instead obtained at home during daily living. In many populations, the use of iodine-containing supplements is recommended during pregnancy to ensure adequate iodine intake [40]. It can be speculated if the time span from most recent iodine supplement intake prior to spot urine sampling could influence UIC and the results of iodine status evaluation.

1.3.2. DURING BREASTFEEDING

Maternal intake of iodine-containing supplements is often recommended during breastfeeding to ensure adequate iodine supply of the mother and the breastfed infant [40]. Acute intake of high doses of iodine has been shown to affect breast milk iodine concentration (MIC) [41]. It can be speculated if also most recent iodine supplement prior to breast milk sampling could influence MIC and the results of iodine status evaluation. It is often difficult to obtain a breast milk sample or a urine sample from the breastfed infant, and it may be convenient to obtain a urine sample from the mother. It has been considered whether maternal UIC can be used as a proxy for iodine supply to the breastfed infant, but correlations between UIC and MIC have not been consistent [42,43]. Maternal fluid intake may influence UIC and a proxy for fluid intake is the urinary creatinine concentration [44]. It can be speculated if maternal fluid intake may differently affect maternal UIC and MIC.

1.4. OBJECTIVE OF THE PHD THESIS

The objective of the PhD thesis was to evaluate iodine status in Danish pregnant and breastfeeding women after the introduction of the mandatory iodine fortification of salt in Denmark, and to study details on the transport of iodide from mother to child as well as challenges in the evaluation of urinary iodine status in pregnant and breastfeeding women. Data were retrieved from a previous investigation performed before the mandatory iodine fortification of salt [17,19] and from a new regional investigation performed after the introduction of the mandatory iodine fortification of salt in Denmark (Fig. 1-1).

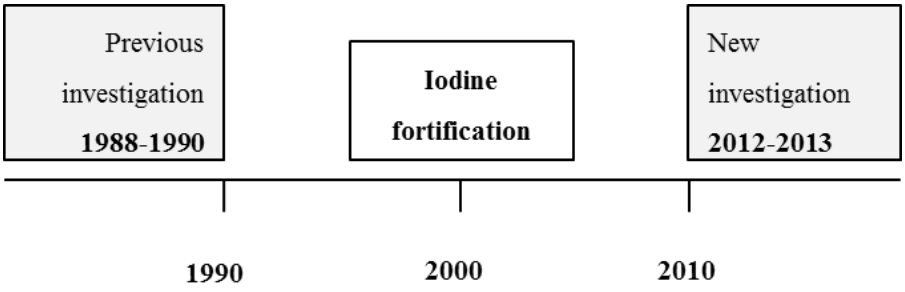

Fig. 1-1. Illustration of the investigations before and after the Danish mandatory iodine fortification of salt which was introduced in the year 2000.

1.4.1. SPECIFIED STUDY OBJECTIVES

To use data from a previous Danish investigation performed before the introduction of the mandatory iodine fortification of salt at a time with a higher frequency of smoking in Danish pregnant women to evaluate if placental iodide transport is autoregulated (paper 1).
To perform a new regional Danish investigation in an area with previously moderate iodine deficiency to evaluate the use of iodine-containing supplements and urinary iodine status in Danish pregnant women after the introduction of the mandatory iodine fortification of salt (paper 2).
To include information on time of most recent iodine supplement intake prior to spot urine sampling, urine samples obtained both in the hospital and at home as well as urine samples from non-pregnant members of the household to study challenges in the evaluation of urinary iodine status in pregnancy (paper 3).
To perform a regional Danish investigation in an area with previously moderate iodine deficiency to evaluate the use of iodine-containing supplements and urinary iodine status in Danish breastfeeding women after the introduction of the mandatory iodine fortification of salt (paper 4).
To include information on time of most recent iodine supplement intake prior to breast milk and spot urine sampling as well as measurement of maternal urinary creatinine concentration to study challenges in the evaluation of iodine status during breastfeeding (paper 4).

Table 1-1. Specified study objectives of the PhD thesis.

CHAPTER 2. BACKGROUND

2.1. SOURCES OF DIETARY IODINE

Iodine (I) is a mineral and a micronutrient required in humans for the synthesis of thyroid hormones [6]. The healthy human adult contains 15-20 mg iodine of which around 70% is stored in the thyroid gland [45]. The main sources of dietary iodine vary between countries and may also vary within the same country [7]. Fish, seafood and in particular seaweed are rich in iodine, thus, in populations with a high intake of fish, these food items are the major source of iodine. Milk and dairy products also contain iodine and these are the main sources of iodine in many populations including Denmark due to a generally high intake of these food items [7]. Finally, drinking water is an important source of iodine in some populations. The content of iodine in drinking water may vary within a country as is the case in Denmark [8,46] with a generally higher content of iodine in drinking water in East Denmark than in West Denmark divided by the great Belt (Fig. 2.1). However, with the exception that the content of iodine in drinking water in Skagen (the most northern part of West Denmark) is high [8].

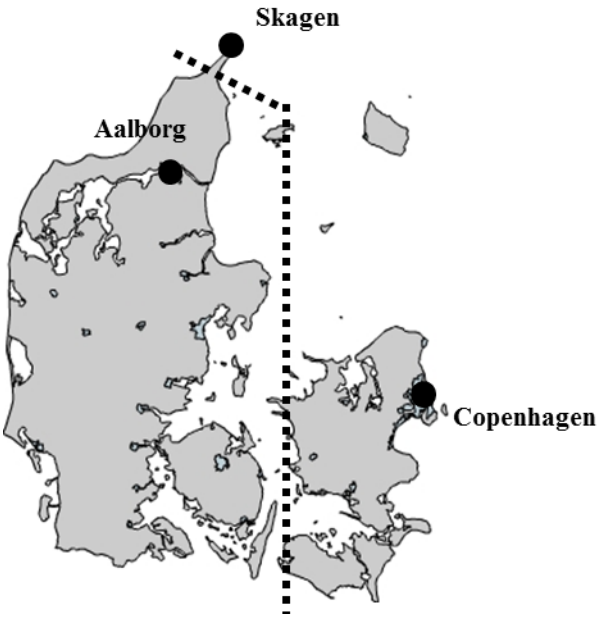

Fig. 2-1. Map of Denmark. The dotted line illustrates the division by the Great Belt into East Denmark with a higher content of iodine in drinking water and West Denmark with a lower content of iodine in drinking water.

2.2. IODINE METABOLISM

2.2.1. GASTROINTESTINAL ABSORPTION

Ingested iodine in other forms than iodide (e.g. iodate) is converted into iodide (I^-) before it is absorbed. NIS is expressed on the apical surface of the enterocytes in the small intestine and mediates active uptake of iodide in rats [26]. NIS is also expressed in the gastric mucosa on the basolateral surface of the epithelial cells mediating the secretion of iodide from the blood to the gastric lumen, but whether the gastric mucosa is also capable of iodide uptake is not clarified [47]. Iodide is rapidly and almost completely absorbed. After oral administration of radioiodine in euthyroid individuals, it rapidly appeared in the circulating blood and reached the maximum level within two hours from ingestion (Fig. 2-2) [48]. Organic iodine is less completely absorbed. Absorption of an oral dose of Levothyroxine varies (e.g. depending on food intake) and has been reported up to 80% [49].

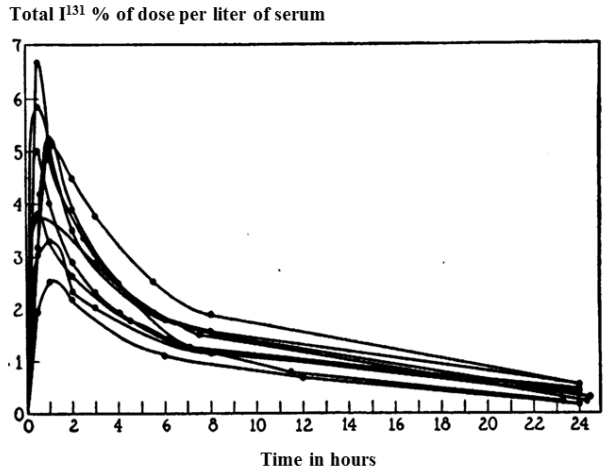

Fig. 2-2. Oral administration of radioiodine in nine euthyroid subject. Reproduced from [48] *with permission.*

2.2.2. PLASMA INORGANIC IODIDE

After gastrointestinal absorption, organic iodine is primarily present in the blood as tetraiodothyronine (T4) [50]. Plasma inorganic iodide (PII) is the pool from which iodide is distributed (Fig. 2-3). The concentration of PPI is proportional to dietary iodine intake [51], and the size of the PII pool is primarily balanced between a) iodine intake and gastrointestinal absorption, b) uptake of iodide in the thyroid gland and metabolism of thyroid hormones, and c) renal excretion of iodide. Only small amounts of iodide are excreted in faeces, sweat and via respiration [51].

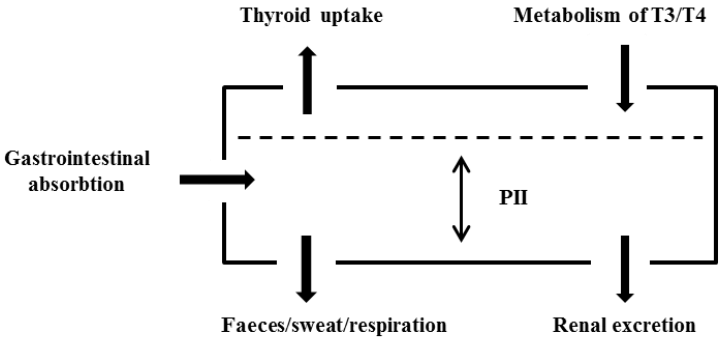

Fig. 2-3. Inputs and outputs from the plasma inorganic iodide (PII) pool.

2.2.3. THYROID UPTAKE

In euthyroid individuals with an adequate intake of iodine, the uptake of iodide by the thyroid gland approximates 1/3 of an administered dose of radioiodine [52]. Transport of iodide from plasma into the thyroid gland is active and mediated by NIS which is located at the basolateral membrane of the thyroidal follicular cells (Fig. 2-4). From the cells, iodide is transferred to the colloidal follicle lumen at the apical membrane, and the chloride-iodide transporter (Pendrin) is thought to be involved in this transport [53].

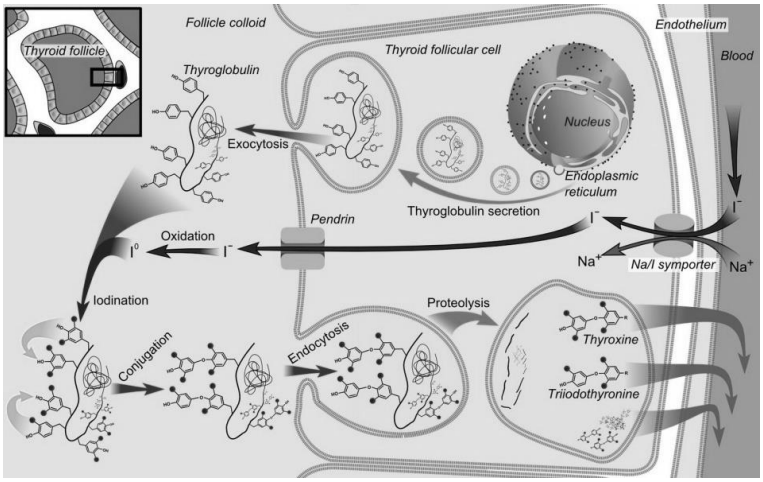

Fig. 2-4. Uptake of iodide in the thyroid gland and synthesis of thyroid hormones.

In the colloidal follicle lumen, iodide is oxidized and incorporated in Tg resulting in the synthesis of monoiodotyrosine (MIT) and diiodotyrosine (DIT) [54]. This

process is catalyzed by the thyroid peroxidase (TPO) enzyme which also catalyzes the formation of triiodothyronine (T3) (DIT+MIT) and T4 (DIT+DIT). When thyroid hormones are released, Tg is endocytosed in the follicular cells, and T3 and T4 are released from Tg by proteolysis before being secreted into the blood. T4 is only produced in the thyroid gland, whereas T3 (80%) is produced by deiodination of T4 in extrathyroidal tissues. Metabolism of T3 and T4 by deiodinases releases iodide to the PII pool (Fig. 2-3) [54].

2.2.4. RENAL EXCRETION

Iodide is mainly excreted in the urine and > 90% of ingested iodine appears in the urine [51,55]. How iodide is excreted in the kidneys is not clarified in detail, but glomerular filtration and tubular reabsorption are presumed to be mechanisms involved [56]. The tubular reabsorption was previously assumed to be passive [56], but NIS expression has been demonstrated in the tubular system of the human kidney and active transport mediated by NIS has been proposed [24]. After oral administration of radioiodine in euthyroid individuals (Fig. 2-5), 2/3 of the dose was excreted in the urine within 48 hours and approximately half of the dose excreted in the urine was excreted within six hours [52]. Clearance is defined as the volume of plasma from which a substance is completely removed per unit time [57]. For the renal clearance of iodide this equals the urinary iodide excretion rate (UIC multiplied by the urine flow rate) divided by the PII concentration. The renal clearance of iodide was shown to be constant when a radioactive tracer with different quantities of carrier iodine was administered to 13 patients with exophthalmic goiter [58]. Thus, when the urine flow rate is unchanged, UIC is expected to fluctuate according to the PII concentration.

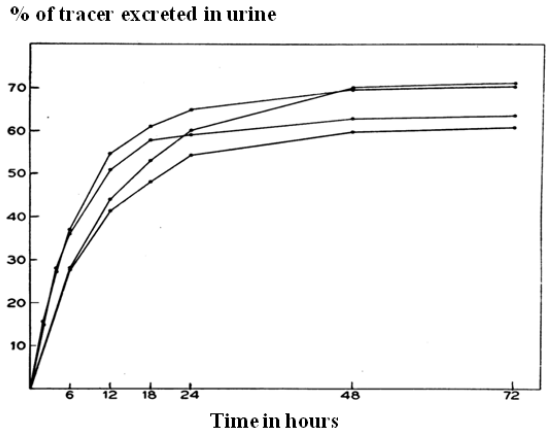

Fig. 2-5. Urinary excretion of radioiodine in four euthyroid male subjects. Reproduced from [52] *with permission.*

2.3. EVALUATION OF IODINE STATUS

Iodine status in a population can be assessed from different methods (Table 2-1).

Method
Thyroid size
Serum thyroglobulin (Tg)
Serum thyroid stimulating hormone (TSH)
Urinary iodine excretion
Dietary assessment of iodine intake

Table 2-1. Methods to assess iodine status in a population.

2.3.1. THYROID SIZE

Historically, evaluation of thyroid size by inspection and palpation was the method by which goiter prevalence in a population was estimated and iodine status was evaluated. However, in mild iodine deficiency, the evaluation of thyroid size by thyroid ultrasonography is preferable [38]. Thyroid size is a long-term indicator of iodine status (months to years) and although thyroid ultrasonography is non-invasive and quickly performed, the method requires training and differences in technique can produce interobserver variation [59].

2.3.2. SERUM THYROGLOBULIN

Tg is a thyroid-specific protein exclusively synthesized in the thyroid gland. Serum Tg is a valid marker of iodine deficiency in population studies [34,35], but a high serum Tg concentration is not a specific sign of iodine deficiency and may also be present in various thyroid disorders and due to physiological changes in pregnancy [60]. The need for measurement of thyroglobulin antibodies (Tg-Ab) to examine possible interference with serum Tg should be considered [61].

2.3.3. SERUM THYROID STIMULATING HORMONE

Iodine deficiency tends to lower T4 and increase serum thyroid stimulating hormone (TSH), but TSH is not a sensitive marker of iodine deficiency in schoolchildren and adults, including pregnant women, because it is often remained within the normal range [1]. On the contrary, TSH tends to be lower with age in populations with mild to moderate iodine deficiency due to thyroid autonomy [62]. It has been suggested that TSH is a more sensitive indicator of iodine deficiency in neonates due to low iodine content in the neonatal thyroid gland and a high turnover of iodine [3], but this may be hampered by the use of iodine-containing skin disinfection in mothers at delivery [63].

2.3.4. URINARY IODINE EXCRETION

Urinary iodine excretion is an indicator of recent iodine intake (hours-days). In individuals, daily urinary iodine excretion varies considerably [64,65], and a single spot urine sample cannot be used to diagnose iodine deficiency in an individual [65]. The golden standard to quantify urinary iodine excretion is to collect urine in a full 24-hour sample [44], but for determination of iodine status in individuals, more than one 24-hour sample is preferable [64,65], and for determination of iodine status in a population, the collection of urine over 24 hours is often troublesome and may not be complete [66]. WHO, the United Nations Children's Fund (UNICEF), and the International Council for the Control of Iodine Deficiency Disorders (ICCIDD) recommend the median UIC from spot urine samples to determine iodine status in a population (Table 2-2) [3].
UIC is calculated per urine volume and varies by fluid intake [44]. Creatinine is a product of muscle metabolism which is excreted in the urine at a relative constant rate, and it has been suggested to use the iodine/creatinine ratio (µg iodine/gram creatinine) to adjust for variation in urine volume [44]. But creatinine excretion varies with age and gender and a further adjustment has been proposed in which the iodine/creatinine ratio is multiplied by an estimated age- and gender specific 24-hour creatinine excretion to calculate the estimated 24-hour urinary iodine excretion (µg iodine/24 hours) [67]. Another aspect is the variation in UIC during the day. In particular, UIC in a fasting morning spot sample tend to be lower [64].

Median UIC (µg/l)	Iodine intake	Iodine deficiency
< 20	Insufficient	Severe
20-49	Insufficient	Moderate
50-99	Insufficient	Mild
100-199	Adequate	-
200-299	Above requirements	-
≥ 300	Excessive	-

Table 2-2. Assessment of population iodine status from median urinary iodine concentration (UIC) in schoolchildren ≥ 6 years and non-pregnant adults [3].

2.3.5. DIETARY ASSESSMENT OF IODINE INTAKE

Dietary assessment methods aim to quantify the habitual iodine intake. It is often difficult due to day-to-day variation [64], but the most significant dietary sources of iodine can be determined. One method is the food frequency questionnaire, which assesses the frequency and portion sizes of iodine-containing foods within a defined time frame [68]. Another method is the 24-hour food diary which assesses intake of iodine-containing foods in the previous 24 hours [69].

2.4. IODINE DEFICIENCY & EXCESS

The recommended daily intake of iodine is 150 µg in non-pregnant and non-lactating adults [3]. Both insufficient and excessive iodine intake may lead to the development of thyroid disease and the relation between iodine intake and thyroid disease in a population is U-shaped [70]. Worldwide iodine status in 2013 [5] indicated that substantial progress has been made in the elimination of iodine deficiency mainly through programs of universal salt iodization. Approximately 70% of all households worldwide have access to adequately iodized salt (versus 10% in 1990) [5]. Among countries with available data on UIC in schoolchildren, 111 countries had adequate iodine status (versus 67 in 2003), 9 countries had moderate iodine deficiency, 21 countries had mild iodine deficiency and 10 countries had excessive intake of iodine [5]. However, subgroups (e.g. specific dietary habits, no use of iodized salt, pregnancy and breastfeeding) may still be iodine deficient in countries classified as iodine sufficient.

2.4.1. IODINE DEFICIENCY

Iodine deficiency disorders refer to *'all the consequences of iodine deficiency in a population that can be prevented by ensuring that the population has an adequate iodine intake'* [3]. Iodine deficiency is associated with a higher frequency of goiter and thyroid multinodularity [71]. The mechanisms involved in goitrogenesis are presumably secondary to iodide autoregulation which increases the activity and the growth of the thyroid gland [70]. Severe iodine deficiency can cause hypothyroidism [70]. In less severe iodine deficiency, the thyroid gland is able to keep thyroid hormone synthesis sufficient due to an increased activity, but this hyperactivity may lead to the development of thyroid autonomy and a higher rate of toxic multinodular goiter [72].
Iodine deficiency during pregnancy and breastfeeding may affect both the mother and the fetus/newborn. Depending on the degree of iodine deficiency, iodine supply may be insufficient for thyroid hormone synthesis resulting in maternal and fetal/neonatal hypothyroxinemia (low T4, normal TSH) or hypothyroidism (low T4 and high TSH) [1].

2.4.2. IODINE EXCESS

The upper tolerable level of iodine intake in non-pregnant and non-lactating adults has been set to 1,100 µg/day [6]. Sources of excess iodine include food items (e.g. seaweed), excessive iodization of salt, iodine-containing supplements, medications (e.g. amiodarone), and contrast agents [6]. The acute Wolff-Chaikoff effect was described in 1948 [73]. In rats exposed to high levels of iodine, a reduction in the synthesis of thyroid hormones was observed. The reduction was only transient (escape from the Wolff-Chaikoff effect), which may be caused by a reduced

expression of NIS [31]. Failure to escape from the Wolff-Chaikoff effect may lead to hypothyroidism, especially in individuals with thyroid autoimmunity or previous thyroid disease [70]. Hence, before the iodine fortification of salt in Denmark, the incidence of overt hypothyroidism was highest in East Denmark with the highest iodine intake [74] and was predominantly spontaneous autoimmune hypothyroidism [75]. Hyperthyroidism may develop in susceptible individuals (e.g. autonomous thyroid nodules, relapse of Graves' disease) following high intake of iodine [70].
Excessive intake of iodine during pregnancy and breastfeeding is less studied than iodine deficiency, but in China where iodine content of drinking water is high, excessive iodine intake was associated with maternal subclinical hypothyroidism (normal T4, high TSH) in late pregnancy [76]. Fetal and neonatal exposure to excessive amounts of iodine through placental transfer or breast milk can cause neonatal hypothyroidism [77].

2.4.3. IODINE & BRAIN DEVELOPMENT

The most serious and adverse effect of iodine deficiency is developmental brain damage [78]. Brain development is initiated in the very early pregnancy and continues throughout pregnancy and during the first years of life (Fig. 2-6) [79].

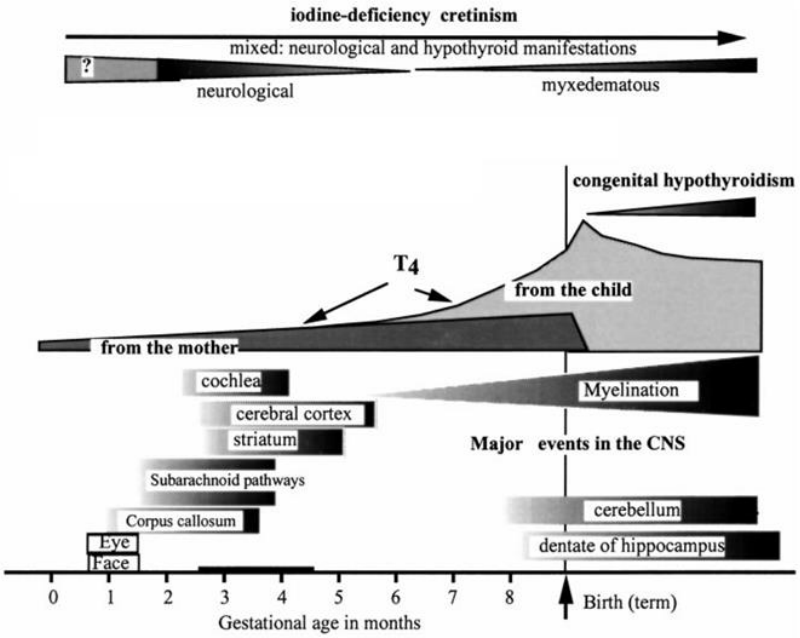

Fig. 2-6. Illustration of events during early brain development. Reproduced from [79] *with permission.*

Thyroid hormones are essential regulators of brain development and involved in myelination, cell differentiation and migration [2]. T3 is the active hormone and acts by binding to intracellular receptors which are increasingly present in the fetal brain from gestational week 10 [2]. In the brain, 80% of T3 is generated locally from T4 by the type 2 iodothyronine deiodinase (D2), and D2 activity is regulated by T4 [2]. Before the onset of fetal thyroid hormone production, thyroid hormones involved in the regulation of early fetal brain development are of maternal origin (Fig. 2-6). But evidence suggests that maternal thyroid hormones are also transferred to the fetus after the onset of fetal thyroid hormone production [79]. In newborns unable to synthesize T4 due to a total defect in organification, T4 (which must be of maternal origin) was measured in cord blood [80]. Similarly, the paucity of neurological symptoms in newborns with congenital hypothyroidism due to failure of the thyroid gland, indicate a protective role of maternal thyroid hormones throughout pregnancy [78].

Severe iodine deficiency can cause cretinism which is characterized by profound mental and physical disabilities [81]. Two clinical types of cretinism have been described [82]. The neurological cretinism is the most common type and characterized by irreversible neurological deficits such as spasticity and squint. The myxedematous cretinism is less common and dominated by signs of hypothyroidism with growth retardation and dry and thickened skin. Clinical characteristics often overlap between the two types, and it has been proposed that the clinical picture of cretinism results from two temporally different events caused by iodine deficiency (Fig. 2-6) [79]. The first is iodine deficiency *in utero* resulting in maternal hypothyroxinemia and the neurological features of cretinism. The second is the duration and severity of hypothyroidism after birth where prompt replacement therapy and iodine supplement will improve the symptoms.

The neurodevelopmental consequences of mild to moderate iodine deficiency are less evident. One way to study this is to look at the impact of maternal iodine supplementation in pregnancy. Several studies have evaluated neurodevelopmental outcomes in children less than two years, but as reviewed in detail [83], studies were mainly observational and results were divergent with a lack of evidence. Another approach is the retrospective design with definition of exposure groups from maternal urinary iodine status in pregnancy. Two longitudinal observational studies from Australia [84] and the United Kingdom [85] assessed neuro-developmental outcomes in children age 8-9 years according to maternal UIC in the pregnancy. In these studies, poorer educational outcomes and lower child IQ were observed in the group of children born to mothers who had median UIC below the recommended range in pregnancy.

The association between maternal excessive iodine intake and brain development is less extensively studied, but evidence from studies in rats suggest that both lack and excess of iodine may affect early neurodevelopment [86].

CHAPTER 3. IODINE, PLACENTA & NIS

3.1. NIS-MEDIATED IODIDE TRANSPORT

NIS is a transmembrane glycoprotein which couples the inward transport of two Na^+ ions along the electrochemical gradient (actively generated by the Na^+/K^+ ATPase) with the inward transport of one I^- ion against its electrochemical gradient [22]. The gene encoding NIS was cloned in 1996 [21] and this has been followed by molecular characterization of NIS, investigations of NIS regulation, NIS expression in extrathyroidal tissues and the pathophysiological role of NIS (e.g. NIS mutation leading to congenital iodide transport defect [22]).

3.1.1. REGULATION OF NIS

TSH stimulates NIS-mediated iodide transport into the thyroid gland (e.g. via regulation of NIS expression) [22], but also other types of NIS regulation exist including autoregulation by iodide (escape from the Wolff-Chaikoff effect) which is shown to be mediated by downregulation of NIS expression [31]. Competitive inhibitors of NIS have been indentified such as the anions perchlorate (ClO_4^-) and thiocyanate (SCN^-) [22]. Perchlorate is among others a component of fireworks, matches and auto airbag inflation systems and environmental exposure is thought to be ubiquitous [87]. It is a more potent inhibitor of NIS than thiocyanate [88], but the low level of exposure found in the environment has not been convincingly related to adverse effects on thyroid function [87].
In some populations (e.g. the Democratic Republic of Congo), the cassava plant is the major source of thiocyanate [36] and a high intake of cassava has been causally linked to a high prevalence of endemic goiter [89] and cretinism [90]. Another source of thiocyanate is rapeseed in feeds for cows which decreases the excretion of iodine into milk [91]. As dairy products constitute a major part of iodine intake in many populations, the feeding of cows may influence the iodine status of the population. The main source of thiocyanate in many countries including Denmark is tobacco smoking [36]. Cyanide in tobacco smoke is toxic and is detoxified in the liver to thiocyanate. The amount of cyanide produced in tobacco smoking is variable [92], and thiocyanate is not solely derived from smoking, thus, other markers of smoking such as the nicotine metabolite cotinine are preferable [93].
NIS has been identified in a number of extrathyroidal tissues including the salivary gland, choroid plexus, ciliar body of the eye, sweat glands [22], gastric mucosa [47], intestine [26], kidney [24] and the lactating mammary gland [23], and NIS-mediated extrathyroidal iodide transport has been proposed. It appears, however, that the local regulation of NIS might differ between tissues. In some extrathyroidal tissues, NIS activity seems to be autoregulated by iodide similar to the regulation of NIS in the thyroid gland. One example of this is the regulation of NIS-mediated

absorption of iodide in the intestine. In a study in rats [94], the intestinal NIS-mediated iodide transport was autoregulated by downregulation of NIS expression in iodine excess. The importance of such regulation in humans remains to be clarified. In other extrathyroidal tissues, no autoregulation of NIS seems to take place. One example of this is the transport of iodide into breast milk in the lactating mammary gland. In a Danish clinical study [19] performed before the introduction of the Danish iodine fortification of salt, cotinine in urine and serum was used to classified mothers as smokers (n=50) and non-smokers (n=90). Smoking mothers had 50% lower breast milk iodine content on day five postpartum than non-smoking mothers, and the iodine content of their neonates' urine was considerably lower (Fig. 3-1). Serum thiocyanate levels were higher in smoking mothers, and results were compatible with thiocyanate inhibition of NIS-mediated transport of iodide into breast milk. Results suggested that NIS in the lactating mammary gland is not autoregulated by iodide. If autoregulation was present, such difference in breast milk iodine content between smoking and non-smoking mothers was not to be expected.

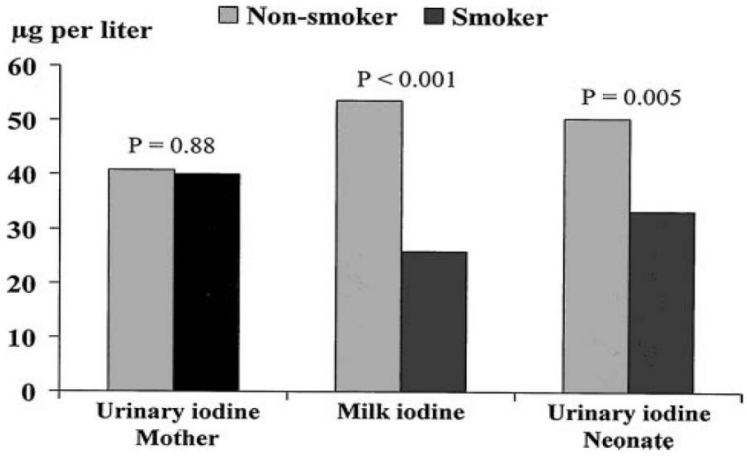

Fig. 3-1. Median maternal urinary iodine concentration, breast milk iodine concentration and neonate urinary iodine concentration stratified by maternal smoking status. Samples were obtained on day five after delivery. Reproduced from [19] *with permission.*

3.1.2. PLACENTAL IODIDE TRANSPORT

The human placenta is essential for fetal development and provides direct contact between the maternal and the fetal circulation. One important function is the transplacental transport of gases, nutrients, and other molecules including the transport of thyroid hormones and iodide. Classically, the human placenta was considered impermeable to the transport of maternal thyroid hormones, but the

study by Vulsma et al. [80] demonstrating that neonates unable to synthesize thyroid hormones had measureable T4 in cord serum, and studies showing the presence of T4 and T3 in fetal tissues before the onset of fetal thyroid hormone production [27,28] have provided evidence, that maternal thyroid hormones are transported across the placenta.

Studies of the placenta from euthyroid women undergoing elective caesarean section have demonstrated that the human placenta contains iodine [95-97]. The net amount of maternal iodide transported into and across the placenta in pregnancy can be summarized by iodine deposited in the placenta (15-30 µg [97], assuming placental weight of 600 grams at birth [98]), in amniotic fluids (10-30 µg [99,100]), in the fetal thyroid gland at birth (100-300 µg [101]), and in fetal blood (10-15 µg [102], assuming mean birth weight of 3.500 grams [103], 10% of body is blood, 50% of blood is serum, and 65% iodine content of T4). When these amounts are summarized relative to the period of pregnancy where placental iodide transport primarily takes place, the net placental deposit and transfer of iodide to the fetus is approximately 2 µg iodine per day.

NIS expression has been demonstrated in the human placenta after the gene encoding NIS was cloned in 1996 [104-109]. Immunohistochemistry or polymerase chain reaction was used to demonstrate NIS in placental tissue samples and *in vitro* in cell cultures of cytotrophoblast cells and choriocarcinom cells. Human choriocarcinom cells have been used as an *in vitro* model of placenta to study the functional role of NIS in placental iodide transport [25]. In this study [25], NIS was detected, radioiodide uptake in the cells was inhibited by perchlorate, iodide and thiocyanate compatible with NIS-mediated iodide uptake, and confocal microscopy revealed that NIS was distributed at the maternal side of the cells. In the same study [25] expression of the chloride/iodide transporter (Pendrin) at the fetal side of the cell was shown suggesting that iodide is released via Pendrin.

The regulation of NIS activity in placenta has been investigated in rats [32] and *in vitro* in choriocarcinom cells [33]. Both types of experimental studies suggested that NIS in placenta is autoregulated by iodide. In rats on a low iodine diet, NIS was up regulated in the placenta [32], and iodide inhibited NIS expression and iodide uptake in choriocarcinom cells [33].

3.2. AUTOREGULATION OF PLACENTAL IODIDE TRANSPORT (PAPER 1)

The first paper in the PhD thesis addressed the regulation of iodide transport in the human placenta. Previous studies have shown that NIS mediates iodide transport in the human placenta, and that this transport is inhibited by thiocyanate. Studies in rats and *in vitro* have suggested that NIS in placenta is autoregulated by iodide, but the extent of autoregulation is still to be elucidated in a clinical setting.

3.2.1. STUDY OBJECTIVE

The aim of the study was by an indirect method to evaluate if placental iodide transport is autoregulated in humans. We assumed that iodide transport in the placenta is mediated by NIS and that this transport is inhibited by thiocyanate. The source of thiocyanate was maternal smoking in pregnancy, and we examined the impact of thiocyanate on the degree of iodine deficiency in the mother and the fetus. As a marker of iodine deficiency we used Tg in maternal serum (at arrival for delivery) and in cord serum (at delivery).
Maternal iodine status and smoking status were expected to influence serum Tg (Table 3-1). Maternal iodine deficiency was expected to increase both maternal and cord serum Tg due to insufficient supply of iodine to the maternal and the fetal thyroid gland. Thiocyanate from maternal smoking was expected to inhibit NIS-mediated iodide transport in the maternal thyroid gland, in placenta and in the fetal thyroid gland. The inhibition of NIS in the thyroid gland was expected to influence maternal and cord serum Tg in parallel. On the other hand, the inhibition of NIS in the placenta would reduce the transport of maternal iodide to the fetus and increase cord serum Tg exclusively. However, if NIS-mediated iodide transport in placenta is autoregulated, compensatory mechanisms would keep the iodide transport to the fetus sufficient despite thiocyanate inhibition of NIS, and the changes in maternal and serum Tg caused by maternal smoking would be more similar (Table 3-1).

	Maternal serum Tg	**Cord serum Tg**
Maternal iodine deficiency	↑	↑
Maternal smoking		
No autoregulation of NIS in placenta	↑	↑↑↑
Autoregulation of NIS in placenta	↑	↑

Table 3-1. Illustration of the hypothetical impact of maternal iodine deficiency and maternal smoking on maternal and cord serum thyroglobulin (Tg) concentration.

3.2.2. STUDY DESIGN

The study was part of a cross-sectional study of iodine intake in Danish pregnant women performed in the years 1988-1990 before the introduction of the Danish mandatory iodine fortification of salt. The reasons for the use of previously collected data were the higher frequency of maternal smoking and the lower frequency of iodine supplement use. The higher frequency of maternal smoking was imperative to study our hypothesis, since the low frequency of smoking in Danish pregnant women today would make it very difficult to study our hypothesis in recently collected data. The lower frequency of iodine supplement use was also important in the way that the effect of a competitive inhibitor increases when the substrate concentration (in this case iodide) is low.

The pregnant women were consecutively recruited in five Danish hospitals when they arrived for delivery, and 140 pregnant women and their newborns were included in the study. Maternal serum was sampled on the day of arrival for delivery and cord serum immediately after birth. On day five after delivery, a spot urine sample and a breast milk sample were obtained from the mother and urine was collected from the newborn. Serum was analyzed for thiocyanate, cotinine, TSH, total T4, total T3, free T4, Tg, and Tg-Ab. Urine was analyzed for iodine and cotinine, and breast milk was analyzed for iodine. The pregnant women were asked about intake of iodine-containing supplement at the time of inclusion and classified as smokers or non-smokers from cotinine in serum and urine which showed consistent results with high or low cotinine concentrations in mother-child pairs [19]. The possible influence of thiocyanate not originating from tobacco smoking has previously been evaluated and was not apparent [19]. Passive smoking has not been associated with higher thiocyanate levels in pregnant women and newborns [110].

3.2.3. STUDY RESULTS

To examine our hypothesis, we analyzed maternal and cord serum Tg stratified in four groups by maternal iodine supplement intake and smoking status. Mean maternal and cord serum Tg were higher when the mother did not use iodine-containing supplements. Mean maternal and cord serum Tg were also higher when the mother was smoking, but the mean Tg ratio 'cord serum/maternal serum' was similar in the smoking and non-smoking group. Thus, maternal smoking increased the risk of iodine deficiency in pregnant women and their newborns, but serum Tg increased to a similar degree in mother and child.

3.2.4. STUDY DISCUSSION

The study aimed to examine the regulation of human placental iodide transport in a clinical setting. Following our hypothesis, study results were compatible with autoregulation of NIS-mediated iodide transport in placenta since the degree of iodine deficiency caused by maternal smoking was similar in the mother and the fetus. However, our hypothesis was based upon underlying assumptions and evidence for or against our hypothesis should be considered. Hill's viewpoints of causation (Table 3-2) are mainly used in the evaluation of causality in 'traditional' epidemiological studies, but they are also applicable in more general [111].
We investigated the association between maternal smoking in pregnancy and iodine deficiency in the mother and the fetus as evaluated by serum Tg in maternal and cord serum. The assumptions were that a) NIS is present in placenta, b) NIS mediates placental iodide transport, and c) NIS is inhibited by thiocyanate from maternal smoking. Considering *strength, specificity, and temporality*, it is well-established that thiocyanate is a competitive inhibitor of NIS in the thyroid gland

[22], and population studies have shown that smoking increases the risk of goiter with the strongest association in areas with the most pronounced iodine deficiency [112,113]. NIS is expressed in the fetal thyroid gland [114], and the concentration of thiocyanate was similar in maternal and cord serum suggesting that thiocyanate reaches and crosses the placenta [19]. NIS expression in the human placenta has been demonstrated in experimental studies [104-109], and dose-dependent inhibition of NIS-mediated placental iodide transport by both thiocyanate and perchlorate has been shown *(biological gradient)* [25,88,109].

Hill's viewpoints	**Description**
Strength	Strong association between exposure and outcome
Specificity	Exposure associates with one specific outcome
Temporality	Outcome occurs after the exposure
Biological gradient	Dose-response between exposure and outcome
Experimental evidence	Similar findings in experimental studies
Consistency	Results repeatingly shown in different populations
Analogy	Alternative explanations
Plausibility	Plausible mechanism between exposure and outcome
Coherence	In line with the natural history of the outcome

Table 3-2. Hill's viewpoints of causation [111].

Experimental evidence from studies in rats and *in vitro* suggests that NIS-mediated placental iodide transport is autoregulated by iodide [32,33]. Several *in vitro* studies have investigated the regulatory role of the pregnancy-associated hormone human chorionic gonadotropin (hCG) in the thyroid gland [115] and in the placenta [33,109,116]. Results suggested that hCG increases iodide uptake in both tissues via increased expression of NIS, and the regulation of placental NIS by iodide might be via modulation of the hCG mediated NIS expression [33]. In studies of placental tissue samples, increasing expression of NIS was observed in first trimester samples [117], whereas no difference was observed between first trimester and term placenta [104,106]. However, *in vitro* both NIS and hCG expression were higher in cells obtained from first trimester than from term placenta [104].

Still, many details on the regulation of placental iodide transport remains to be elucidated. Other regulatory mechanisms have been proposed including the transcription factor Paired-Box Gene 8 (PAX-8) which is expressed both in the thyroid gland and in the placenta [118], the oxygen level in placenta [119], oxytocin [109], and maternal thyroxine-binding globulin (TBG) [108].

No previous studies have evaluated the hypothesis of autoregulation of placental iodide transport in a clinical setting and further clinical studies are needed to determine the *consistency* of our results. In a study from France [120], cord serum Tg was higher in smoking mothers who did not use iodine-containing supplements, but results were not presented so that the relative impact of maternal smoking on maternal and cord serum Tg could be determined.

Although many criteria are in favor of our hypothesis, the possibility of other alternative explanations (*analogy)* needs to be addressed. Our hypothesis was based on the assumption that placental iodide transport is mediated by NIS, but we cannot exclude that other iodide transporter not inhibited by thiocyanate may be involved in placental iodide transport e.g. the chloride/iodide transporter (Pendrin) [104], and the sodium-multivitamin transporter (SMVT) [121,122]. However, experimental studies of placental iodide transport have indicated kinetic and inhibitory characteristics compatible with a significant role of NIS [25,109].

The consequence of fetal iodine deficiency may be severe. Considering *plausibility and coherence*, it seems reasonable that the placental transport of iodide is autoregulated to protect the developing fetus against iodine deficiency. The clinical type of brain damage occurring in populations with a high intake of thiocyanate from cassava may support the hypothesis of placental autoregulation. In the Democratic Republic of Congo with a high frequency of endemic cretinism, the majority of cases had myxedematous cretinism [90]. This type of cretinism is thought to be caused by neonatal hypothyroidism. Thus, the autoregulation of placental iodide transport may have kept the iodine supply to the fetus sufficient by overcoming thiocyanate inhibition of NIS. On the other hand, no autoregulation of NIS-mediated iodide transport into breast milk is apparent, and the iodine supply to the breastfed infant may be seriously impaired by thiocyanate exposure [19].

CHAPTER 4. IODINE & PREGNANCY

Maternal iodine intake in pregnancy should cover not only the need of the pregnant woman but also the need of the developing fetus, and the consequences of inadequate maternal iodine intake may be severe. Pregnant women are at risk of being iodine deficient, and specific recommendations for this population subgroup have been established.

4.1. IODINE METABOLISM IN PREGNANCY

Pregnancy induces a number of physiological changes both in the thyroid gland and in renal function which result in an altered metabolism of iodine. The thyroid gland is challenged with an increasing demand of thyroid hormone synthesis in pregnancy [123]. TBG is the main thyroid hormone transport protein, and in early pregnancy elevated estrogen levels lead to an increase in circulating TBG and a concomitant increase in total serum T4 (Fig. 4-1). hCG is a glycoprotein increasingly secreted by the placenta in the first trimester of pregnancy (Fig. 4-1). hCG has stimulatory effect on the TSH-receptor with a concomitant increase in free T4 and decrease in TSH around the time of the hCG peak (Fig. 4-1).

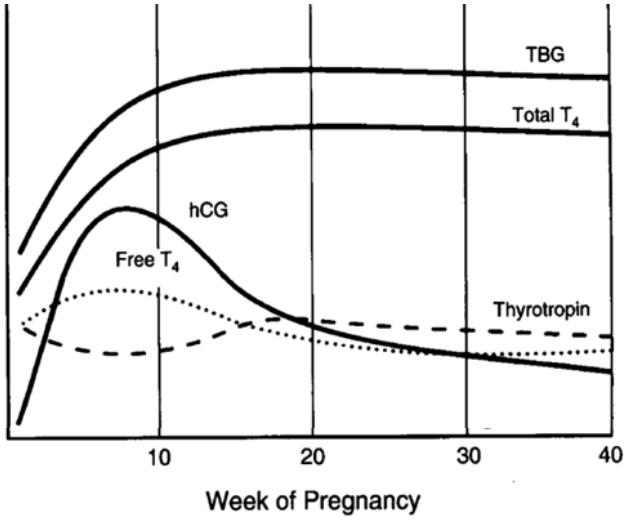

Fig. 4-1. Relative changes in maternal thyroid function in pregnancy. Reproduced from [123] *with permission, copyright Massachusetts Medical Society.*
TBG; Thyroxine-binding globulin, T4; tetraiodothyronine, hCG; human chorionic gonadotropin, Thyrotropin; thyroid stimulating hormone.

There is a need for transfer of thyroid hormones to the fetus and a change in the peripheral metabolism of maternal thyroid hormones in pregnancy [124]. Placenta contains D2 which catalyzes the conversion of T4 to T3, but it also contains abundant type 3 iodothyronine deiodinase (D3), which catalyzes the conversion of T4 to reverse-T3 and T3 to T2 [125]. D3 activity increases in early pregnancy and is higher than D2 activity throughout pregnancy [124].
Physiological changes occur in the kidneys in pregnancy [126]. There is an increase in the renal plasma flow which is 75% higher in midpregnancy [126] and an increase in the glomerular filtration rate (GFR) which is 50% higher than the non-pregnant state at the end of the first trimester and maintained high throughout pregnancy [127]. In terms of iodine metabolism this leads to an increased renal clearance of iodide and a lower PII concentration in pregnancy (Fig. 4-2) [128].

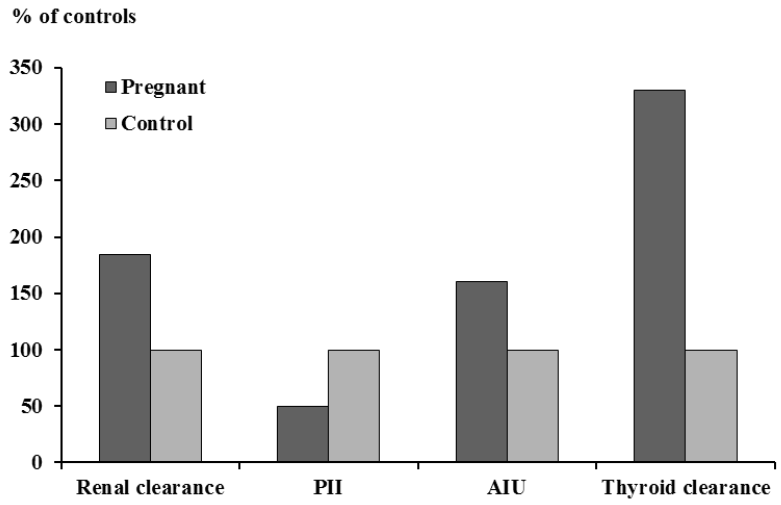

Fig. 4-2. Relative changes in maternal renal iodine clearance, plasma inorganic iodide (PII), thyroid absolute iodine uptake (AIU) and thyroid clearance of iodine in pregnant women vs. controls. Data from [128].

The increased thyroid hormone production in pregnancy can be approximated from the increase in Levothyroxine dose in pregnant women with known hypothyroidism prior to pregnancy [129]. In these women, the dose of Levothyroxine is increased by around 50%, corresponding to a 50% increase in the absolute iodine uptake (AIU) in the thyroid gland (Fig. 4-2). However, as illustrated in Fig. 4-2, the thyroid clearance of iodide is three times higher. Thus, the lower PII makes is more difficult for the thyroid gland to keep iodine uptake sufficient for thyroid hormone synthesis [128]. The normal thyroid gland is able to compensate for the increased demands, but if the function of the thyroid gland is impaired due to thyroid disease or if the iodine intake is insufficient, signs of thyroidal stress may develop.

It should be stressed that results presented in Fig. 4-2 were from a study performed in Scotland, where iodine status was inadequate [130]. The study included 13 pregnant women and 13 controls and used indirect measurement of PII after intravenous injection of radioiodine [128]. In a study of 16 pregnant women with more than adequate iodine intake and direct measurement of PII, no decrease in PII during the pregnancy was observed [131].

4.2. RECOMMENDATIONS IN PREGNANCY

4.2.1. DAILY IODINE INTAKE

Due to the physiological changes in pregnancy, the recommended daily iodine intake is higher than in non-pregnant adult (Table 4-1).

Authority	µg iodine/day
WHO, UNICEF, ICCIDD 2007 [3]	250
US Institute of Medicine 2001 [132]	220
European Food Safety Authority 2014 [133]	200
Nordic Nutrition Recommendations 2012 [134]	175

Table 4-1. Recommended daily iodine intake in pregnancy by different authorities.

All authorities listed agreed on a daily intake of 150 µg iodine/day in non-pregnant adults, whereas the estimate of the additional iodine requirements in pregnancy varied between authorities.

4.2.2. URINARY IODINE CONCENTRATION

The range of median UIC indicating sufficient iodine intake in a population of pregnant women is different from non-pregnant adults (Table 4-2). The median cut-off indicating adequate iodine intake in pregnant women is 150 µg/l, whereas in schoolchildren ≥ 6 years and non-pregnant adults it is 100 µg/l.

Median UIC (µg/l)	Iodine intake
< 150	Insufficient
150-249	Adequate
250-499	Above requirements
≥ 500	Excessive

Table 4-2. Assessment of population iodine status from median urinary iodine concentration (UIC) in pregnant women [3].

4.3. PREVIOUS DANISH STUDIES

Data on urinary iodine excretion in Danish pregnant women before the introduction of the Danish mandatory iodine fortification of salt have been reported in three investigations (Table 4-3) which all revealed that Danish pregnant women were iodine deficient.

Author, year of publication	City	n	GA	Median UI
Pedersen et al., 1988 [13]	Randers	20	34-38	52 µg/g creatinine[1]
Pedersen et al., 1993 [14]	Randers	54	17-18	53 µg/l
Nohr et al., 2000 [18]	Aalborg	66	~ 11	51 µg/l[2]

GA; gestational age
[1]Only reported as iodine/creatinine ratio.
[2]All women were thyroid peroxidase antibody positive. Subgroup with no iodine supplement intake prior to study inclusion (n=32) had median UIC: 46 µg/l.

Table 4-3. Previous data on median urinary iodine (UI) excretion in Danish pregnant women with no use of iodine-containing supplements in pregnancy.

Compared with non-pregnant controls in the same area, urinary iodine excretion was at the same level, but serum Tg was considerably higher in pregnant women [13]. The findings called for studies investigating the impact of iodine supplementation in pregnancy. In an intervention study by Pedersen et al. [14], 54 pregnant women were randomized in gestational week 17-18 to 200 µg iodine/day until 12 months postpartum (n=28) or controls (n=26). Serum TSH, serum Tg and thyroid size increased significantly during pregnancy in the control group, whereas these changes were ameliorated in the iodine supplemented group. As expected, UIC increased and was significantly higher during pregnancy in the iodine supplemented group (gestational week 37; median UIC iodine supplemented group: 108 µg/l vs. controls: 40 µg/l). Similar increase in UIC after iodine supplementation in pregnancy was observed in the study by Nohr et al. [18] (gestational week 35; median UIC iodine supplemented group: 105 µg/l vs. controls: 53 µg/l).
Considering the use of iodine-containing supplements in pregnancy, an investigation in five cities in Denmark [15] in the years 1988-1990 showed that approximately one third of Danish pregnant women used iodine-containing supplements when asked upon arrival for delivery (range 20-50%). One of the concerns about iodine supplementation in pregnancy is the aggravation of thyroid autoimmunity and development of postpartum thyroid dysfunction (PPTD). This was investigated in a Danish study by Nohr et al. [18] in which 66 thyroid peroxidase antibody (TPO-Ab) positive women were recruited in gestational week 11 and randomized to 150 µg iodine supplementation in pregnancy (n=20), in pregnancy and postpartum (n=22) or no iodine supplementation (n=24). Altogether 55% of the women developed PPTD and there was no significant difference in the frequency or severity and duration of PPTD in the three groups.

4.4. IODINE STATUS IN DANISH PREGNANT WOMEN (PAPER 2)

The second paper in the PhD thesis addressed the iodine status in Danish pregnant women. Before the mandatory iodine fortification of salt, Danish pregnant women were iodine deficient with signs of thyroidal stress. The fortification of salt has improved the iodine status in the Danish population in general, but no investigation of iodine intake in Danish pregnant women specifically has been performed after the introduction of the mandatory iodine fortification of salt.

4.4.1. STUDY OBJECTIVE

The study objective was to investigate the use of iodine-containing supplements and urinary iodine status in Danish pregnant women living in an area of Denmark with previously moderate iodine deficiency. The study was a regional investigation in the part of Denmark with the lowest iodine content in drinking water and previously most severe iodine deficiency.

4.4.2. STUDY DESIGN

The study was a cross-sectional investigation. The pregnant women were recruited when they arrived for obstetric ultrasound at Aalborg University Hospital. Aalborg University Hospital is located in the North Denmark Region and the yearly number of births was 3,251 in 2012 corresponding to 63.7% of births in the North Denmark Region and 5.6% of births in Denmark [135]. In Denmark, pregnant women are offered routine obstetric ultrasound in gestational week 11-14 (estimation of the nuchal fold thickness as part of the trisomi 13, 18 and 21 risk assessment) and in gestational week 19-21 (screening for fetal malformations). The rate of participation is high and in 2012 it was above 90% for both examinations [136]. In addition to the two routine ultrasound examinations, ultrasound is performed on specific indication (e.g. suspicion of deviant fetal growth, placenta position, fetal head position, cervical length, and flow in the maternal-fetal circulation). We recruited pregnant women arriving for routine ultrasound examination around gestational week 12, 20 and pregnant women arriving for ultrasound around gestational week 30. All pregnant women recruited, had a scheduled time of ultrasound between 8.20 am and 11.30 am (50% of the ultrasounds scheduled in one day) which was the period when staff was available for inclusion.
Ten days prior to the start of a study inclusion week, a booking list of planned obstetric ultrasounds was retrieved from the Obstetric Department. From this list, pregnant women with scheduled ultrasound were selected for study inclusion. When multiple pregnancy or a need for translator was specified in the booking list, the women were not selected. When the number of women available for inclusion in one day was larger than staff could handle, selection was made by gestational

age, secondly by random. One week prior to the scheduled obstetric ultrasound, a letter was mailed to the pregnant women selected for inclusion. The letter included information about the study and the study questionnaire (Appendix A) which they were asked to complete and bring to the ultrasound examination together with any dietary supplement in current use. Upon arrival in the Obstetric Department, the pregnant women willing to participate delivered the questionnaire and were asked to make a spot urine sample. The questionnaire was reviewed and information on intake of iodine-containing supplements including time of most recent iodine supplement intake prior to the urine sampling was obtained by interview.

The questions in the questionnaire were adapted from the questions used in the population-based Danish investigation on iodine intake and thyroid disease (DanThyr). The DanThyr study group adapted the questionnaires from the 'Glostrup Population Studies' [137] and the 'MONICA studies' [138] and self-constructed the questions concerning thyroid disease, as previously described [62]. Smoking questions from the MONICA study have been validated by the MONICA study group and questions concerning thyroid disease were validated by the DanThyr study group [62]. The questions about dietary habits were collected for future studies and not included in this PhD thesis.

Obstetric data (the Astraia database) which are registered at the first pregnancy visit in general practice or at the time of obstetric ultrasound were obtained for each participant including ethnicity, ultrasound determined gestational age, pre-pregnancy height and weight, smoking status, and parity.

Data registration was performed manually in SPSS by two of the investigators using the same data registration protocol. Double data entry of the questionnaire (Appendix A) was performed in a 5% sub sample (1,020 data fields) and revealed a high agreement between the two individuals (99.9%).

The iodine laboratory was certified by the U.S. Centers for Disease Control and Prevention EQUIP program, which includes measurement of external controls three times a year. UIC was determined by the cerium/arsenite method after alkaline ashing as described by Wilson van Zyl in 1967 and modified as previously described [139]. After thawing and brief mixing of the samples, alkaline ashing to combust organic material is followed by evaporation to dryness after which the samples are resuspended in water for iodine measurement. A standard iodine solution with a known concentration of iodine is used to make the standard curve which plots different iodine concentrations against the absorbance determined by spectrophotometry using the reagents cerium and arsenite. For analyses of the samples, arsenite and then cerium are added and the absorbance is determined after an exact time interval. Using the standard curve, the iodine concentration corresponding to the measured absorbance can be determined. The method has been evaluated as described in the method section of paper 2 and 3.

4.4.3. STUDY RESULTS

Altogether 340 pregnant women were informed about the study by mail prior to obstetric ultrasound, 245 pregnant women gave informed consent to participate upon arrival in the Obstetric Department, and 238 women delivered a spot urine sample. The frequency of self-reported iodine supplement use at the time of inclusion was 84.1%. Overall, median UIC was 101 µg/l and stratified by iodine supplement intake, median UIC was considerably lower in the group of pregnant women with no intake of iodine-containing supplements (68 µg/l vs. 109 µg/l in iodine supplement users). Maternal education qualifying for a profession and lower maternal age were predictors of iodine supplement use in multivariate analysis.

In paper 2, the pregnant women were grouped according to the type of obstetric ultrasound they attended (gestational week 10-15, 19-21 and 28-37). Another way to examine gestational age is to look at trimesters of pregnancy. In Denmark, the first trimester of pregnancy is defined as the first 12 weeks of pregnancy calculated from the first day of the last menstrual period; second trimester as the 13th to the 28th week and third trimester as the remaining pregnancy period [140]. Gestational age by trimester was not a significant predictor of iodine supplement use (Table 4-4). When results of urinary iodine evaluation were stratified by trimester, both median UIC and urinary creatinine concentration were higher in third trimester (Table 4-4), similarly in multivariate linear regression including other maternal predictors (age, education, iodine supplement use etc.). However, when urinary creatinine concentration was used to calculate estimated 24-hour urinary iodine excretion, no difference by trimester was observed (Table 4-4).

	Trimester of pregnancy			
	1st	2nd	3rd	p
Gestational week (range)	10-12	13-28	29-37	
Pregnant women (n)	47	167	24	
Iodine supplement use (%)	85.1	81.9	92.0	0.5
Urinary iodine concentration (µg/l)	103	94	140	0.01
Urinary creatinine concentration (mmol/l)	6.5	6.1	10.4	0.01
Estimated 24-hour urinary iodine excretion (µg/24 hours)	146	154	147	0.96

Table 4-4. Median urinary iodine concentration, urinary creatinine concentration and estimated 24-hour urinary iodine excretion in Danish pregnant women stratified by trimesters of pregnancy.

4.4.4. STUDY DISCUSSION

Danish pregnant women living in an area of Denmark with previously moderate iodine deficiency were still iodine deficient after the introduction of the mandatory iodine fortification of salt. Both iodine supplement users and non-users had median UIC below the range recommended in pregnancy. Iodine supplement non-users had mild iodine deficiency, whereas when iodine-containing supplements were used, the median UIC was within the range recommended in non-pregnant adults.

The use of iodine-containing supplements in pregnancy had considerably increased compared with a previous study. In our study population, ~85% used iodine-containing supplements whereas previously ~35% were iodine supplement users when they arrived for delivery [15]. In Denmark, no official recommendations for iodine supplement intake in pregnancy exist, but there are recommendations for intake of folic acid, vitamin D and iron which are often combined in a multivitamin pill [11]. The pregnant women all obtained iodine in a multivitamin pill, and the majority of iodine supplement non-users used dietary supplements, but these supplements did not contain iodine either because it was a single vitamin and/or mineral or because it was a multivitamin pill not containing iodine.

For the assessment of predictors of iodine supplement intake our study was limited by sample size. The groups were small in the stratified analyses, and results of the multivariate analysis were subject to some uncertainty. Only sparse data are available on predictors of iodine supplement in pregnancy. In a study from Australia, the main predictors of iodine supplement use in pregnancy were general dietary supplement use and knowledge of the importance of iodine [141]. In the general population, a Danish study found that higher educational level versus primary school only was a significant predictor of iodine supplement use [142], in line with our findings for iodine supplement use in pregnancy. Also Danish studies of preconceptional folic acid and multivitamin use [143] and iron supplement use in pregnancy [144], reported higher educational level as a significant predictor. Lower maternal age was the other independent predictor of iodine supplement use in our study. For the use of dietary supplements in the Danish population [142], in Danish pregnancy planners [143] and for the use of iron in Danish pregnant women [144], higher maternal age was a significant predictor. However, it can be speculated if pregnant women with higher age and parity are less focused on following recommendations in pregnancy than nulliparous, younger women. Larger studies are needed to clarify predictors and possible interactions in detail.

Dietary factors may influence iodine status of pregnant women and depending on the main dietary sources of iodine in a population; women with specific dietary habits may be more vulnerable to a low iodine intake [145,146]. The dietary data collected will be investigated in future studies. The habit of buying organic milk was associated with higher maternal age and higher educational level, but was not an independent predictor of iodine supplement intake. The incentive to include this variable was studies reporting a lower iodine content of organic milk [46,147]. Future studies including dietary data should investigate this aspect.

Internal validity is the extent to which results are correctly collected and analyzed within the study population. Concerning information bias, the data collection was performed using a questionnaire in which the majority of questions have previously been validated. The information letter was mailed to the pregnant woman one week prior to study inclusion and information was kept low grade to avoid influencing iodine supplement use prior to study inclusion. The intake of iodine-containing supplements was confirmed by interview at the time of study inclusion. We cannot exclude that misclassification of iodine supplement intake or other variables occurred, however, such misclassification is most likely to be non-differential. The urine samples were kept separately in the Obstetric Department and clearly marked to avoid the use of urine test strips which can be a source of iodine contamination [148]. The iodine laboratory is certified with several yearly external blind controls and urine samples were randomly measured including internal controls.
Concerning selection bias, the rate of participation among women invited was high, but we cannot exclude that participants might have differed from non-participants. To elaborate on this, we obtained permission from the Danish Health and Medicine Authority to view the medical records of a random sample of pregnant women scheduled for obstetric ultrasound in the same hospital the following year. Maternal characteristics were compared with the pregnant women included in our study (Table 4-5). There was no significant difference in gestational age, obstetric consultation secondary to obstetric ultrasound, pre-pregnancy BMI, smoking, and area of living (the city of Aalborg versus outside of Aalborg). Significant differences were observed in maternal age, parity and ethnicity (Table 4-5).

	Study participants		**Random sample**		**p[1]**
	n = 245		n = 107		
Maternal age, years	**n**	**%**	**n**	**%**	
< 25	27	11.0	12	11.2	0.034
25-35	180	73.5	66	61.7	
> 35	38	15.5	29	27.1	
Parity[2]	**n**	**%**	**n**	**%**	
1	130	53.1	43	41.0	0.022
2	90	36.7	41	39.0	
≥ 3	25	10.2	21	20.0	
Ethnicity	**n**	**%**	**n**	**%**	
Caucasian	242	98.8	97	90.6	< 0.001
Non-Caucasian	3	1.2	10	9.4	

[1]Result of the Chi-square test: study participants vs. random sample.
[2]Previous still- and live births including index pregnancy. Missing values (n=2) not included.

Table 4-5. Maternal characteristics which were significantly different between participants in our investigation (2012) and a random sample of pregnant women arriving for obstetric ultrasound at Aalborg University Hospital in the same period the following year (2013).

The pregnant women included in our study tended to be younger and were more often expecting their first child. As expected, pregnant women with another ethnicity than Danish were underrepresented, because the questionnaire was in Danish and was not mailed to the woman if the booking list indicated a need for translator. The random sample included pregnant women arriving for obstetric ultrasound before (n=74) and after (n=33) noon. In such comparison, women living in the city of Aalborg tended to be overrepresented before noon.

It was a priori decided to exclude women treated for thyroid disease. The hyper- and hypothyroid state influences iodine metabolism and Levothyroxine contains 65% iodine [52]. We excluded women with gastrointestinal disease including gastric bypass, although a recent study showed that iodine absorption was not influenced by bariatric surgery [149].

External validity is the extent to which results are applicable to a larger study population than the one examined. We performed a regional investigation and we cannot exclude that the iodine status of Danish pregnant women in the Eastern part of Denmark with a higher content of iodine in drinking water is different. Concerning the use of iodine-containing supplements, the previous national investigation of pregnant women in five cities in Denmark did not report large geographical discrepancies [15]; neither did the investigation of the general Danish population in East and West Denmark [20].

UIC shows a wide variation and a large number of spot urine samples are required for precise estimation of the median UIC in a population [65]. The total number of pregnant women included in our study was appropriate; however, in the stratified analyses, the numbers tended to be small.

It has been discussed if gestation-specific reference intervals for UIC are needed. To elaborate on this, we performed stratified analyses of urinary measurements by trimester in our study population (Table 4-4), and we identified other studies reporting median value of UIC in each of the three trimesters (Table 4-6).

No consistent pattern in median UIC by trimester of pregnancy was observed when comparing different studies (Table 4-6). In our Danish study (Table 4-4), median UIC and urinary creatinine concentration varied in parallel between trimester of pregnancy and estimated 24-hour urinary iodide excretion did not differ between trimesters. Only the studies from Japan in Table 4-6 [150,151] reported creatinine adjusted measurements (urinary iodine/creatinine ratio) which were either lower in the first trimester compared with later trimesters or similar in the three trimesters.

Considering what determines UIC in pregnancy, there is some iodine retention due to the increased maternal T4 pool (TBG increase, tissue expansion and transfer of T4 to the fetus) and the transfer of iodide to the fetus, but it can be calculated that this represents only a few µg iodine per day. The renal clearance of iodide is increased from early pregnancy, but this is followed by a lower PII concentration and a new steady state is expected. Thus, the main determinants of UIC in pregnancy appear to be the dietary iodine intake and the fluid intake, and the diverse pattern of median UIC by trimesters of pregnancy (Table 4-6) may reflect differences in iodine intake and/or fluid intake during the pregnancy.

First author	Year	Country	n	Median UIC (µg/l)			p[1]
				1st[2]	2nd[2]	3rd[2]	
Cross-sectional data							
Caldwell [152]	2013	United States	176	109	128	172	< 0.05
Fuse [151]	2013	Japan	563	227	259	205	ns
Raverot[3] [153]	2012	France	100	69	91	91	< 0.05
Pettigrew [154]	2011	New Zealand	170	41	39	37	ns
Garcia-Solis [155]	2011	Mexico	294	273	285	231	ns
Andersson [156]	2010	Switzerland	648	116	166	156	ns
Rezvanian [157]	2002	Iran	90	206	233	184	ns
Smyth[3] [158]	1997	Ireland	115	135	122	122	< 0.05[4]
Longitudinal data							
Fuse [151]	2013	Japan	65	216	136	148	< 0.05
Ainy[3] [159]	2007	Iran	298	193	159	141	< 0.05
Smyth [160]	2005	Sri Lanka	19	194	104	74	ns[4]
Kung [161]	2000	Hong Kong	230	107	115	124	< 0.05
Smyth[3] [158]	1997	Ireland	38	158	122	115	< 0.05[4]
Pedersen[3] [14]	1993	Denmark	26	51	42	40	ns
Mixed cross-sectional and longitudinal data							
Fuse [150]	2011	Japan	683	221	208	193	ns
Stillwell [162]	2008	Tasmania	431	109	64	74	< 0.05

[1]Result of the comparison by trimesters.
[2]Trimesters of pregnancy (1st, 2nd, 3rd).
[3]Specified in the study that the participants did not use iodine-containing supplements.
[4]Result of the comparison to non-pregnant controls.

Table 4-6. Median urinary iodine concentration (UIC) reported in different studies stratified by trimester of pregnancy (1st, 2nd, 3rd).

4.5. CHALLENGES IN EVALUATION OF IODINE STATUS IN PREGNANCY (PAPER 3)

The third paper in the PhD thesis addressed challenges in the evaluation of iodine status in pregnancy. More specifically, it focused on factors that may influence urinary iodine status in a population of pregnant women when evaluated by spot urine samples. Certain aspects characterize studies in pregnant women compared with studies in non-pregnant population groups e.g. urine samples are often obtained during a routine hospital visit and iodine supplement use is often recommended.

4.5.1. STUDY OBJECTIVE

The main study of iodine status in Danish pregnant women was performed in a routine manner to strengthen the comparability with other studies. In the present study, we aimed to investigate if results of such evaluation would be different if spot urine sampling was instead performed at home or if time of most recent iodine supplement intake prior to spot urine sampling was considered. In addition to this we aimed to compare urinary iodine status of pregnant women with that of their household members when spot urine sampling was performed under similar conditions.

4.5.2. STUDY DESIGN

The study was supplementary to the investigation of iodine intake in Danish pregnant women, and data were collected in two ways (Table 4-7). For method 1, the male partner was recruited at the same time as the pregnant woman in the hospital. For method 2, vials for sampling were brought home and the pregnant woman and members of the household made a non-fasting spot urine sample at home as close in time as possible. Thus, pregnant women participating with the household at home (method 2) delivered two urine samples; one in the hospital at inclusion and one another day at home (Table 4-7).

Method	Hospital urine sampling	At home urine sampling	Samples from each pregnant woman
1	Pregnant woman Male partner		n=1
2	Pregnant woman	Pregnant woman Household members	n=2

Table 4-7. Illustration of urine samples collected in the hospital and at home.

Urine samples from home were sent by mail immediately after collection. All male partners and children completed a questionnaire (Appendix B and C) constructed similar to the questionnaire for pregnant women. All participants were informed to note detailed information on iodine-containing supplements including time of most recent iodine supplement intake prior to spot urine sampling.

4.5.3. STUDY RESULTS

Individual comparison of the spot urine sample in the hospital and at home from 66 pregnant women showed that UIC and urinary creatinine concentration, but not 24-hour estimated urinary iodine excretion, were higher when sampling was at home. To further investigate these findings, we looked into the time of sampling in a *posthoc* analysis. Samples in the hospital were obtained before noon, whereas at home the majority of women had sampled in the afternoon/evening. When analyses were stratified by time of sampling at home before/after 5 pm, UIC was higher at home only when sampling at home was at or after 5 pm. The time span from most recent iodine supplement intake to spot urine sampling influenced UIC with the highest median value when iodine supplement intake was the same day. Urinary iodine status in the pregnant women versus male partners and children was ascertained by looking at the median UIC and by individual comparison between household members. Median UIC was not significantly different between pregnant women, male partners and children (Table 4-8).

	All		**Iodine supplement**		**No iodine supplement**	
	n	Median UIC (µg/l)	n	Median UIC (µg/l)	n	Median UIC (µg/l)
Pregnant women	68	134	59	136	9	98
Male partners	67	115	10	136	57	110
Children	51	126	13	151	38	121

Table 4-8. Median urinary iodine concentration (UIC) in pregnant women, male partners and children sampling at home. Pregnant women vs. male partners vs. children (Kruskal-Wallis test): all (p=0.1), iodine supplement (p=0.5), no iodine supplement (p=0.4). Results were similar when analyses were restricted to households with participation from the pregnant woman, the male partner and 1-3 children.

The use of iodine-containing supplements was much more frequent in pregnant women than in male partners and children (Table 4-8). Thus, in the majority of the female-male couples, only the pregnant woman used iodine-containing supplement. In this group, UIC was higher in the pregnant women than in the male partner, but in the groups were both or none used iodine-containing supplements no difference in UIC was observed.

4.5.4. STUDY DISCUSSION

The study was a pilot investigation designed to examine challenges in the evaluation of urinary iodine status in pregnancy. The sample size was limited and results should be corroborated in other studies, but the study poses a number of important challenges which optimally should be considered and reported in the evaluation of urinary iodine status in pregnancy.

The intention to include urine samples both during routine hospital visit and at home emerged from the examination of studies evaluating urinary iodine status in pregnancy. In the vast majority of studies, pregnant women were recruited during a routine hospital visit. Our investigation had several aims, and consequently the pregnant women were asked to sample urine at home at the same time as the other household members. Time of home sampling was not pre-specified to increase the rate of participation (except that it should be non-fasting). We were not able to distinguish between the role of sampling location and time of sampling in the present investigation. In our *posthoc* analyses it appeared that time of sampling at home was often in the afternoon or evening whereas sampling in the hospital was always scheduled before noon. We observed that UIC was influenced by time of sampling in line with some [64,163] and in contrast to other studies [164]. To evaluate if sampling location influences UIC, a study needs to be designed in which the sampling time is similar in the two locations and the timing of food and drink intake in relation to urine sampling should preferable be specified.

When urinary creatinine concentration was used to calculate estimated 24-hour iodine excretion, the time dependent difference in urinary iodine excretion disappeared. Hydration status can be a confounder in the examination of UIC [165]. We observed that both UIC and urinary creatinine concentration were higher when sampling was performed later in the day. To calculate the estimated 24-hour urinary iodine excretion, the urinary iodine/creatinine ratio is multiplied by a measure of 24-hour urinary creatinine excretion to account for age and gender specific values of urinary creatinine excretion. As specified in the method section of paper 3, we used previous measures of 24-hour urinary creatinine excretion in Danish pregnant women, Belgian men and German children. Another approach is to use the formula developed for the general Danish population with an individual estimation based on age, height and weight [166]. Using this formula for the male partners, the mean 24-hour urinary creatinine excretion was 1.62 g creatinine/24 hours and not considerably different from the value applied (1.74 g creatinine/24 hours [167]).

The investigation was designed to include as many non-pregnant household members as possible. Thus, vials were provided for sampling at home mainly if the couple already had children at home, otherwise the male partner participated in the hospital. Due to this design, pregnant women sampling both in the hospital and at home tended to be older with a higher parity, but gestational week at inclusion, educational level and use of iodine-containing supplements was not different from women participating in the hospital only.

The intention to include information on time of most recent iodine supplement intake prior to spot urine sampling emerged from the association between iodine intake and urinary iodine excretion (Fig. 2-5). Examination of studies evaluating urinary iodine status in pregnancy revealed that use of iodine-containing supplements is often recommended, but information on time of most recent iodine supplement intake prior to urine sampling often not included. We obtained information in four categories; the same day, the day before, several days ago or non-user. The stratification made the groups rather small, and we had to collapse the categories 'several days ago/non-user'. Despite these limitations, results suggest that the time span from iodine supplement intake to spot urine sampling should be considered, but larger studies are needed to corroborate results.

The intention to include urine samples from non-pregnant members of the household emerged from the discussion on whether iodine status of pregnant women can be evaluated from data on non-pregnant population groups. Wong et al. [39] examined 48 surveys with median UIC of pregnant women and schoolchildren and 26 surveys with median UIC of pregnant and non-pregnant women. The authors showed that when median UIC of schoolchildren or non-pregnant adults indicated adequate iodine intake, pregnant women had inadequate iodine intake in approximately half of the surveys. In the majority of surveys [39], data were obtained from the World Health Organization Vitamin and Mineral Nutrition Information System and no details on time and location of urine sampling were reported. Four surveys were identified by literature review [39] including a household study from Thailand [168] which has been followed by a study in India using the same design [169]. In these studies [168,169], median UIC was higher in the children than in the pregnant women, whereas in our Danish study, median UIC of the pregnant women was not significantly different from that of the children. In the study from India [169], the pregnant women and children shared all meals. We cannot exclude dietary differences among household members in our Danish study, but all household members were instructed to perform spot urine sampling at home at the same time. Urine sampling conditions were not controlled in the studies from Thailand [168] and India [169], and it can be speculated if differences in time and location of spot urine sampling could explain part of the disparity in median UIC observed between pregnant women and children.

We observed a more frequent use of iodine-containing supplements in the pregnant women than in members of their household. The use of iodine-containing supplements was self-reported, and in general urinary iodine excretion was higher when iodine supplement use was reported. However, the impact of iodine supplementation on median UIC appeared less pronounced in the male partners, and we cannot exclude discrepancies in the self-reported iodine supplement use.

Further studies are needed to clarify the relationship between urinary iodine status of pregnant women and that of non-pregnant population groups, and studies performed in different populations with different dietary habits and iodine status are needed. Optimally, both time of spot urine sampling, intake of iodine-containing supplements and dietary patterns should be considered in the study design.

CHAPTER 5. IODINE & BREASTFEEDING

Maternal iodine intake during breastfeeding should cover not only the need of the mother, but also the need of the breastfed infant, and the consequences of inadequate maternal iodine intake may be severe. Breastfeeding women are at risk of being iodine deficient, and specific recommendations for this population subgroup have been established.

5.1. IODINE METABOLISM DURING BREASTFEEDING

Breastfeeding is associated with changes in maternal iodine metabolism (Fig. 5-1) [170]. During breastfeeding, around 40-45% of ingested iodine is excreted into breast milk (Fig. 5-1) and consequently < 90% of ingested iodine is excreted in the urine. NIS is expressed in the lactating mammary gland and mediates the transport of iodide into breast milk [23]. The transport is inhibited by thiocyanate from maternal smoking, but in contrast to transport of iodide in the thyroid gland and the placenta, the transport of iodide into breast milk is not autoregulated [19].

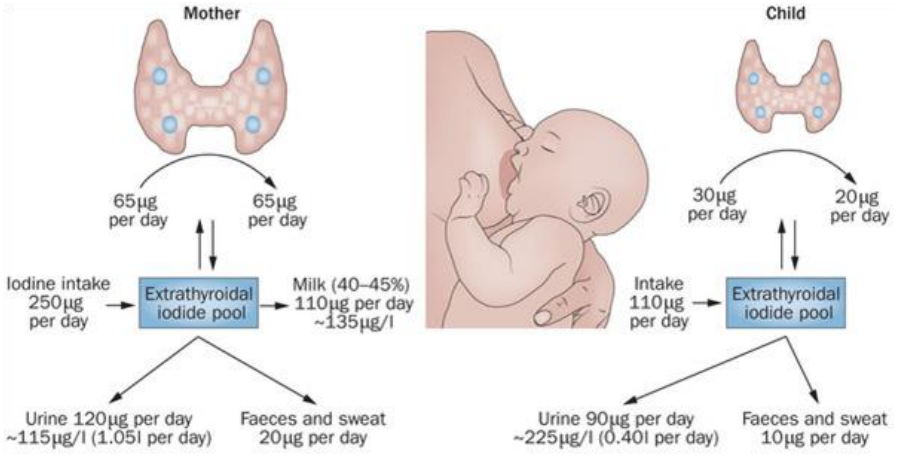

Fig. 5-1. Iodine metabolism during breastfeeding. Reproduced from [170] *with permission.*

5.2. RECOMMENDATIONS DURING BREASTFEEDING

5.2.1. DAILY IODINE INTAKE

Different authorities have made recommendations for daily iodine intake in breastfeeding women and children < 2 years (Table 5-1). For breastfeeding women, the recommendation from WHO, UNICEF, ICCIDD and the European Food Safety Authority are similar to the recommendations in pregnancy. For children < 2 years, some authorities divide their recommendations into shorter age intervals taking into account changes in body weight and urine volume during the first years of life.

	Breastfeeding mother	**Child < 2 years**
Authority	µg/day	µg /day
WHO, UNICEF, ICCIDD 2007 [3]	250	90
US Institute of Medicine 2001 [132]	290	90-130[1]
European Food Safety Authority 2014 [133]	200	70-90[2]
Nordic Nutrition Recommendations 2012 [134]	200	50-70

[1]0-6 months: 110 µg/day, 7-12 months: 130 µg/day, 1-3 years: 90 µg/day.
[2]7-11 months: 70 µg/day, 1-3 years: 90 µg/day.

Table 5-1. Recommended daily iodine intake by different authorities in breastfeeding women and children < 2 years.

5.2.2. URINARY IODINE CONCENTRATION

In breastfeeding women and children < 2 years, the recommendations for median UIC are similar to the recommendations for non-pregnant adults and children ≥ 6 years (Table 5-2). Iodine requirements are increased during breastfeeding, but the fraction of ingested iodide excreted in the urine is lower due to the transport of iodide into breast milk. To ensure an adequate supply of iodine to the exclusively breastfed infant of 90 µg/day it can be estimated that MIC should be ≥ 110 µg/l assuming an average breast milk volume of 0.8 l/day [171]. When the infant is not or only partly breastfed, iodine is obtained via infant formulas.

	Breastfeeding women	**Children < 2 years**
Median UIC (µg/l)	Iodine intake	Iodine intake
< 100	Insufficient	Insufficient
≥ 100	Adequate	Adequate

Table 5-2. Assessment of population iodine status from median urinary iodine concentration (UIC) in breastfeeding women and children < 2 years old [3] .

5.3. PREVIOUS DANISH STUDIES

Iodine status in Danish breastfeeding women and their newborns has been evaluated before the introduction of the mandatory iodine fortification of salt (Table 5-3 and 5-4). Median values were in general low and below the level recommended, but higher in iodine supplement users.

Author, year of publication	Sampling	Median UIC	
	Time after delivery	+ Iodine	No iodine
Pedersen et al., 1993 [14]	1 week	45 µg/l	30 µg/l
	6 months	115 µg/l	55 µg/l
	10 months	85 µg/l	45 µg/l
Nohr et al., 1993 [15]	5 days	58 µg/l	35 µg/l
Nohr et al., 2000 [18]	7 months	75 µg/l	43 µg/l

Table 5-3. Data on median urinary iodine concentration (UIC) in Danish breastfeeding women stratified by iodine supplement intake.

Author, year of publication	Median MIC		Median UIC child	
	+ Iodine	No iodine	+ Iodine	No iodine
Pedersen et al., 1993 [14]	41 µg/l	28 µg/l	64 µg/l	27 µg/l
Nohr et al., 1993 [16]	57 µg/l	34 µg/l	61 µg/l	32 µg/l

Table 5-4. Data on median breast milk iodine concentration (MIC) in Danish breastfeeding women and median urinary iodine concentration (UIC) in their breastfed infants stratified by iodine supplement intake. Samples were obtained five days after delivery.

Data on iodine content of infant formulas in Denmark are available from one previous investigation in which the median iodine concentration was 57 µg/l [8].

5.4. IODINE STATUS IN DANISH BREASTFEEDING WOMEN (PAPER 4)

The first part of the fourth paper in the PhD thesis addressed iodine status in Danish breastfeeding women. Danish breastfeeding women and their newborns were iodine deficient before the mandatory iodine fortification of salt, but no specific investigation of iodine intake in Danish breastfeeding women has been performed after the introduction of the mandatory iodine fortification of salt.

5.4.1. STUDY OBJECTIVE

The study objective was to investigate the use of iodine-containing supplements and urinary iodine status in Danish breastfeeding women living in an area of Denmark with previously moderate iodine deficiency.

5.4.2. STUDY DESIGN

The study was a follow-up investigation of the women initially recruited in pregnancy. Participants who had given birth to a live-born child were contacted by phone in the postpartum period and a telephone interview was performed about intake of iodine-containing supplements, breastfeeding, and smoking. The women were asked to deliver a spot urine sample and a breast milk sample and/or a sample of prepared infant formula to the hospital. They were instructed to make the urine and breast milk sample non-fasting as close in time as possible. MIC was measured using the same method as for UIC and the analysis of MIC was evaluated as described in the method section of paper 4.

5.4.3. STUDY RESULTS

Altogether 209 women participated in a telephone interview postpartum, 183 of the women were partly or exclusively breastfeeding their child and 127 breastfeeding women delivered a spot urine and a breast milk sample. The frequency of iodine supplement use was 47% in the entire group and in the subgroup of breastfeeding women. Median maternal UIC was below the recommended range, and although higher in iodine supplement users, it was also below recommendations in this group. Median MIC was below the recommended range, but higher in iodine supplement users, where the median value was just within the recommendations. Median iodine concentration of infant formulas was 122 µg/l (range 62-167 µg/l).

5.4.4. STUDY DISCUSSION

In a regional investigation, Danish breastfeeding women were still iodine deficient after the introduction of the mandatory iodine fortification of salt and the content of iodine in breast milk was below the level recommended. The use of iodine-containing supplements was less frequent during breastfeeding than in pregnancy and iodine deficiency was most severe in the ~50% of the mothers who did not use iodine-containing supplements during breastfeeding. No official recommendations for dietary supplements during breastfeeding exist in Denmark [12], whereas during pregnancy other recommendations often lead to the use of a multivitamin pill. One of the supplements was recommended by the manufacturer especially for breastfeeding women, but for reasons unknown, it contained only 45 µg iodine/day. The supplement was used by 7% of the women included in our study, and although

the group was small, results suggested that iodine intake was lower in this group compared with women using supplements that contained 150-175 µg iodine/day.
The follow-up design induced the possibility that the women had more knowledge about iodine, and that the frequency of iodine supplement use was higher than in the general population. However, the information about iodine and the consequences of iodine deficiency was kept very low grade when the women were investigated in pregnancy, and they were not at this point informed about the postpartum investigation, but they accepted a subsequent contact by phone. The interview was performed at median 22 days after birth (range 9-146 days). No significant difference in the use of iodine-containing supplements was observed by the time of interview, but data were cross-sectional and we cannot exclude that some women used iodine-containing supplements only partly during breastfeeding. Women are encouraged to breastfeed their newborn child, but due to different circumstances it may not always be possible [12]. The public focus on breastfeeding could create bias in the information obtained by interview, but such misclassification is expected to be non-differential. The frequencies of breastfeeding are difficult to compare, but results seem compatible with general Danish data on breastfeeding four months after birth where 60% are fully breastfeeding, 25% are partly breastfeeding and 15% are not breastfeeding [12].
In our study, breast milk samples were obtained from two weeks to five months after birth (90% within three months) and we did not observe significant changes in MIC with time from birth. For the first month variation, a higher content of iodine was found in colostrum decreasing to stable levels by 10 days postpartum [172]. During the first 6-month period of breastfeeding, both a decreasing trend [173,174] and stable levels have been reported [175].
The iodine laboratory was certified by the U.S. Centers for Disease Control and Prevention EQUIP program. The recovery of added iodine to breast milk was 93.6% (SEM 1.04%), which would underestimate MIC with ~6%. No correction for this disparity was made in the analyses.
Aalborg University Hospital covers obstetric ultrasound in a large geographical area in the North Denmark Region. In the postpartum investigation, the women sampled urine and breast milk at home and delivery of the samples to the hospital was mainly possible for women living relatively close to the hospital. Following this, the number of women participating in the telephone interview was high, but the number of women delivering samples was lower. The women who did not participate in the telephone interview tended to be younger, more often nulliparous and to have lower educational level, but the use of iodine-containing supplements in pregnancy was similar to the women included. Compared with women who participated in the telephone interview only, the women who participated in the interview and delivered samples tended to be older with higher educational level, but the frequency of iodine supplement use was similar. Since our postpartum investigation was a follow-up study of the pregnancy cohort, women with other ethnicity than Danish were underrepresented, and the investigation was regional implying that results may not be generalized to other regions of Denmark.

5.5. CHALLENGES IN EVALUATION OF IODINE STATUS DURING BREASTFEEDING (PAPER 4)

The second part of the fourth paper in the PhD thesis addressed challenges in the evaluation of iodine status during breastfeeding. As in pregnancy, certain aspects characterize studies of breastfeeding women which may challenge the interpretation of the results. One way to evaluate iodine status of newborns is to collect spot urine samples. It is, however, often difficult to obtain such urine samples from the newborns, and it has been considered whether maternal UIC could be used as a proxy for iodine supply to breastfed infants. But results have been inconclusive, and we speculated how maternal fluid intake would influence maternal UIC and MIC.

5.5.1. STUDY OBJECTIVE

The main study of iodine status in Danish breastfeeding women was performed in a routine manner to strengthen the comparability with other studies. In the present study, we aimed to investigate if results of such evaluation would be different if time of most recent iodine supplement intake prior to breast milk sampling and details on breast milk sampling were considered. We also evaluated how maternal fluid intake would influence maternal UIC and MIC as part of assessing whether iodine supply to exclusively breastfed infants can be evaluated from maternal UIC.

5.5.2. STUDY DESIGN

The study was part of the investigation of iodine status in Danish breastfeeding women. The 127 women who delivered a spot urine and a breast milk sample were asked to note detailed information on time of most recent iodine supplement intake prior to sampling and details about breast milk sampling (one or both breasts, before or after breastfeeding). A small group of women (n=13) was instructed to sample breast milk both before and after breastfeeding the child. Urinary creatinine concentration was measured and used as a proxy for maternal fluid intake.

5.5.3. STUDY RESULTS

The time span from most recent iodine supplement intake to breast milk sampling influenced MIC in a dose-dependent trend with the highest median MIC when iodine supplement intake was the same day prior to sampling. On the other hand, no significant trend in median UIC was observed ($p=0.072$). For the sampling of breast milk in relation to breastfeeding of the child, results were not consistent. In independent group-wise comparison, no difference in MIC was observed between sampling from one versus both breasts and between sampling before versus after breastfeeding. On the other hand, individual comparison of MIC in the subgroup of women who sampled breast milk both before and after breastfeeding suggested that

MIC was slightly higher in samples made before breastfeeding of the child. A strong correlation was observed between maternal UIC and urinary creatinine concentration, whereas maternal urinary creatinine concentration did not correlate with MIC. When urinary creatinine concentration was used to estimate 24-hour urinary iodine excretion, the correlation between maternal urinary iodine excretion and breast milk iodine excretion was stronger.

5.5.4. STUDY DISCUSSION

Results of the present investigation suggested that 40-45% of maternal ingested iodine is excreted into breast milk based on the mean ratio between 24-hour breast milk iodine excretion and maternal 24-hour urinary iodine excretion (Fig. 5-1). The transport of iodide into breast milk is mediated by NIS [23], and no autoregulation seems to take place. In smoking mothers, MIC was considerably lower than in non-smoking mothers compatible with thiocyanate mediated inhibition of NIS [19], and in a study from Korea where it is common to serve seaweed soup to new mothers, the iodine content of colostrum (2-5 days postpartum) was high [176].

We observed that breast milk iodine content was influenced by time of most recent iodine supplement intake with the highest median value when iodine supplement intake was the same day prior to sampling. In the group of women with iodine supplement intake the same day, the time span from iodine supplement intake to sampling ranged from 0.25 to 13 hours with a median of 3 hours, but no correlation between the time span in hours and MIC was observed. In a study by Leung et al. [41], acute intake of a high dose of iodine was associated with a rapid increase in MIC with peak within 6 hours. The observation period was 8 hours, and UIC remained stable during the period, in line with our finding that median MIC, but not maternal UIC was influenced by time of most recent iodine supplement intake prior to sampling. The groups were relatively small in our stratified analyses, and further studies are needed to corroborate results, but the findings encourage that details on iodine supplement intake during breastfeeding are collected and reported.

MIC varies within and among individuals [177] and it can be speculated if the time of sampling in relation to breastfeeding of the child could influence results. This aspect has previously been ascertained in studies of eight [178] and 30 [179] breastfeeding women in which no difference in iodine content of breast milk was observed before/after breastfeeding and sequentially during breastfeeding. However, data are limited, and our results were not conclusive (inter- versus intraindividual comparison). More data are needed considering the method of breast milk sampling for determination of iodine content.

A sufficient number of breast milk samples is often difficult to obtain, and it may be even more difficult to obtain a urine sample from the newborn for determination of UIC. Therefore, it has been discussed if maternal UIC can be used as a proxy for iodine supply to the breastfed infant, but reports are few and not consistent (Table 5-5). Our findings are in line with the study from Australia by Chan et al. [42] in which no correlation was observed between MIC and UIC, but a significant

correlation to MIC was observed when UIC was adjusted by the urinary creatinine concentration. Creatinine is excreted in the urine at a relatively constant rate, and urinary creatinine concentration can be used as a proxy for maternal fluid intake [44]. We observed a strong correlation between maternal urinary creatinine concentration and UIC. On the other hand, no correlation was observed between urinary creatinine concentration and MIC suggesting that maternal fluid intake does not influence MIC. The relationship between breast milk and fluid intake has mainly been ascertained to evaluate if an increase in fluid intake increases breast milk production which studies did not suggest [180,181].

First author	**Country**	Year	**n**	**r**	**p**
Correlation with urinary iodine concentration					
Ordookhani [182]	Iran	2007	42	0.43	0.004
Bazrafshan [183]	Iran	2005	100	0.44	< 0.001
Chan [42]	Australia	2003	49	0.19	0.2
Correlation with urinary iodine/creatinine ratio					
Chan [42]	Australia	2003	49	0.52	< 0.001
Johnson [173]	New Zealand	1990	93	0.44	< 0.01

Table 5-5. Previous studies reporting correlation between breast milk iodine concentration and maternal urinary iodine concentration or maternal urinary iodine/creatinine ratio.

We used urinary creatinine concentration to estimate 24-hour iodine excretion. Following this, the correlation with breast milk iodine content was stronger than for UIC alone. Hydration status can be a confounder when looking at UIC alone [165], and our results suggest that maternal estimated 24-hour iodine excretion could be a better proxy for iodine supply to the breastfed infant than UIC. It should be stressed that the calculation of estimated 24-hour iodine excretion is based upon assumptions, and the golden standard is to measure iodine excretion in a full 24-hour urine collection. This is, however, often not possible in a population study. We used the mean urinary creatinine excretion previously measured in a small group of Danish pregnant women (mean 1.09 g/24-hours) [13] to estimate 24-hour iodine excretion during breastfeeding. However, when we looked into the urinary iodine excretion measured in a Belgian population study [167] for women age 25-34 years, it was very similar to the one applied (1.22 g/24 hours) and from a study in New Zealand of selenium status during pregnancy and lactation, 24-hour urinary creatinine excretion could be estimated and was not significantly different between pregnancy and the postpartum period [184]. Correlation analysis is a useful method to examine the relationship between two continuous variables, but it should be noted that the correlation coefficient measures the extent of a linear relationship and that it can be influenced by outliers in the data.

CHAPTER 6. PERSPECTIVES

Iodine is an essential micronutrient for human health. The crucial role of thyroid hormones in early brain development emphasizes the importance of adequate iodine intake in pregnant and breastfeeding women to cover the need of the developing fetus and the breastfed infant. Meanwhile, pregnant and breastfeeding women constitute population subgroups that are vulnerable to iodine deficiency, and both iodine deficiency and iodine excess may interfere with the function of the thyroid gland. Adequate iodine intake in a population relies on a valid assessment of iodine status and efforts to ensure sufficient iodine intake e.g. programs of universal salt iodization and/or individual iodine supplementation.

6.1. NATIONAL PERSPECTIVE

The Danish mandatory iodine fortification of salt was introduced in the year 2000 and urinary iodine status in the Danish population had improved when evaluated in 2004-2005 (median UIC 101 µg/l) [9]. However, a small decrease was observed in the most recent investigation in 2008-2010 (median UIC 83 µg/l) suggesting that the current level of iodine fortification of salt in Denmark is not sufficient [20].
Our regional investigation of pregnant and breastfeeding Danish women supports a need for a modest increase in iodine added to salt in Denmark as both iodine supplement users and non-users had median UIC below the level recommended. Median UIC was higher in pregnant and breastfeeding women with a use of iodine-containing supplements, and the use of iodine-containing supplements should be officially recommended to pregnant and breastfeeding women in Denmark.
Our investigation was regional and to increase the external validity, data are now being collected from Danish pregnant women living in East Denmark. Our results were limited by sample size in some of the stratified analyses, and the larger combined study population will make it possible to explore predictors of iodine supplement intake and urinary iodine status in more detail and to analyze the dietary data. The supplementary data collection in East Denmark also aims to include a larger number of women of different ethnic origin.
One reason to be cautious about the level of iodine fortification is the risk of excess iodine intake in children. The number of children in our study was limited, and a Danish schoolchildren survey is needed. Such data would also add to the discussion on whether iodine status in non-pregnant subgroups can be used to evaluate iodine status in pregnant women. Another way to explore this in more details would be to compare the pregnant women randomly included in the Danish population studies (DanThyr) with non-pregnant women in the same age and region [10].

6.2. INTERNATIONAL PERSPECTIVE

Besides human efforts to ensure adequate iodine intake, endogenous mechanism are involved in iodine metabolism. In the thyroid gland, NIS mediated iodide transport is autoregulated to keep iodine uptake sufficient for thyroid hormone synthesis, and studies in rats, *in vitro* and our clinical data suggest that similar autoregulation may take place in the placenta. Such mechanism seems biologically plausible to protect the fetus from iodine deficiency during the period of early brain development, but further studies are needed to investigate details on the regulation of placental iodide transport and to clarify the exact role of NIS and possibly other iodide transporters.

A valid assessment of iodine status precedes human efforts to ensure adequate iodine intake and is also imperative in studies which aim to look at the long-term consequences of iodine deficiency in pregnancy for neurocognitive development of the child. Although the results of our pilot investigation are not conclusive, they pose a number of challenges in the evaluation of urinary iodine status in pregnant and breastfeeding women and encourage further studies to report and consider methodological details.

The adverse consequences of severe iodine deficiency during pregnancy and breastfeeding are well-established, and in such regions iodine supplementation is indisputable. The worldwide efforts primarily through implementation of programs of universal salt iodization have considerably decreased the prevalence of iodine deficiency and eradicated severe iodine deficiency. Thus, the dilemma in many populations today is whether iodine supplementation during pregnancy and breastfeeding is beneficial in mild-moderate iodine deficiency, and which quantity of iodine the supplement should contain to avoid excess iodine intake. One of the concerns is that iodine supplementation in pregnancy may aggravate thyroid autoimmunity, but no increased risk of PPTD was observed in a Danish randomized study [18]. Randomized studies showing positive effects of iodine supplementation to pregnant women with mild to moderate iodine deficiency on child development are lacking. It has been proposed that randomized controlled trials of iodine supplementation should be performed [185]. However, others have argued that such studies would be unethical because iodine supplementation is often recommended by authorities [40]. For iodine supplementation after birth of the child, the transport of iodine via breast milk is significant, and maternal iodine supplementation is preferable when breastfeeding is possible as this will benefit both the mother and the child [170,186].

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APPENDICES

Appendix A. Questionnaire for pregnant women

Appendix B. Questionnaire for household members ≥15 years

Appendix C. Questionnaire for household members <15 years

Appendix D. Paper 1

Appendix E. Paper 2

Appendix F. Paper 3

Appendix G. Paper 4

Appendix A. Questionnaire for pregnant women

Undersøgelse af jodindtag blandt gravide

Spørgeskema gravide

I det følgende vil vi stille dig en række spørgsmål af betydning for jodindtaget.

Alle besvarede spørgsmål vil blive behandlet fortroligt ifølge reglerne om tavshedspligt for hospitalsansatte.

De fleste spørgsmål besvares ved at sætte en cirkel om tallet ud for den svar mulighed, du selv synes er den mest rigtige. Enkelte spørgsmål besvares med ord eller tal på de markerede linjer.

Obs! Der er spørgsmål på begge sider.

A. Indledende oplysninger om dig

1. Din fødselsdato _____
2. Din højde _____ cm
3. Din aktuelle vægt _____ kg

B. De følgende spørgsmål omhandler sociale forhold

1. Hvor bor du? _____(postnummer, by)

   Har du altid boet i Jylland? 1. ja 2. nej

   Hvis nej, hvor mange år har du boet i Jylland _____ år

   Hvor boede du før _____

2. Hvor mange personer bor der i din husstand inkl. dig selv _____ (antal)

   Antal husstandsmedlemmer < 15 år _____ 15-18 år _____ > 18 år _____

3. Bor du sammen med en anden voksen?
   1. bor med ægtefælle/samlever
   2. bor alene
   3. andet _____

4. Hvad er din etniske baggrund?
   1. dansk
   2. anden etnisk baggrund end dansk,
   angiv hvilken _____

5. Er du født i Danmark? 1. ja 2. nej

   Hvis nej, hvor er du født _____

   Hvor mange år har du boet i Danmark _____år

C. De følgende spørgsmål omhandler graviditet og fødsel

1. Har du tidligere været gravid? 1. ja 2. nej
   Hvis ja, hvor mange gange tidligere? _____ gange
   Alder ved første graviditet _____ år

2. Har du tidligere født? 1. ja 2. nej
   Hvis ja, hvor mange gange? _____ gange
   Alder ved første barns fødsel _____ år

D. De følgende spørgsmål omhandler sygdomme i skjoldbruskkirtlen

1. Har din læge nogensinde fortalt dig, at du havde:
   for lavt stofskifte 1. ja 2. nej
   for højt stofskifte 1. ja 2. nej
   struma (forstørret skjoldbruskkirtel) 1. ja 2. nej
   knude i skjoldbruskkirtlen 1. ja 2. nej

2. Har du nogensinde været i behandling for:
   for lavt stofskifte 1. ja, nu 2. ja, tidligere 3. nej, aldrig
   for højt stofskifte 1. ja nu 2. ja, tidligere 3. nej, aldrig
   Hvis ja, skriv hvilken behandling og hvornår _____
   _____
   _____
   _____

E. De følgende spørgsmål omhandler tobak

1. Ryger du?
   1. ja, dagligt – gå videre til spørgsmål 3
   2. ja, lejlighedsvist (mindre end en cigaret, cerut, cigar eller et pibestop dagligt)
   3. nej

   Hvis du ikke ryger nu, har du røget tidligere?
   1. ja, dagligt
   2. ja, lejlighedsvist (mindre end en cigaret, cerut, cigar eller et pibestop dagligt)
   3. nej – fortsæt til spørgsmål 6

Hvilket år holdt du op med at ryge? _____ (årstal)

Hvis rygeophør inden for det seneste år, hvilken måned? _____ (måned)

2. Hvis du kun ryger/har røget lejlighedsvis, hvor ofte ryger/røg du? _____ dage ugentligt

3. Hvor meget ryger du i gennemsnit? _____ cigaretter dagligt
   Skriv gerne flere steder. _____ cerutter dagligt
   _____ cigarer dagligt
   _____ gram pibetobak ugentligt

4. Hvor gammel var du, da du begyndte at ryge dagligt? _____ år
5. Hvor mange år har du røget regelmæssigt? _____ år

6. Hvor mange timer om dagen plejer du at opholde dig i et rum, hvor der bliver røget?
   1. mere end 5 timer om dagen
   2. 1 til 5 timer om dagen
   3. ½ til 1 time om dagen
   4. stort set aldrig

F. De følgende spørgsmål omhandler arbejds- og uddannelsesforhold

1. Hvilken erhvervstilknytning har du?
   1. er i erhverv
   2. har været i erhverv
   3. har aldrig været i erhverv

2. Hvad er din hovedbeskæftigelse for tiden?
   1. selvstændig
   2. medhjælpende ægtefælle
   3. lønmodtager
   4. studerende/elev/lærling
   5. på bistandshjælp, kontanthjælp
   6. førtidspensionist
   7. hjemmegående husmor
   8. arbejdsløs på arbejdsløshedsdagpenge
   9. på sygedagpenge
   10. andet _____

3. Hvad er din stillingsbetegnelse _____

4. Hvor mange år har du gået i skole?
   1. Folkeskole eller tilsvarende (7-10 års skolegang)
   2. Gymnasial uddannelse: gymnasium, HF og lignende (10-12 års skolegang)

5. Har du eller er du i gang med en erhvervs- eller videregående uddannelse? 1. ja, har gennemført
2. ja, er i gang med en
3. nej

Hvis ja, hvilken af følgende uddannelser? 1. Erhvervsfaglig uddannelse
2. Kort videregående uddannelse (1-2 år)
3. Mellemlang videregående uddannelse (3-4 år)
4. Lang videregående uddannelse (5 år el. mere)
5. Anden uddannelse _____

G. De følgende spørgsmål omhandler kostvaner

1.	Er du vegetar?	1. ja	2. nej	
2.	Har du ændret kostvaner under din nuværende graviditet?	1. nej	2. ja, lidt	3. ja, meget

Hvis du har ændret kostvaner, har du ændret kostens indhold af:

fedt	1. samme	2. mere	3. mindre
fisk	1. samme	2. mere	3. mindre
fedtstof på brød	1. samme	2. mere	3. mindre
fedtfattige produkter	1. samme	2. mere	3. mindre
kød	1. samme	2. mere	3. mindre
mælkeprodukter	1. samme	2. mere	3. mindre
grøntsager	1. samme	2. mere	3. mindre
frugt	1. samme	2. mere	3. mindre
brød	1. samme	2. mere	3. mindre
salt	1. samme	2. mere	3. mindre

3.	Bruger du normalt salt ved madlavning?	1. ja	2. nej	
	Hvis ja, hvor meget:	1. meget	2. moderat	3. lidt
	Salter du normalt maden ved bordet?	1. ja	2. nej	
	Hvis ja, hvor meget salter du:	1. meget	2. moderat	3. lidt

4.	Hvilken slags salt har I for tiden i hjemmet:		
	Almindeligt bordsalt	1. ja	2. nej
	Specialsalt (fx Middelhavssalt eller Læsø salt)	1. ja	2. nej
	Hvis ja, skriv hvilken specialsalt:	_____	

5. Hvor ofte spiser du brød?

1. Aldrig eller sjældent
2. 1-3 gange om måneden
3. 1-3 gange om ugen
4. Hver dag

Hvis du spiser brød hver dag, er det da

1. 1-2 gange om dagen
2. 3 eller flere gange om dagen

6. Bager du selv dit brød? 1. altid 2. ofte 3. sjældent 4. aldrig
   Køber du brød hos bageren? 1. altid 2. ofte 3. sjældent 4. aldrig
   Køber du brød i supermarked el. lign.? 1. altid 2. ofte 3. sjældent 4. aldrig
   Får du brød andre steder fra?
   Skriv hvor _____

7. Hvor ofte spiser du fisk?

1. Aldrig eller sjældent
2. 1-3 gange om måneden
3. 1-3 gange om ugen
4. Hver dag

Hvis du spiser fisk hver dag, er det da

1. 1-2 gange om dagen
2. 3 eller flere gange om dagen

8. Hvor ofte spiser du ost?

1. Aldrig eller sjældent
2. 1-3 gange om måneden
3. 1-3 gange om ugen
4. Hver dag

Hvis du spiser ost hver dag, er det da

1. 1-2 gange om dagen
2. 3 eller flere gange om dagen

9. Hvor ofte indtager du mælkeprodukter? (mælk, yoghurt etc.)

1. Aldrig eller sjældent
2. 1-3 gange om måneden
3. 1-3 gange om ugen
4. Hver dag

Hvis du indtager mælkeprodukter hver dag, er det da

1. 1-2 gange om dagen
2. 3 eller flere gange om dagen

10. Køber du overvejende økologisk mælkeprodukter? 1. ja 2. nej

H. Medicin, kosttilskud, vitaminer m.m.

1. Skriv venligst her, hvad du får af medicin (præparatnavn, dosis)

_____
_____
_____
_____
_____
_____

2. Skriv venligst her, hvad du tager af kosttilskud, vitaminer og lign. (præparatnavn, dosis)

_____
_____
_____
_____
_____
_____
_____

3. Hvis du tager kosttilskud, vitaminer og lign. tog du også disse *før* din nuværende graviditet? 1. ja, alle 2. ja, nogle 3. nej, ingen

4. Hvis du *ikke* tog alle kosttilskud, vitaminer og lign. *før* din nuværende graviditet, skriv venligst hvilke du er begyndt med i forbindelse med graviditeten og ca. hvornår i graviditeten, du begyndte at tage disse kosttilskud

_____
_____
_____
_____
_____
_____

Ved behov for supplerende oplysninger, skriv gerne

Tlf.: _____

E-mail: _____

Tak for hjælpen!

Medbring venligst spørgeskemaet samt evt. medicin, kosttilskud, vitaminer og lign. til undersøgelsen.

Appendix B. Questionnaire for household members ≥15 years

Undersøgelse af jodindtag blandt gravide og deres husstand

Spørgeskema husstand (≥15 år)

I det følgende vil vi stille dig en række spørgsmål af betydning for jodindtaget.

Alle besvarede spørgsmål vil blive behandlet fortroligt ifølge reglerne om tavshedspligt for hospitalsansatte.

De fleste spørgsmål besvares ved at sætte en cirkel om tallet ud for den svar mulighed, du selv synes er den mest rigtige. Enkelte spørgsmål besvares med ord eller tal på de markerede linjer.

Obs! Der er spørgsmål på begge sider.

A. Indledende oplysninger om dig

1. Din fødselsdato _____
2. Din højde _____ cm
3. Din aktuelle vægt _____ kg
4. Køn: 1. Kvinde 2. Mand

B. De følgende spørgsmål omhandler sociale forhold

1. Har du altid boet i Jylland? 1. ja 2. nej

   Hvis nej, hvor mange år har du boet i Jylland _____ år

   Hvor boede du før _____

2. Hvad er din etniske baggrund?
   1. dansk
   2. anden etnisk baggrund end dansk,
   angiv hvilken _____

3. Er du født i Danmark? 1. ja 2. nej

   Hvis nej, hvor er du født _____

   Hvor mange år har du boet i Danmark _____år

C. De følgende spørgsmål omhandler sygdomme i skjoldbruskkirtlen

1. Har din læge nogensinde fortalt dig, at du havde:

for lavt stofskifte	1. ja	2. nej
for højt stofskifte	1. ja	2. nej
struma (forstørret skjoldbruskkirtel)	1. ja	2. nej
knude i skjoldbruskkirtlen	1. ja	2. nej

2. Har du nogensinde været i behandling for:

for lavt stofskifte	1. ja, nu	2. ja, tidligere	3. nej, aldrig
for højt stofskifte	1. ja nu	2. ja, tidligere	3. nej, aldrig

Hvis ja, skriv hvilken behandling og hvornår _____

_____

_____

_____

D. De følgende spørgsmål omhandler tobak

1. Ryger du?
   1. ja, dagligt – gå videre til spørgsmål 3
   2. ja, lejlighedsvist (mindre end en cigaret, cerut, cigar eller et pibestop dagligt)
   3. nej

   Hvis du ikke ryger nu, har du røget tidligere?
   1. ja, dagligt
   2. ja, lejlighedsvist (mindre end en cigaret, cerut, cigar eller et pibestop dagligt)
   3. nej – fortsæt til spørgsmål 6

   Hvilket år holdt du op med at ryge? _____ (årstal)
   Hvis rygeophør inden for det seneste år, hvilken måned? _____ (måned)

2. Hvis du kun ryger/har røget lejlighedsvis, hvor ofte ryger/røg du? _____ dage ugentligt

3. Hvor meget ryger du i gennemsnit?
   Skriv gerne flere steder.
   _____ cigaretter dagligt
   _____ cerutter dagligt
   _____ cigarer dagligt
   _____ gram pibetobak ugentligt

4. Hvor gammel var du, da du begyndte at ryge dagligt? _____ år
5. Hvor mange år har du røget regelmæssigt? _____ år

6. Hvor mange timer om dagen plejer du at opholde dig i et rum, hvor der bliver røget?
   1. mere end 5 timer om dagen
   2. 1 til 5 timer om dagen
   3. ½ til 1 time om dagen
   4. stort set aldrig

E. De følgende spørgsmål omhandler arbejds- og uddannelsesforhold

1. Hvilken erhvervstilknytning har du?
   1. er i erhverv
   2. har været i erhverv
   3. har aldrig været i erhverv

2. Hvad er din hovedbeskæftigelse for tiden?
   1. selvstændig
   2. medhjælpende ægtefælle
   3. lønmodtager
   4. studerende/elev/lærling
   5. på bistandshjælp, kontanthjælp
   6. førtidspensionist
   7. hjemmegående husmor/husfar
   8. arbejdsløs på arbejdsløshedsdagpenge
   9. på sygedagpenge
   10. andet _____

3. Hvad er din stillingsbetegnelse _____

4. Hvor mange år har du gået i skole?
   1. Folkeskole eller tilsvarende (7-10 års skolegang)
   2. Gymnasial uddannelse: gymnasium, HF og lignende (10-12 års skolegang)

5. Har du eller er du i gang med en erhvervs- eller videregående uddannelse?
   1. ja, har gennemført
   2. ja, er i gang med en
   3. nej

   Hvis ja, hvilken af følgende uddannelser?
   1. Erhvervsfaglig uddannelse
   2. Kort videregående uddannelse (1-2 år)
   3. Mellemlang videregående uddannelse (3-4 år)
   4. Lang videregående uddannelse (5 år el. mere)
   5. Anden uddannelse _____

F. De følgende spørgsmål omfatter kostvaner

1. Er du vegetar? 1. ja 2. nej

2. Bruger du normalt salt ved madlavning? 1. ja 2. nej
   Hvis ja, hvor meget: 1. meget 2. moderat 3. lidt

3. Salter du normalt maden ved bordet? 1. ja 2. nej
Hvis ja, hvor meget salter du: 1. meget 2. moderat 3. lidt

4. Hvilken slags salt har I for tiden i hjemmet:
Almindeligt bordsalt 1. ja 2. nej
Specialsalt (fx Middelhavssalt eller Læsø salt) 1. ja 2. nej
Hvis ja, skriv hvilken specialsalt: _____

5. Hvor ofte spiser du brød?
1. Aldrig eller sjældent
2. 1-3 gange om måneden
3. 1-3 gange om ugen
4. Hver dag

Hvis du spiser brød hver dag, er det da
1. 1-2 gange om dagen
2. 3 eller flere gange om dagen

6. Bager du selv dit brød? 1. altid 2. ofte 3. sjældent 4. aldrig
Køber du brød hos bageren? 1. altid 2. ofte 3. sjældent 4. aldrig
Køber du brød i supermarked el. lign.? 1. altid 2. ofte 3. sjældent 4. aldrig
Får du brød andre steder fra?
Skriv hvor _____

7. Hvor ofte spiser du fisk?
1. Aldrig eller sjældent
2. 1-3 gange om måneden
3. 1-3 gange om ugen
4. Hver dag

Hvis du spiser fisk hver dag, er det da
1. 1-2 gange om dagen
2. 3 eller flere gange om dagen

8. Hvor ofte spiser du ost?
1. Aldrig eller sjældent
2. 1-3 gange om måneden
3. 1-3 gange om ugen
4. Hver dag

Hvis du spiser ost hver dag, er det da
1. 1-2 gange om dagen
2. 3 eller flere gange om dagen

9. Hvor ofte indtager du mælkeprodukter? (mælk, yoghurt etc.)
   1. Aldrig eller sjældent
   2. 1-3 gange om måneden
   3. 1-3 gange om ugen
   4. Hver dag

   Hvis du indtager mælkeprodukter hver dag, er det da
   1. 1-2 gange om dagen
   2. 3 eller flere gange om dagen

10. Køber du overvejende økologisk mælkeprodukter? 1. ja 2. nej

G. Medicin, kosttilskud, vitaminer m.m.

1. Skriv venligst her, hvad du får af medicin (præparatnavn, dosis)
   _____
   _____
   _____
   _____
   _____
   _____

2. Skriv venligst her hvad du tager af kosttilskud, vitaminer og lign. (præparatnavn, dosis)
   _____
   _____
   _____
   _____
   _____
   _____
   _____

H. Det følgende spørgsmål skal først besvares når urinprøven er lavet

1. Hvis du tager vitamin/mineral tilskud, hvornår har du sidst taget dette forud for urinprøven?
   1. samme dag 2. dagen før 3. flere dage før

Ved behov for supplerende oplysninger, skriv gerne

Tlf.: _____

E-mail: _____

Tak for hjælpen!

Appendix C. Questionnaire household members <15 years

Undersøgelse af jodindtag blandt gravide og deres husstand

Spørgeskema husstand (<15 år)

I det følgende vil vi stille en række spørgsmål af betydning for jodindtaget.

Alle besvarede spørgsmål vil blive behandlet fortroligt ifølge reglerne om tavshedspligt for hospitalsansatte.

De fleste spørgsmål besvares ved at sætte en cirkel om tallet ud for den svar mulighed, du selv synes er den mest rigtige. Enkelte spørgsmål besvares med ord eller tal på de markerede linjer.

Obs! Der er spørgsmål på begge sider.

A. Indledende oplysninger om barnet

1. Barnets fødselsdato _____
2. Barnets højde _____ cm
3. Barnets aktuelle vægt _____ kg
4. Køn: 1. Pige 2. Dreng

B. De følgende spørgsmål omhandler sociale forhold

1. Har barnet altid boet i Jylland? 1. ja 2. nej

   Hvis nej, hvor mange år har barnet boet i Jylland _____ år

   Hvor boede barnet før _____

2. Hvad er barnets etniske baggrund?
   1. dansk
   2. anden etnisk baggrund end dansk, angiv hvilken _____

3. Er barnet født i Danmark? 1. ja 2. nej

   Hvis nej, hvor er barnet født _____

   Hvor mange år har barnet boet i Danmark _____ år

C. De følgende spørgsmål omhandler sygdomme i skjoldbruskkirtlen

1. Har din læge nogensinde fortalt dig, at barnet havde:

for lavt stofskifte	1. ja	2. nej
for højt stofskift	1. ja	2. nej
struma (forstørret skjoldbruskkirtel)	1. ja	2. nej
knude i skjoldbruskkirtlen	1. ja	2. nej

2. Har barnet nogensinde været i behandling for:

for lavt stofskifte	1. ja, nu	2. ja, tidligere	3. nej, aldrig
for højt stofskifte	1. ja nu	2. ja, tidligere	3. nej, aldrig

Hvis ja, skriv hvilken behandling og hvornår _____

_____

_____

_____

D. De følgende spørgsmål omfatter kostvaner

1. Er barnet vegetar?	1. ja	2. nej	
2. Salter barnet normalt maden ved bordet?	1. ja	2. nej	
Hvis ja, hvor meget salter barnet:	1. meget	2. moderat	3. lidt

3. Hvor ofte spiser barnet brød?

1. Aldrig eller sjældent
2. 1-3 gange om måneden
3. 1-3 gange om ugen
4. Hver dag

Hvis barnet spiser brød hver dag, er det da

1. 1-2 gange om dagen
2. 3 eller flere gange om dagen

4. Hvor ofte spiser barnet fisk?

1. Aldrig eller sjældent
2. 1-3 gange om måneden
3. 1-3 gange om ugen
4. Hver dag

Hvis barnet spiser fisk hver dag, er det da

1. 1-2 gange om dagen
2. 3 eller flere gange om dagen

5. Hvor ofte spiser barnet ost?

1. Aldrig eller sjældent
2. 1-3 gange om måneden
3. 1-3 gange om ugen
4. Hver dag

Hvis barnet spiser ost hver dag, er det da

1. 1-2 gange om dagen
2. 3 eller flere gange om dagen

6. Hvor ofte indtager barnet mælkeprodukter (mælk, yoghurt etc.)

1. Aldrig eller sjældent
2. 1-3 gange om måneden
3. 1-3 gange om ugen
4. Hver dag

Hvis barnet indtager mælkeprodukter hver dag, er det da

1. 1-2 gange om dagen
2. 3 eller flere gange om dagen

E. Medicin, kosttilskud, vitaminer m.m.

1. Skriv venligst her, hvad barnet får af medicin (præparatnavn, dosis)

_____
_____
_____
_____
_____
_____

2. Skriv venligst her hvad barnet tager af kosttilskud, vitaminer og lign. (præparatnavn, dosis)

_____
_____
_____
_____
_____
_____
_____

F. Det følgende spørgsmål skal først besvares når urinprøven er lavet

1. Hvis barnet tager vitamin/mineral tilskud, hvornår har barnet sidst taget dette forud for urinprøven?
   1. samme dag 2. dagen før 3. flere dage før

Tak for hjælpen!

Appendix D. Paper 1

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CLINICAL STUDY

Thyroglobulin in smoking mothers and their newborns at delivery suggests autoregulation of placental iodide transport overcoming thiocyanate inhibition

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(Correspondence should be addressed to S L Andersen; Email: stine.a@rn.dk)

Abstract

Background: Placental transport of iodide is required for fetal thyroid hormone production. The sodium iodide symporter (NIS) mediates active iodide transport into the thyroid and the lactating mammary gland and is also present in placenta. NIS is competitively inhibited by thiocyanate from maternal smoking, but compensatory autoregulation of iodide transport differs between organs. The extent of autoregulation of placental iodide transport remains to be clarified.
Objective: To compare the impact of maternal smoking on thyroglobulin (Tg) levels in maternal serum at delivery and in cord serum as markers of maternal and fetal iodine deficiency.
Methods: One hundred and forty healthy, pregnant women admitted for delivery and their newborns were studied before the iodine fortification of salt in Denmark. Cotinine in urine and serum classified mothers as smokers ($n=50$) or nonsmokers ($n=90$). The pregnant women reported on intake of iodine-containing supplements during pregnancy and Tg in maternal serum at delivery and in cord serum were analyzed.
Results: In a context of mild-to-moderate iodine deficiency, smoking mothers had significantly higher serum Tg than nonsmoking mothers (mean Tg smokers 40.2 vs nonsmokers 24.4 μg/l, $P=0.004$) and so had their respective newborns (cord Tg 80.2 vs 52.4 μg/l, $P=0.006$), but the ratio between Tg in cord serum and maternal serum was not significantly different in smokers compared with nonsmokers (smoking 2.06 vs nonsmoking 2.22, $P=0.69$).
Conclusion: Maternal smoking increased the degree of iodine deficiency in parallel in the mother and the fetus, as reflected by increased Tg levels. However, placental iodide transport seemed unaffected despite high thiocyanate levels, suggesting that thiocyanate-insensitive iodide transporters alternative to NIS are active or that NIS in the placenta is autoregulated to keep iodide transport unaltered.

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Introduction

Thyroid hormones are essential for early growth and brain development, and iodine is required for thyroid hormone synthesis (1, 2). In the early weeks of pregnancy, the developing fetus relies on maternal thyroid hormones, but endogenous fetal thyroid hormone production contributes increasingly from the beginning of the second trimester and is dependent on placental transport of iodide from the maternal to the fetal circulation (3).

Placenta contains iodine (4, 5, 6) and evidence suggests that a number of iodide transporters are involved in placental iodide transport. Placental tissue and cell culture studies have demonstrated that the sodium iodide symporter (NIS) is expressed in different types of placental cells (7, 8, 9) and a functional role of NIS in placental iodide transport has been proposed (10, 11). NIS is a member of the SLC5 family (SLC5A5) of sodium solute symporters and it is well known that NIS mediates the active transport of iodide into the thyroid gland (12). NIS is also expressed in the lactating mammary gland (13) mediating the active transport of iodide into breast milk (14) and in some other extra-thyroidal tissues including the intestine (15, 16).

Another member of the SLC5 family (SLC5A6), the sodium multivitamin transporter (SMVT), has also been demonstrated in various tissues including the intestine (17) and the placenta (18). SMVT shares high sequence similarity with NIS (12) and has been demonstrated to mediate iodide transport in SMVT-expressing oocytes (19).

In addition to SLC5 family transporters, sodium-independent iodide transporters have also been

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proposed to play a role in placental iodide transport. Pendrin is a chloride–iodide transporter expressed in the placenta and in the thyroid gland (7, 20).

One way to study iodide transport in humans is to observe the effect of a known iodide transport inhibitor, such as thiocyanate (21). In humans, thiocyanate (SCN^-) stems from various sources, but in many populations, the most important source is tobacco smoking. Thiocyanate competitively inhibits NIS-mediated iodide transport in the thyroid gland; however, the reduced thyroid iodide uptake is compensated by iodide autoregulation that tends to keep thyroid iodide uptake sufficient for thyroid hormone synthesis (12, 14, 22). On the other hand, the increased thyroid activity associated with autoregulation leads to increased serum thyroglobulin (Tg) and increased risk of goiter in smokers (22, 23).

By contrast, autoregulation of NIS in the lactating mammary gland seems minimal or absent. Breast milk iodine content parallels urinary iodine excretion over a wide range of concentrations (24), and iodine supplements lead to dose-dependent increases in milk iodine content both in domestic animals (25) and in breastfeeding women (26). In accordance with this, we previously showed increased risk of iodine deficiency in breast-fed newborns of smoking mothers with no signs of NIS autoregulation in the lactating mammary gland (14).

The aim of this study was to compare the impact of maternal smoking on Tg levels in maternal serum at delivery and in cord serum. We studied a unique cohort of iodine-deficient pregnant women with a high frequency of smoking and their newborns. Tg in maternal serum at delivery and in cord serum was used as a marker of iodine deficiency (27, 28, 29), and we examined the impact of thiocyanate from maternal smoking on the degree of iodine deficiency in the mother and in the fetus by comparing serum Tg in smoking and nonsmoking mothers and cord serum Tg.

Materials and methods

Study design and study population

This is a cross-sectional study carried out from November 1988 to March 1990 in five different cities in Denmark (14, 24, 30, 31). As the time of study enrollment was before the mandatory Danish iodine fortification of salt introduced in the year 2000 (32), the population had in general mild (East Denmark) to moderate (West Denmark) iodine deficiency with the majority of the women under study living in an area of moderate iodine deficiency (78.6%). A total of 152 healthy pregnant women with no history of thyroid disease, no visible goiter, and no recent exposure to excess iodine and their newborn children were studied. The pregnant women were consecutively recruited when admitted for delivery after uncomplicated pregnancy in the Departments of Obstetrics in each of the five cities (Copenhagen, $n=30$; Aarhus, $n=30$; Ringkøbing, $n=30$; Randers $n=29$; and Aalborg $n=33$). Six women were subsequently excluded from this study due to intermittent intake of iodine supplements, and another six women were excluded due to signs of a change in smoking status before and after delivery, thus leaving 140 pregnant women and their 140 newborn

Table 1 Characteristics of the mothers and their newborns.

		Iodine supplements[a] ($n=47$)			No iodine supplements[b] ($n=93$)		
	All ($n=140$)	Smoking ($n=16$)	Nonsmoking ($n=31$)	P^c	Smoking ($n=34$)	Nonsmoking ($n=59$)	P^d
Maternal age (years)							
Mean	27.3	27.4	28.2	0.60[e]	27.6	26.7	0.32[e]
S.D.	4.5	4.6	4.7		5.2	4.0	
Parity							
Median	1	1	1	0.82[f]	1	1	0.29[f]
Range	1–5	1–3	1–3		1–4	1–5	
Gestational age (weeks)							
Mean	40.0	39.8	40.2	0.44[e]	40.1	40.0	0.63[e]
S.D.	1.5	1.9	1.4		1.6	1.4	
Birth weight (g)							
Mean	3520	3302	3699	0.01[e]	3367	3574	0.04[e]
S.D.	473	498	442		461	452	

[a]Maternal daily intake of vitamin/mineral supplements containing iodine.
[b]No maternal daily intake of vitamin/mineral supplement containing iodine.
[c]Statistical comparison of smokers and nonsmokers within the iodine supplement group.
[d]Statistical comparison of smokers and nonsmokers within the no iodine supplement group.
[e]Independent sample *t*-test.
[f]Mann–Whitney *U* test.

children in the final study population. Informed consent was obtained from each participant and the study was approved by the Local Ethics Committee.

Data collection

When the pregnant women were admitted for delivery, detailed information was obtained on intake of iodine-containing vitamin and mineral supplements and the women were instructed to continue their previous vitamin and mineral supplementation during the puerperal period. All women intended to breastfeed their newborn child. Blood samples ($n=138$) were taken from the pregnant women by standard puncture of a cubital vein shortly after admission for delivery. Closure of the umbilical cord was performed within the first minute after delivery, and mixed cord blood ($n=133$) was sampled from the placental part shortly after. After sampling, blood was centrifuged and serum was stored at -20 °C until analyses. One cord serum sample had a limited amount of serum, which precluded some of the analyses.

On day 5 after delivery, a breast milk sample ($n=136$) and a morning spot urine ($n=140$) was collected from the mother and a urine sample was collected in a small self-adhesive plastic bag (Coloplast baby urine collector; Coloplast, Espergærde, Denmark) from the newborn child ($n=135$). Urine samples were stored at -20 °C until analyses.

Laboratory procedures

Classification of smokers was performed by measurements of the nicotine metabolite cotinine in serum (Immulite 2000 Nicotine Metabolite Assay; analytical sensitivity 5 μg/l, cutoff to distinguish smokers 25 μg/l) and urine (double antibody RIA Diagnostic Products Cooperation; analytical sensitivity 9 μg/l, cutoff to distinguish smokers 500 μg/l), as described previously in detail (14). In participants, a clear separation of smokers ($n=50$) and nonsmokers ($n=90$) was obtained both when evaluated by cotinine in maternal serum when admitted for delivery (median (range) smokers 164 (36–>600) vs nonsmokers <5 (<5–24) μg/l) and in cord serum at delivery (164 (32–>600) vs <5 (<5–22) μg/l) as well as in urine from the mother on day 5 *postpartum* (3480 (537–10 500) vs 53 (10–218) μg/l). Differences between smokers and nonsmokers

Table 2 Thyroid function parameters and iodine status in mothers and their newborns stratified by maternal smoking status. TSH, thyroglobulin, urinary iodine, and milk iodine were log transformed for calculation of geometric mean and 95% CI.

	Mothers				Newborns			
	All ($n=140$)	Smoking[a] ($n=50$)	Nonsmoking[a] ($n=90$)	P[b]	All ($n=140$)	Smoking[c] ($n=50$)	Nonsmoking[c] ($n=90$)	P[d]
TSH[e] (mU/l)								
Mean	2.07	2.05	2.08	0.85	8.07	7.21	8.60	0.12
95% CI	1.89–2.26	1.77–2.37	1.86–2.33		7.25–8.98	6.01–8.64	7.52–9.83	
T_3[f] (nm/l)								
Mean	2.39	2.47	2.35	0.16	0.84	0.86	0.84	0.55
95% CI	2.32–2.47	2.34–2.60	2.25–2.45		0.80–0.88	0.79–0.93	0.79–0.88	
T_4[f] (nm/l)								
Mean	177	175	178	0.58	162	169	159	0.054
95% CI	176–183	166–184	171–186		157–167	160–177	153–165	
Free T_4[e] (pmol/l)								
Mean	8.35	8.01	8.53	0.075	12.27	12.57	12.09	0.16
95% CI	8.07–8.61	7.49–8.54	8.22–8.84		11.94–12.60	11.92–13.24	11.73–12.46	
Thyroglobulin[g] (μg/l)								
Mean	22.9	30.4	19.5	0.004	50.0	62.9	44.0	0.009
95% CI	19.7–26.5	23.4–39.4	16.4–23.2		43.9–57.0	50.2–78.7	37.6–51.4	
Urinary iodine[h] (μg/l)								
Mean	40.6	40.1	40.8	0.88	43.5	33.3	50.4	0.006
95% CI	36.0–45.7	34.1–47.1	34.6–48.1		37.8–50.1	26.8–41.5	42.1–60.3	
Milk iodine[i] (μg/l)								
Mean	41.4	26.0	53.8	<0.001	NA	NA	NA	
95% CI	35.8–47.8	20.7–32.6	45.5–63.5					

NA, not applicable.
[a]Mothers classified as smokers or nonsmokers from cotinine in serum and urine.
[b]Statistical comparison of smoking and nonsmoking mothers (independent sample *t*-test).
[c]Newborns of smoking and nonsmoking mothers.
[d]Statistical comparison of newborns of smoking and nonsmoking mothers (independent sample *t*-test).
[e]Maternal serum samples ($n=138$; smokers $n=49$, nonsmokers $n=89$). Cord serum samples ($n=133$; smoking mother $n=48$, nonsmoking mother $n=85$).
[f]Maternal serum samples ($n=138$; smokers $n=49$, nonsmokers $n=89$). Cord serum samples ($n=132$; smoking mother $n=47$, nonsmoking mother $n=85$).
[g]Maternal serum samples ($n=137$; smokers $n=49$, nonsmokers $n=88$). Cord serum samples ($n=131$; smoking mother $n=47$, nonsmoking mother $n=84$).
[h]Newborn urine samples ($n=135$; smoking mother $n=48$, nonsmoking mother $n=87$).
[i]Breast milk samples ($n=136$; smokers $n=49$, nonsmokers $n=87$).

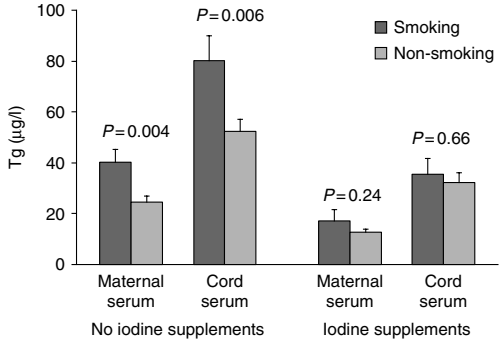

Figure 1 Thyroglobulin (Tg) concentrations in maternal and cord serum stratified by maternal smoking status and maternal intake of iodine supplements (no maternal iodine supplements, Tg maternal serum smoking 40.2 (95% CI 31.1–52.0) vs nonsmoking 24.4 (19.9–30.0) and cord serum 80.2 (63.0–102.1) vs 52.4 (43.5–63.1) µg/l; maternal iodine supplements, Tg maternal serum smoking 17.0 (10.1–28.7) vs nonsmoking 12.6 (9.7–16.4) and cord serum 35.4 (24.4–51.4) vs 32.1 (24.8–41.7) µg/l). Log-transformed serum Tg concentrations were used for calculation of geometric mean and statistical comparison; *P* values are results of independent sample *t*-test. Bars represent +1 S.E.M.

were substantiated by measurement of thiocyanate in maternal and cord serum by a manual method (33). Thiocyanate concentrations were considerably higher in smokers than in nonsmokers (mean (S.D.), maternal serum: smokers 84.9 (25.4) vs nonsmokers 54.7 (18.2); cord serum: smoking mother 94.6 (31.9) vs nonsmoking mother 48.3 (15.5) µmol/l) (14). Iodine in urine and breast milk was measured by the colorimetric method of the Sandell–Kolthoff reaction after alkaline ashing, as described previously (34).

Tg in maternal and cord serum was determined by an immunoluminometric assay (Behringwerke, Marburg, Germany; detection limit <1 µg/l), including recovery measurements. Tg antibodies (Tg-Ab) were measured by a very sensitive radioimmunoprecipitation assay (detection limit 20 U/l; Medical Research Council standard reference code A 65193), as described previously (31, 35). Tg-Ab was detectable in six maternal serum samples (4.4%) and 14 cord serum samples (10.6%). Because Tg-Ab may influence Tg measurements (36), serum samples with Tg-Ab values more than 200 U/l (maternal serum $n=1$, cord serum $n=2$) were excluded from serum Tg analyses. Tg-Ab levels up to 200 U/l have previously been shown not to interfere with serum Tg measurements using this assay (37), and our results were consistent when limiting the analyses to samples without detectable Tg-Ab (data not shown). Thyroid function parameters were measured in maternal and cord serum: TSH by an immunoluminometric assay (Berilux, Behringwerke), total thyroxine (T_4) and total tri-iodothyronine (T_3) by an RIA (Farmos, Turko, Finland), and free T_4 by a two-step method (RIA-gnost-FT$_4$, Behringwerke), as described previously (31).

Statistical analyses

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Statistics version 19) and Stata 11 (StataCorp., College Station, TX, USA). Concentrations of TSH, Tg, breast milk iodine, and urinary iodine showed log-normal distribution, and logarithmically transformed concentrations or ratios between concentrations were used for calculating geometric means and making statistical comparisons. We used independent sample *t*-test or Mann–Whitney *U* test when comparing either mothers or newborns stratified by maternal smoking and/or intake of iodine supplements, whereas Tg levels in mother and child were compared using paired *t*-tests. We also evaluated serum Tg in multivariate linear regression models using logarithmically transformed serum Tg concentrations and the ratio between cord serum Tg and maternal serum Tg as dependent variables and maternal smoking, maternal intake of iodine supplements, and other variables plausibly related to serum Tg as potential explaining variables. We considered possible interaction between maternal smoking and maternal intake of iodine supplements by including an interaction term (smoking x iodine supplement intake) in the models. A 5% level of statistical significance was chosen.

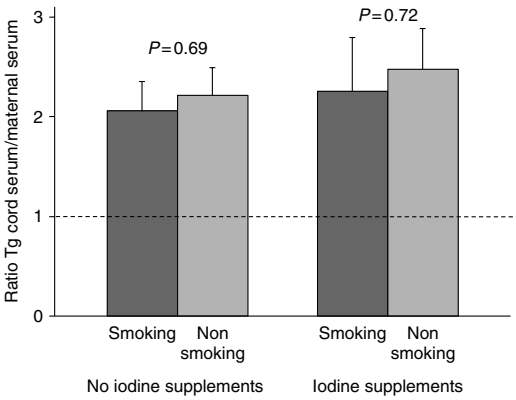

Figure 2 Ratio between thyroglobulin (Tg) in cord serum and maternal serum stratified by maternal smoking status and maternal intake of iodine supplements (no maternal iodine supplements, ratio Tg smoking 2.06 (95% CI 1.56–2.71) and nonsmoking 2.22 (1.76–2.80); maternal iodine supplements, ratio Tg smoking 2.26 (1.42–3.59) and nonsmoking 2.48 (1.82–3.40)). Log-transformed ratios in individual pairs of mother and child were used for calculation of geometric mean and statistical comparison; *P* values are results of independent sample *t*-test. Bars represent +1 S.E.M.

Results

Thyroid function and iodine status in the mothers and their newborns

Smoking and nonsmoking mothers with and without a daily intake of iodine-containing supplements had similar age, parity, and gestational age whereas the birth weight of the newborns was lower in children born to smoking mothers (Table 1). The frequency of maternal smoking in the iodine and no iodine supplement groups was similar (no iodine supplements, 36.6% smokers; iodine supplements, 34.0% smokers) and the frequency of intake of iodine supplements in mothers was independent of maternal smoking status (smoking mothers, 32.0% iodine supplements; nonsmoking mothers, 34.4% iodine supplements). All iodine supplements contained 150 μg iodine/day.

Thyroid hormone levels in maternal serum collected shortly before delivery and in cord serum collected at delivery showed a similar pattern in smoking and nonsmoking mothers as well as in their newborns (Table 2). Urinary iodine was similar in smoking and nonsmoking mothers (ratio smoking/nonsmoking 0.98, 95% CI 0.76–1.26), whereas in the breast-fed newborns, urinary iodine was significantly lower in newborns of smoking mothers (ratio smoking/nonsmoking 0.66, 95% CI 0.50–0.88). In addition, breast milk iodine was reduced to approximately half in smokers (ratio smoking/nonsmoking 0.48, 95% CI 0.37–0.64).

Tg in maternal and cord serum

Serum Tg showed different results depending on maternal intake of iodine supplements and/or maternal smoking status. Intake of iodine supplements in mothers was associated with a lower Tg in maternal serum (14.0 vs 29.3 μg/l if no iodine supplements, $P<0.001$) and in cord serum (31.1 vs 61.6 μg/l, $P<0.001$). Maternal smoking (Table 2) was associated with considerably higher Tg in maternal serum (ratio smoking/nonsmoking 1.56, 95% CI 1.15–2.10) and in cord serum (ratio smoking/nonsmoking 1.43, 95% CI 1.10–1.86).

However, the impact of maternal smoking on serum Tg was most striking in the no iodine supplement group when stratified by both maternal smoking and maternal intake of iodine supplements (Fig. 1). In the no iodine supplement group, smoking mothers had higher serum Tg (ratio smoking/nonsmoking 1.64, 95% CI 1.18–2.29) and cord serum Tg in newborns of smoking mothers was higher (ratio smoking/nonsmoking 1.53, 95% CI 1.14–2.06). In the group taking iodine supplements, the difference in serum Tg between smoking and nonsmoking mothers was statistically nonsignificant (ratio smoking/nonsmoking 1.35, 95% CI 0.82–2.23) and cord serum Tg was similar (ratio smoking/nonsmoking 1.10, 95% CI 0.71–1.72).

In general, Tg was higher in cord serum (mean ratio cord serum/maternal serum 2.24, 95% CI 1.94–2.58, $P<0.001$). To evaluate the degree of iodine deficiency in smoking mothers and their newborns, we compared serum Tg ratios, cord serum relative to maternal serum, in individual pairs of mother and child (Fig. 2). The ratios were similar in smokers and nonsmokers independent of maternal intake of iodine supplements.

The associations between serum Tg in mother and fetus, maternal intake of iodine supplements, and maternal smoking status were also studied in multivariate linear regression models (Table 3), which further included maternal age at delivery, gestational age, parity, and area of living as a proxy variable for iodine intake (West Denmark with moderate iodine deficiency vs East Denmark with mild iodine deficiency). The models corroborated our findings in the stratified analyses. To evaluate possible interaction between maternal intake of iodine supplements and maternal smoking, we included an interaction term (smoking×iodine supplement intake), which was not statistically significant in any of the models ($P>0.1$).

Table 3 Predictors of serum Tg in multivariate linear regression models. Serum Tg and ratio Tg cord/maternal serum were log transformed before analysis. Results are exponentiated β and 95% CI respectively.

Dependent variables	Explanatory variables	Multivariate[a] β (95% CI)
Maternal serum Tg	Iodine supplements[b]	0.48 (0.36–0.64)
	Smoking[c]	1.52 (1.15–2.00)
Cord serum Tg	Iodine supplements[b]	0.57 (0.44–0.73)
	Smoking[c]	1.38 (1.08–1.77)
Ratio Tg cord/maternal serum	Iodine supplements[b]	1.16 (0.85–1.57)
	Smoking[c]	0.93 (0.69–1.26)

Tg, thyroglobulin.
[a]Models included maternal age (years), gestational age (weeks), parity, and area of living: West Denmark with moderate iodine deficiency (reference) vs East Denmark with mild iodine deficiency.
[b]Maternal daily intake of iodine-containing supplements; yes/no (reference).
[c]Maternal smoking status classified from cotinine in serum and urine; smoker/nonsmoker (reference).

Discussion

Study rationale and principal findings

Iodide autoregulation of NIS in the thyroid gland is well known (12), whereas in the lactating mammary gland, no autoregulation of NIS seems to occur (14). To evaluate autoregulation of placental iodide transport, we used Tg in maternal and cord serum at delivery as markers of iodine deficiency in the mother and the fetus respectively (27, 28, 29).

Thiocyanate inhibits NIS-mediated iodide transport in cultured placental cells (11). In contrast to inhibition of NIS in the thyroid, which affects iodide uptake in both the maternal and the fetal thyroid, impaired placental iodide transport would affect the iodide supply to the fetus exclusively. We assumed that thiocyanate would inhibit NIS and possibly other transporters involved in placental iodide transport, and we hypothesized the following scenarios: if no autoregulation of iodide transport takes place in the placenta, thiocyanate from maternal smoking would lead to a particular worsening of fetal iodine deficiency with a relatively higher increase in cord serum Tg. On the other hand, if there is autoregulation of placental iodide transport, we would observe the same degree of smoking-induced Tg changes in the mother and the fetus.

Tg was higher in cord serum than in maternal serum, which is a normal finding not related to iodine deficiency (38). Tg in maternal and cord serum increased to a similar degree as indicated by similar ratios between cord serum and maternal serum Tg in smoking and nonsmoking mothers and in accordance with the identical levels of thiocyanate in maternal and cord serum. Thus, placental iodide transport seemed unaffected in smoking mothers, suggesting autoregulation of placental iodide transport similar to autoregulation of NIS in the thyroid gland. Thyroid function was not affected by maternal smoking, neither in the mother nor in the fetus, but maternal smoking led to high serum Tg in both, which is consistent with autoregulation of NIS in the thyroid.

On the other hand, the data collected on day 5 *postpartum* were related to iodide transport in the lactating mammary gland and not in the placenta. As previously published (14), breast milk iodine content and urinary iodine in the newborns on day 5 *postpartum* were low if the mother was a smoker, corresponding to lack of NIS autoregulation in the lactating mammary gland.

Previous studies

Studies of placental tissue samples have demonstrated that placenta contains iodine (4, 5, 6), and *in vitro* cell culture studies have suggested a functional role of NIS (10, 11) and SMVT (19) in placental iodide transport. In addition, the chloride–iodide transporter pendrin has been proposed to play a role (11).

Autoregulation of placental NIS in iodine-deficient rats was found by Schrøder van der Elst *et al.* (39), and Li *et al.* (40) demonstrated in the BeWo (human trophoblast) cell line that iodide suppressed NIS expression with a decrease in iodide uptake. They suggested that iodide inhibition of NIS expression might be through inhibition of human chorionic gonadotrophin (hCG) action on NIS expression (40). Thus, our results are consistent with previous findings of similarities between the iodide autoregulation of NIS in the thyroid and in the placenta.

Unlike iodide autoregulation, NIS in the placenta and in the thyroid may be regulated differently by other mechanisms. For example, TSH enhances NIS expression in the thyroid (12) whereas hCG enhances NIS expression in the placenta (10). Also, the paired-box transcription factor *PAX8* may affect thyroidal and placental NIS differently (41), and recently, a regulatory role of placental O_2 concentrations on NIS expression in placenta has been investigated (42). In addition, the functional role of pendrin in iodide transport might differ between the placenta and the thyroid (7).

Another transporter present in the placenta and involved in iodide transport is the SMVT. SMVT mediates the uptake of the micronutrients pantothenate, biotin, and lipoate in placental cells (43), and in SMVT-expressing oocytes, this transport was inhibited by iodide (19). Moreover, SMVT-mediated sodium-dependent iodide transport was demonstrated. Notably, the transport was insensitive to perchlorate (ClO_4^-), which is a known competitive NIS inhibitor (12), but the effect of thiocyanate was not investigated (19).

Burns *et al.* (5, 6) recently reported results indicating that placenta is not only involved in transport but also stores iodine and they suggested that this storage may protect against fetal iodine deficiency. It is unknown whether maternal smoking affects placental iodine content.

Strengths and limitations

This study is to our knowledge the first study evaluating autoregulation of placental iodide transport in a clinical setting. The number of pregnant women studied in the stratified analyses was rather low, but at the time of enrollment, the frequency of smoking among pregnant women in Denmark was much higher than today (44), making it very difficult to repeat the study. Also, as the study was conducted before the mandatory iodine fortification of salt in Denmark (32), the pregnant women suffered from mild-to-moderate iodine deficiency, which makes iodide transport by NIS or other placental iodide transporters more sensitive to the competitive inhibition by thiocyanate.

Thiocyanate crosses placenta as indicated by similar serum thiocyanate levels in maternal and cord serum (14). Thus, it seems unlikely that our results would be explained by lack of thiocyanate access to placental

iodide transporters. It has been demonstrated that thiocyanate inhibits NIS-mediated iodide transport in cultured placental cells (11) and structural similarities between NIS and SMVT exist (12); however, it cannot be excluded that placental iodide transport is primarily mediated by transporters and/or mechanisms not affected by thiocyanate. On the other hand, *in vivo* findings in rats (39) and *in vitro* findings in placental cultured cells of iodide-dependent regulation of NIS expression in the placenta (40) are consistent with our findings. Further studies are needed to clarify exact roles of different transporters in placental iodide transport.

We found higher serum Tg in smoking mothers and their newborns compared with the nonsmoking groups, but the effect of smoking was only significant in the group without maternal intake of iodine supplements. This observation is consistent with the biochemical characteristics of competitive inhibition; its effect is reduced by higher substrate concentration (45), in this case iodide.

Tg in maternal serum collected at the time of admission for delivery and in cord serum collected at delivery was used as a marker of iodine deficiency in mother and fetus respectively. Serum Tg was previously shown to be a sensitive marker of iodine deficiency in the general population (28, 29) and in pregnancy (27). Tg is a thyroid-specific protein but is also released from the thyroid gland due to other stimuli than iodine deficiency. However, none of the participants suffered from thyroid disease.

Conclusion

In a cohort of iodine-deficient pregnant women, maternal smoking increased serum Tg in both mother and fetus as a sign of aggravating iodine deficiency. However, the degree of iodine deficiency varied in parallel between smoking mother and fetus with no signs of a particular worsening in the fetus. The results therefore suggest autoregulation of placental iodide transport similar to the thyroid but in contrast to the lactating mammary gland, which further substantiates that iodide transporters might display tissue-specific autoregulation and inhibitory profiles. Moreover, it may indicate that iodide transporters other than NIS, insensitive to thiocyanate inhibition, are active in the human placental iodide transport.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Appendix E. Paper 2

Iodine deficiency in Danish pregnant women

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ABSTRACT

INTRODUCTION: Maternal iodine requirements increase during pregnancy. Studies performed before the introduction of mandatory iodine fortification of salt in Denmark in 2000 showed that pregnant women with no intake of iodine-containing supplements were moderately iodine-deficient and showed signs of thyroidal stress. We investigated the intake of iodine-containing supplements and urinary iodine excretion in Danish pregnant women after the introduction of iodine fortification of salt.

MATERIAL AND METHODS: We conducted a cross-sectional study between June and August 2012 in an area of Denmark where iodine deficiency had previously been moderate. Pregnant women coming to Aalborg University Hospital for obstetric ultrasound were recruited consecutively. Participants filled in a questionnaire and handed in a spot urine sample for measurement of iodine and creatinine.

RESULTS: Among the pregnant women included (n = 245), 84.1% reported an intake of iodine-containing supplements, and compared with those not taking iodine supplements the median urinary iodine concentration was significantly higher in this group: 109 g/l (25th-75th percentile: 66-191 µg/l). On the other hand, the median urinary iodine concentration was considerably below the recommended level, even for the non-pregnant state in pregnant women with no iodine supplement intake: 68 µg/l (35-93 µg/l), p < 0.001.

CONCLUSION: The majority of pregnant women took iodine-containing supplements, but the subgroup of non-users was still iodine-deficient after the introduction of iodine fortification of salt. Iodine supplement intake during pregnancy in Denmark should be officially recommended.

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TRIAL REGISTRATION: not relevant.

Adequate maternal iodine intake is required for thyroid hormone synthesis, and thyroid hormones, in turn, are essential for foetal growth and development, especially for early brain development [1, 2]. Maternal iodine requirements increase during pregnancy [3]. In their guidelines, the World Health Organization (WHO), the United Nations Children's Fund (UNICEF) and the International Council for the Control of Iodine Deficiency Disorders (ICCIDD) define an adequate intake of iodine during pregnancy as 250 µg/day, which corresponds to a median urinary iodine concentration of 150-249 µg/l in a population of pregnant women [4]. By contrast, a median urinary iodine concentration in the 100-199 µg/l range is considered a sufficient iodine intake in the non-pregnant state [4].

Iodine intake may stem from drinking water and from dietary iodine contents, from food fortified with iodine (typically salt), or from intake of iodine-containing supplements [5]. Denmark was previously iodine-deficient with regional differences caused by regional variation in the levels of iodine in the drinking water: moderate iodine deficiency was observed in Western Denmark and mild iodine deficiency in Eastern Denmark [6, 7]. A mandatory iodine fortification of salt was introduced in the year 2000 [8], and this programme had increased urinary iodine concentration to the lower threshold of the recommended level in the Danish population in 2004-2005 [9]. On the other hand, a recent study performed in 2008-2010 found that urinary iodine concentration had again decreased in the Danish population [10].

In studies of Danish pregnant women before the year 2000, pregnant women with no intake of iodine-containing supplements had a low urinary iodine concentration corresponding to moderate iodine deficiency, increased thyroid volume and high serum thyroglobulin (Tg), and their serum thyroid-stimulating hormone (TSH) levels increased during pregnancy [11, 12]. In a study conducted in five Danish cities [13], 36% of the pregnant women reported an intake of iodine-containing supplements when asked upon arrival for delivery. No previous study has specifically addressed the iodine intake and the use of iodine supplements during pregnancy in Denmark after the introduction of iodine fortification of salt.

The aim of the present study was to investigate if pregnant women living in an area of Denmark with previously moderate iodine deficiency took iodine-containing supplements, to examine predictors of iodine supplement intake and to evaluate iodine intake during pregnancy by measurement of urinary iodine concentration.

MATERIAL AND METHODS

Study population and design

We conducted a cross-sectional study between 13 June and 10 August 2012 in an area of Denmark with previously moderate iodine deficiency [8]. We consecutively recruited healthy, pregnant women referred to Aalborg

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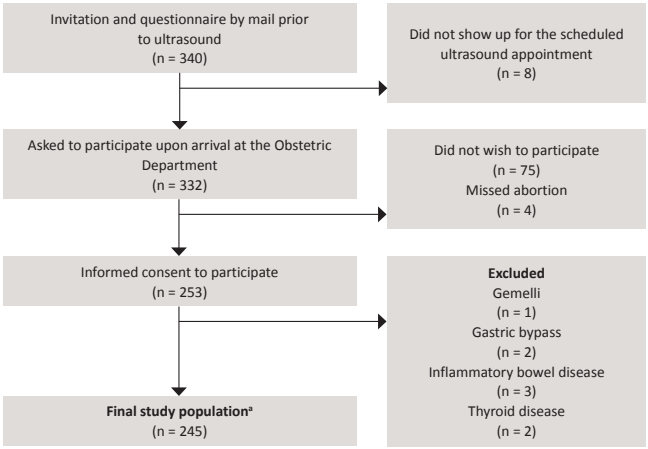
FIGURE 1

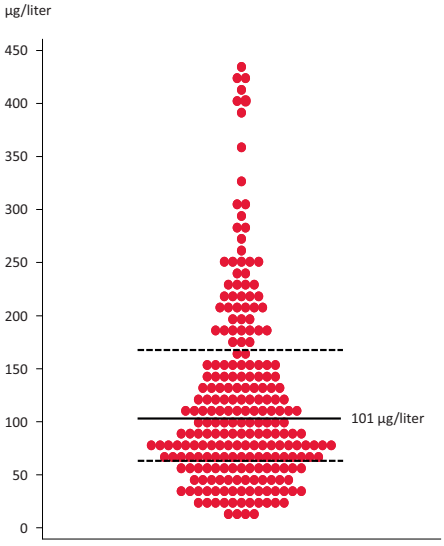
Flow chart illustrating the number of pregnant women invited, participating and included in the final study population.



a) Urinary samples (n = 238).

FIGURE 2

Overall urinary iodine excretion among the pregnant women (n = 238). Horizontal lines illustrate median (solid line) and interquartile range (dashed lines).



Urinary iodine concentrations in range from 460-1000 µg/liter (n=8) not illustrated.
Values were stratified into 40 bands in the range from 7-440 µg/liter; each band corresponding to 10.8 µg/liter.

University Hospital for obstetric ultrasound as part of the antenatal investigation programme [14]. Two of the pregnant women were excluded (**Figure 1**), because they had a diagnosis of hypothyroidism and took levothyroxin replacement therapy at the time of enrollment. None of the remaining women had a history of thyroid disease verified by a doctor.

After providing informed consent, participants were asked to fill out a questionnaire including obstetric history, socio-demographics and smoking habits, and to list detailed information on any medication and intake of vitamin and/or mineral supplements. Information about dietary supplements was verified by interview; and at the time of enrollment, the women were asked to provide a spot urine sample. The time of sampling was between 8 am and 1 pm, except for two women who had sampled the urine at home the same day prior to inclusion into the study (at 6:00 a.m. and 7:10 a.m., respectively). The study protocol was approved by the local ethics committee.

Laboratory procedures

Urine samples were stored at –20 °C until measurement of urinary iodine concentration in runs during the time of study inclusion. Urinary iodine concentrations (µg/l) were determined by the cerium/arsenite method after alkaline ashing, as previously described [15]. The analytical sensitivity was 2 µg/l. The iodine laboratory was certified by the U.S. Centers for Disease Control and Prevention's EQUIP Programme. Urinary creatinine concentrations (µmol/l) were measured immediately after urinary sampling on a Cobas 8,000 system. The equipment was calibrated according to the manufacturer's instructions, and external standards were included.

Statistical analyses

Characteristics of the pregnant women according to intake of iodine containing supplements were compared using Fisher's exact test, and predictors of iodine supplement intake were examined in multivariate logistic regression. Urinary iodine excretion was expressed as spot urine concentration (µg iodine/l), iodine/creatinine ratio (µg iodine/g creatinine) and estimated 24-h iodine excretion (µg iodine/24 h) calculated from the mean 24-h urinary creatinine excretion (1.09 g creatinine/24 h) previously measured in Danish pregnant women [11]. Urinary iodine concentrations showed a skewed distribution, and the results were expressed as medians with 25th and 75th percentiles. Non-parametric tests were used to compare urinary iodine excretion stratified by iodine supplement intake (Mann-Whitney test) and by gestational week (Kruskal-Wallis test). Statistical analyses were performed using Stata 11 (StataCorp, College Station, TX, USA), and a 5% level of significance was chosen.

Trial registration: not relevant.

RESULTS

Study population

A total of 245 women were included in the final study population (Figure 1), corresponding to 73.8% of the women invited to participate upon arrival at the Obstetric Department. The median age at the time of enrollment was 30.5 years (range 18.4-41.2 years), the median gestational age was week 20 (range week 10-37), and approximately half of the women were expecting their first child (**Table 1**).

Use of iodine-containing supplements

Nearly all of the pregnant women used dietary supplements (95.9%) at the time of enrollment, and 206 women (84.1%) reported a regular intake of iodine-containing supplements, whereas 39 women (15.9%) were taking either no vitamin and/or mineral supplements (n = 10) or vitamin and/or mineral supplements not containing iodine (n = 29).

The iodine containing supplements typically contained 175 µg iodine/day (81.1%); less often 150 µg iodine per day (17.0%); and a few women (1.9%) took iodine-containing supplements different from the recommended dose (87.5 and 375 µg iodine per day, respectively). The intake of the iodine supplement was often initiated during pregnancy (75.2%) at median gestational week 6 (range week 1-32), but some of the pregnant women had initiated iodine supplement intake in the year preceding the pregnancy (16.5%) at median 10 weeks before conception (range 2-52 weeks), or more than a year before conception (8.3%). Among iodine supplement users, 38 women reported intake of another iodine-containing supplement before their current supplement, and eight of the current non-users had stopped iodine supplement intake during the pregnancy before study inclusion.

Predictors of iodine supplement intake

In the univariate analyses, only maternal age significantly differed according to intake of iodine-containing supplements (Table 1). In the multivariate analyses including all variables listed in Table 1 as categoricals, lower maternal age (≤ 35 years, p = 0.001) and maternal education, i.e. qualifying for a profession (vocational or higher education versus primary/secondary school only, p = 0.039), were significant predictors of maternal intake of iodine-containing supplements. On the other hand, the total number of years of education did not significantly predict iodine supplement intake.

Urinary iodine excretion

The overall median urinary iodine concentration (**Figure 2**) was just within the recommended range for the non-pregnant state (100-199 µg/l), but below the recommended level during pregnancy (150-249 µg/l). The median urinary iodine concentration was higher in the group of pregnant women reporting an intake of iodine-

TABLE 1

Characteristics of the pregnant women in the final study population at the time of enrollment and according to intake of iodine-containing supplements.

	All		Iodine supplements		No iodine supplements		
	n	%	n	%	n	%	p[a]
Pregnant women	245	100.0	206	84.1	39	15.9	–
Gestational week at enrollment							0.51
10-15	87	35.5	74	35.9	13	33.3	
19-21	133	54.3	109	52.9	24	61.6	
28-37	25	10.2	23	11.2	2	5.1	
Parity[b]							0.47
1	132	53.9	112	54.4	20	51.3	
2	88	35.9	75	36.4	13	33.3	
≥ 3	25	10.2	19	9.2	6	15.4	
Age, years							0.006
< 25	27	11.0	24	11.7	3	7.7	
25-35	180	73.5	157	76.2	23	59.0	
> 35	38	15.5	25	12.1	13	33.3	
Cohabitation							1.00
Living with partner	233	95.1	196	95.2	37	94.9	
Not living with partner	12	4.9	10	4.8	2	5.1	
Ethnicity							1.00
Danish	227	92.7	191	92.7	36	92.3	
Other than Danish	18	7.3	15	7.3	3	7.7	
Education[c]							0.26
Basic	23	9.4	16	7.8	7	17.9	
Low	64	26.1	56	27.2	8	20.5	
Middle	98	40.0	83	40.3	15	38.5	
High	60	24.5	51	24.7	9	23.1	
Occupation							0.48
Employed	181	73.9	153	74.3	28	71.8	
Student	28	11.4	25	12.1	3	7.7	
Unemployed/not a student	36	14.7	28	13.6	8	20.5	
Pre-pregnancy BMI[d], kg/m²							0.36
< 25.0	143	59.3	119	58.6	24	63.2	
25.0-29.9	59	24.5	53	26.1	6	15.8	
≥ 30.0	39	16.2	31	15.3	8	21.0	
Smoking							0.92
Current	14	5.7	12	5.8	2	5.1	
Previous	82	33.5	70	34.0	12	30.8	
Never	149	60.8	124	60.2	25	64.1	
Organic milk[e]							0.26
Yes	79	32.2	63	30.6	16	41.0	
No	166	67.8	143	69.4	23	59.0	

BMI = body mass index.
a) Fisher's exact test: iodine supplements vs. no iodine supplements.
b) Previous live births and stillbirths including index pregnancy.
c) Highest educational level achieved or initiated. General education: "basic" (primary/secondary education only; 9-13 yrs). General education and education qualifying for a profession: "low" (vocational education and training: 9-13 yrs), "middle" (short- or medium cycle higher education: 14-16 yrs), "high" (long-cycle higher education: ≥ 17 yrs).
d) Missing value on BMI (n = 4) not included, 7 women had a BMI < 18.5 kg/m² (all iodine supplement users).
e) Do you mainly buy organic milk? (yes/no). Included as an indicator variable for maternal food-buying habits.

TABLE 2

Urinary iodine concentration, iodine/creatinine ratio and estimated 24-h iodine excretion according to maternal intake of iodine-containing supplements.

	Iodine supplements	No iodine supplements	p[a]
Pregnant women, n[b]	199	39	
Urinary iodine concentration, µg/l, median (25th-75th percentile)	109 (66-191)	68 (35-93)	< 0.001
Iodine/creatinine ratio, µg/g, median (25th-75th percentile)	153 (105-257)	73 (54-100)	< 0.001
Estimated 24-h iodine excretion, µg, median (25th-75th percentile)[c]	167 (114-280)	80 (59-109)	< 0.001

a) Mann-Whitney test: iodine supplements versus no iodine supplements.
b) Pregnant women with no urinary sample (n = 7) not included.
c) Calculated from 24-h urinary creatinine excretion previously measured in Danish pregnant women: 1.09 g creatinine/24 h [11].

containing supplements (**Table 2**) and below the lower recommended level for even the non-pregnant state in the group with no iodine supplement intake. The findings were similar when urinary iodine excretion was expressed as an iodine/creatinine ratio and estimated 24-h iodine excretion (Table 2). There was no significant difference in urinary iodine concentrations among iodine supplement users when stratified by gestational age; median urinary iodine week 10-15 (n = 73): 107 µg/l (25th-75th percentile: 66-197 µg/l), week 19-21 (n = 103): 102 µg/l (65-170 µg/l), week 28-37 (n = 23): 140 µg/l (91-252 µg/l), p = 0.06.

DISCUSSION

Principal findings

More than ten years after the introduction of mandatory iodine fortification of salt in Denmark, pregnant women living in an area previously characterized by moderate iodine deficiency had urinary iodine concentrations below the level recommended during pregnancy. As expected, pregnant women who took iodine-containing supplements had a higher median urinary iodine concentration than pregnant women with no intake of iodine supplements, and the median urinary iodine concentration was within the recommended level for the non-pregnant state in this group. The frequency of iodine supplement intake during pregnancy in Denmark had increased steeply compared with a previous study and was significantly predicted by maternal age and education. However, a subgroup of women still took no iodine supplements during pregnancy, and in this group the median urinary iodine concentration was considerably below the level recommended even for the non-pregnant state.

Iodine supplement use in Danish pregnant women

In a previous study [13] conducted in Denmark before the year 2000, nearly all pregnant women reported taking vitamin and/or mineral supplements (93.4%) when asked upon arrival for delivery, but only 36% reported an intake of iodine-containing supplements (150 µg iodine/day). Thus, the frequency of dietary supplement during pregnancy before the year 2000 was comparable to that observed in our study population (95.9%), but the use of iodine-containing supplements had increased considerably. The reasons for this increase are not completely clear. There are no current official recommendations on the intake of iodine-containing supplements during pregnancy in Denmark. Iodine supplement was obtained by intake of a multivitamin pill, and official recommendations on the intake of folic acid, vitamin D and iron during pregnancy do exist [14]. Thus, it seems likely that iodine supplement intake is incidental to other recommendations during pregnancy in Denmark. The gestational age of the women included in our study ranged 10-37 weeks. No previous study specifically addressed the use of iodine supplements in early pregnancy in Denmark.

Urinary iodine excretion in Danish pregnant women

Several of the previous studies on urinary iodine excretion in Danish pregnant women examined pregnant women living in Western Denmark, an area where moderate iodine deficiency was previously observed. In the studies by Pedersen et al [11, 12], the median urinary iodine concentration was low (approximately 50 and 40 µg/l in gestational weeks 17 and 37, respectively) and serum Tg was high in pregnant women with no intake of iodine-containing supplements. Furthermore, thyroid volume as well as TSH tended to increase during pregnancy. These changes were ameliorated by iodine supplement intake, and urinary iodine increased to 106 µg/l in late pregnancy after iodine supplement intake during the pregnancy [12], which was also shown in a study by Nohr et al [16].

Remember iodine!

Thus, our results indicate that the median urinary iodine concentration has increased in pregnant women with no iodine supplement intake, which corresponds to the general increase in the median urinary iodine concentration after the introduction of iodine fortification of salt in Denmark [9]. However, pregnant women with no intake of iodine-containing supplements still have urinary iodine concentrations substantially below the recommended level for pregnancy [4] and even below the recommended range for the non-pregnant state [4].

In the Danish population, the iodine fortification of salt increased urinary iodine to the lower threshold within the recommended range, but results from the DanThyr study (The Danish investigation on iodine intake and thyroid disease) [8] recently showed a modest decrease in urinary iodine concentration [10]. The present study confirms that the current level of iodization of salt in Denmark is insufficient, i.e. that it will not produce a urinary iodine concentration within the recommended range.

Strengths and limitations

Urinary iodine excretion is a recommended marker of recent iodine intake [4], and spot urine samples can be used in population studies [17]. The relatively large number of pregnant women included in our study increased the precision of the estimated iodine excretion [18]. However, the number of pregnant women in the stratified analyses was limited in some groups.

Our study only included women referred to Aalborg University Hospital, and we cannot exclude that differences in the use of dietary supplements during pregnancy may exist across Denmark. In a previous study performed in five Danish cities, the frequency of iodine supplement intake during pregnancy ranged from 21% to 50% when stratified by city [13]. The level of iodine in drinking water and urinary iodine excretion in the area investigated in our study was previously reported to correspond to the level observed in most parts of Western Denmark (i.e. west of the Great Belt) [6, 7].

We consecutively recruited healthy, pregnant women and the rate of participation was high. However, we cannot exclude some degree of selection bias.

CONCLUSION

Danish pregnant women living in an area previously characterized by moderate iodine deficiency still have urinary iodine concentrations below the recommended level for the pregnant state after the introduction of the mandatory iodine fortification of salt in Denmark. Pregnant women with no intake of iodine-containing supplements are at particular risk of insufficient iodine intake. Results indicate a need for more attention among healthcare professionals to ensure sufficient iodine status in Danish pregnant women. Intake of iodine-containing supplements during pregnancy in Denmark should be officially recommended, and our results may indicate a need for a modest increase in the level of iodine added to salt in Denmark.

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Jodmangel hos gravide

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INTRODUKTION

Jod indgår i syntesen af thyroideahormoner, som er essentielle udviklingsfaktorer, specielt hvad angår hjernens udvikling. Jodbehovet øges under graviditet, og WHO, UNICEF og ICCIDD definerer et tilstrækkeligt jodindtag ved en medianurinjodkoncentration på 150-249 µg/l i en population af gravide. I modsætning hertil vurderes et sufficient jodindtag hos ikkegravide at være en medianurinjodkoncentration på 100-199 µg/l.

I Danmark var der tidligere jodmangel med regionale forskelle: mild jodmangel i det østlige Danmark og moderat jodmangel i det vestlige Danmark. Den obligatoriske jodberigelse af salt blev indført i 2000 og førte til en stigning i medianurinjodkoncentrationen i den danske befolkning målt i 2004-2005, men urinjodkoncentrationen var dog i seneste undersøgelse fra 2008-2010 igen lidt faldende.

Tidligere undersøgelser af jodindtag hos gravide i Danmark ligger alle før den danske jodberigelse af salt. På daværende tidspunkt var det 36% af danske gravide, som tog jodholdigt kosttilskud, og gruppen af gravide, der ikke tog jodholdigt kosttilskud, havde moderat jodmangel og tegn på jodmangelinducerede ændringer i glandula thyroideas funktion og struktur med høj thyroglobulinkoncentration samt stigende koncentration af thyroideastimulerende hormon og stigende thyroideavolumen under graviditeten.

Formålet med vores undersøgelse var at belyse, om gravide i Aalborgområdet i dag tager jodholdigt kosttilskud, samt at vurdere deres jodindtag ved måling af urinjodkoncentration.

Remember iodine!

MATERIALE OG METODER

Vi udførte en tværsnitsundersøgelse ved Aalborg Universitetshospital fra juni til august 2012 og inkluderede gravide, der var henvist til obstetrisk ultralydsskanning som led i et normalt svangreforløb. Undersøgelsen omfattede besvarelse af et spørgeskema, og oplysninger om indtag af kosttilskud blev verificeret ved et interview på inklusionsdagen, hvor også en urinprøve blev afleveret. Urinprøven blev analyseret for jod og kreatinin.

RESULTATER

I alt 245 gravide blev inkluderet i undersøgelsen (graviditetsuge 10-37, medianuge 20). På inklusionstidspunktet angav 84,1% af de gravide, at de indtog et jodholdigt kosttilskud, mens en mindre gruppe ikke tog jodholdigt kosttilskud (15,9%). Alder over 35 år og lavt uddannelsesniveau prædikterede manglende indtag af jodholdigt kosttilskud.

Medianurinjodkoncentrationen var 101 µg/l (25-75-percentiler: 63-167 µg/l) og således under det anbefalede niveau for gravide. Urinjodkoncentrationen var signifikant højere blandt gravide, som tog jodholdigt kosttilskud: median 109 µg/l (25-75-percentiler: 66-191 µg/l), og var i denne gruppe inden for det anbefalede niveau for ikkegravide. Derimod havde gruppen af gravide uden indtag af jodholdigt kosttilskud en medianurinjodkoncentration, der var væsentligt under det anbefalede niveau for ikkegravide: 68 µg/l (25-75-percentiler: 35-93 µg/l), $p < 0,001$.

KONKLUSION

Mange gravide tog jodholdigt kosttilskud, men en undergruppe af gravide gjorde ikke, og denne gruppe havde fortsat jodmangel efter den danske jodberigelse af salt. Der er behov for jodholdigt kosttilskud under graviditet i Danmark, hvilket officielt bør anbefales, og resultaterne bidrager sammenholdt med den seneste befolkningsundersøgelse til overvejelser om, hvorvidt jodtilsætningen til salt i Danmark bør øges.

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INTERESSEKONFLIKTER: Forfatternes ICMJE-formularer er tilgængelige sammen med artiklen på Ugeskriftet.dk.

DANISH MEDICAL JOURNAL: Dette er et resume af en originalartikel publiceret på danmedj.dk som Dan Med J 2013;60(7):A4657.

Appendix F. Paper 3

Clinical Thyroidology / Original Paper

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Challenges in the Evaluation of Urinary Iodine Status in Pregnancy: The Importance of Iodine Supplement Intake and Time of Sampling

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Key Words
Urinary iodine concentration · Urinary creatinine concentration · 24-Hour urinary iodine excretion · Pregnancy · Household · Iodine supplement

Abstract
Objectives: Median urinary iodine concentration (UIC) is the recommended method to evaluate iodine status in pregnancy, but several factors may challenge the interpretation of the results. We evaluated UIC in pregnant women according to (1) sampling in the hospital versus at home, (2) time of the most recent iodine supplement intake prior to sampling, and (3) members of their household. ***Study Design:*** Danish cross-sectional study in the year 2012. Pregnant women (n = 158), their male partners (n = 157) and children (n = 51) provided a questionnaire with detailed information on iodine supplement intake and a spot urine sample obtained in the hospital and/or at home for measurement of UIC and urinary creatinine concentration. ***Results:*** In the pregnant women providing a urine sample both in the hospital and at home (n = 66), individual UIC (p = 0.002) and urinary creatinine concentration (p = 0.042), but not estimated 24-hour urinary iodine excretion (p = 0.79), were higher when sampling was at home. Median UIC was dependent on the time of the most recent iodine supplement intake prior to sampling [same day (n = 79): 150 μg/l (95% CI 131–181 μg/l), the day before (n = 51): 105 μg/l (78–131 μg/l), several days ago/non-user (n = 28): 70 μg/l (56–94 μg/l), p < 0.001]. The pattern was similar in the male partners. Apart from a more frequent iodine supplement intake in pregnancy (87.3% vs. partners 15.9%), no systematic differences were observed in urinary measurements between the pregnant women and their partners. ***Conclusions:*** Time of spot urine sampling and time span from iodine supplement intake to spot urine sampling should be considered when evaluating urinary iodine status in pregnancy.

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Introduction

Population median urinary iodine concentration (UIC) is the recommended method to assess iodine status [1]. UIC in pregnancy is extensively studied and adequate maternal iodine intake is of major concern [2–4]. Guidelines recommend the use of spot urine samples and the estimation of median UIC in a population of pregnant women, and results are compared to the recommended

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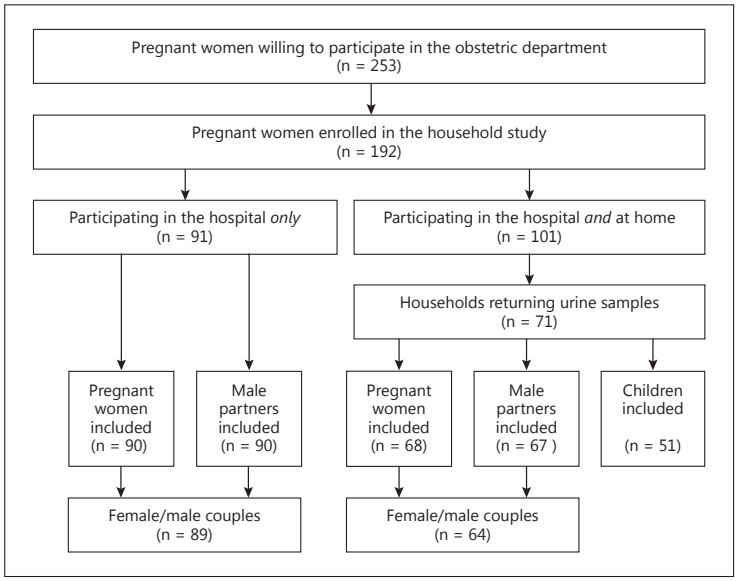

Fig. 1. Flowchart illustrating the selection of participants. Exclusions in the group 'participating in the hospital only' were: no urine sample from the pregnant woman (n = 1) and not a male partner (n = 1). Exclusions in the group 'participating in the hospital and at home' were: pregnant woman with gastric bypass (n = 1), pregnant women with inflammatory bowel disease (n = 1), pregnant woman with inconsistent information on iodine supplement intake between samplings (n = 1), partner did not complete the questionnaire (n = 2), and not a male partner (n = 2).

level during pregnancy (150–249 μg/l) defined by the World Health Organization (WHO), the United Nation Children's Fund (UNICEF) and the International Council for the Control of Iodine Deficiency Disorders (ICCIDD) [1]. Another method to study iodine intake is to measure iodine excretion in a full 24-hour urine collection or to estimate 24-hour iodine excretion based on measurement of both iodine and creatinine concentration in a spot urine sample [5].

Physiological changes occur in both thyroid and renal function during pregnancy which may challenge the interpretation of the results [6]. We previously reported that iodine supplement intake in Danish pregnant women was frequent, but median UIC was low and below the level recommended in pregnancy both in iodine supplement users and non-users, and also when UIC was adjusted by urinary creatinine to estimate 24-hour urinary iodine excretion [7].

In our study [7] and in the majority of studies evaluating iodine status in pregnant women, urine samples are obtained during a routine antenatal hospital visit. We speculated whether urine samples obtained at hospital were representative of daily life in pregnant women. Thus, we expanded our previous study to include both a urine sample obtained during the hospital visit for routine antenatal ultrasound in pregnancy and a urine sample obtained a subsequent day, when the pregnant woman was at home.

It has been reported that the median UIC in family members such as schoolchildren are not representative for UIC in pregnant women [8]. We speculated whether at least some of this difference might be caused by the pregnant women and their family member being investigated under different conditions. To evaluate this hypothesis, we sampled urine for iodine measurements both from the pregnant women and from members of their household under identical conditions.

In our previous study [7], the majority of pregnant women took iodine supplements. Finally, we speculated whether the timing of the most recent iodine supplement intake prior to spot urine sampling would have an influence on the UIC obtained and thus the results of iodine status evaluation.

Materials and Methods

Study Population and Design

The pregnant women enrolled in the present study took part in our investigation on iodine intake during pregnancy in an area of Denmark with previously moderate iodine deficiency [7]. From June 13 to August 10, 2012, we consecutively recruited healthy, pregnant women referred to Aalborg University Hospital for ob-

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Table 1. Characteristics of the pregnant women and their household members at the time of inclusion in the study

	Pregnant women (n = 158)	Partners (n = 157)	Children (n = 51)
Male gender	NA	157 (100.0)	26 (51.0)
Danish ethnicity[a]	151 (95.6)	149 (94.9)	49 (96.1)
Age, years	30 (19–41)	32 (23–49)	6 (1–14)
Weight, kg[b]	67 (48–114)	85 (56–135)	19 (9–70)
Height, cm[c]	169 (151–182)	183 (166–201)	115 (74–169)
BMI[d]	23.4 (17.6–41.0)	25.3 (19.2–39.9)	15.5 (12.7–29.1)
<25	93 (60.0)	70 (45.2)	45 (97.8)
25–29.9	36 (23.2)	69 (44.5)	1 (2.2)
≥30	26 (16.8)	16 (10.3)	0 (0)
Smoking[e]			
Current	6 (3.8)	27 (17.3)	NA
Previous	52 (32.9)	33 (21.2)	NA
Never	100 (63.3)	96 (61.5)	NA
Educational level[f]			
Basic	11 (7.0)	26 (16.6)	NA
Low	21 (13.3)	43 (27.4)	NA
Middle	81 (51.3)	47 (29.9)	NA
High	45 (28.4)	41 (26.1)	NA
Supplement not containing iodine	13 (8.2)	15 (9.6)	2 (3.9)
Iodine supplement	138 (87.3)	25 (15.9)	13 (25.5)
175 μg/day	111	0	0
150 μg/day	24	22	0
70 μg/day	0	0	12
Others[g]	3	3	1

Values are n, n (%) or median (range). [a] Two children had 1 parent of Danish origin and 1 parent of non-Danish origin. [b] Values are pre-pregnancy weight for the pregnant women. Missing values on weight (n = 7) not included. [c] Missing values on height (n = 6) not included. [d] Missing values on BMI (n = 9) not included. Pre-pregnancy BMI for the pregnant women. [e] Missing value on smoking (n = 1) not included. [f] Highest educational level achieved or initiated. 'Basic' (primary/secondary education only; 9–13 years), 'low' (vocational education and training: 9–13 years), 'middle' (short or medium cycle higher education: 14–16 years), 'high' (long cycle higher education: ≥17 years). [g] Pregnant women: 87.5 μg/day (n = 2), 350 μg/day (n = 1); partners: 300 μg/week (n = 1), 300 μg/day (n = 2); children: 75 μg/day (n = 1).

stetric ultrasound as part of the antenatal investigation program (fig. 1). After informed consent, the women were asked to fill out a questionnaire, to list detailed information on dietary supplements and to deliver a spot urine sample.

Members of their household were recruited in one of the following ways: (1) the partner filled out a questionnaire and delivered a spot urine sample in the hospital at the same time as the pregnant woman or (2) only the pregnant woman participated in the hospital and questionnaires and vials for urine sampling were handed out to the pregnant woman and her household members for sampling at home (fig. 1). Thus, 66 pregnant women participating with the household at home delivered a urine sample both at the time of inclusion in the hospital and at home (2 women only delivered a urine sample at home). Participants were instructed to perform non-fasting urine sampling as close in time as possible for all household members and to list information on iodine supplement intake including the time of the most recent supplement intake prior to urine sampling.

The study protocol was approved by the local ethical committee.

Laboratory Procedures

Urine samples were stored at –20°C until measurement of UIC in runs during the time of study inclusion. UIC was determined by the cerium/arsenite method after alkaline ashing, as previously described [9]. The analytical sensitivity was 2 μg/l and the recovery of iodine was 95.5% (SEM 2.4%). When a urine sample (UIC 93.9 μg/l) was measured in triplicates in 18 assays, the intra-assay CV was 2.1% and the inter-assay CV was 2.7% for single determination [10]. The iodine laboratory was certified by the US Centers for Disease Control and Prevention EQUIP program. Urinary creatinine concentrations were measured on a Cobas 8000 system (Roche,

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Table 2. UIC, urinary creatinine concentration and estimated 24-hour iodine excretion in Danish pregnant women and their household members

	All participants			Iodine supplement			No iodine supplement			p[a]
	n	median	IQR	n	median	IQR	n	median	IQR	
UIC, µg/l										
Pregnant women	158	119	67–180	138	130	69–203	20	76	55–132	0.008
Sampling in the hospital	90	105	59–208	79	112	65–213	11	59	21–93	0.021
Sampling at home	68	134	85–177	59	136	93–180	9	98	62–132	0.10
Male partners	157	91	58–124	25	110	74–164	132	91	57–123	0.12
Sampling in the hospital	90	75	51–111	15	91	36–139	75	72	51–108	0.26
Sampling at home	67	115	80–150	10	136	91–175	57	110	77–137	0.23
Children	51	126	102–157	13	151	116–202	38	121	98–150	0.035
Urinary creatinine concentration, mmol/l[b]										
Pregnant women	158	7.6	4.2–12.6	138	7.2	3.9–11.8	20	11.8	5.7–14.2	0.051
Sampling in the hospital	90	6.4	3.4–11.1	79	5.9	3.4–10.6	11	9.1	3.6–14.3	0.30
Sampling at home	68	9.2	4.8–12.8	59	8.1	4.5–12.7	9	13.1	10.8–14.2	0.052
Male partners	157	13.1	8.0–17.4	25	14.6	5.5–17.5	132	13.0	8.1–17.5	0.89
Sampling in the hospital	90	11.5	7.4–16.3	15	13.6	3.4–15.7	75	11.3	7.4–16.4	0.90
Sampling at home	67	14.6	9.6–19.2	10	15.7	12.6–26.7	57	14.6	9.6–18.9	0.62
Children	51	7.2	4.8–10.3	13	6.3	4.7–7.8	38	7.4	5.1–11.6	0.28
Estimated 24-hour iodine excretion, µg/24 h										
Pregnant women[c]	158	164	109–263	138	174	123–278	20	86	56–100	<0.001
Sampling in the hospital	90	164	114–309	79	172	121–346	11	83	54–109	<0.001
Sampling at home	68	159	105–211	60	175	123–228	9	87	77–96	<0.001
Male partners[d]	157	113	85–145	25	136	102–217	132	110	82–137	0.013
Sampling in the hospital	90	105	82–136	15	136	102–323	75	103	81–125	0.020
Sampling at home	67	122	92–160	10	137	94–196	57	122	86–145	0.31
Children[e]	51	63	49–101	13	66	59–103	38	63	48–82	0.19

[a] p value from the Mann-Whitney U test: iodine supplement vs. no iodine supplement. [b] Urinary creatinine: 1 mmol/l = 0.1131 µg/l. [c] Estimated from the 24-hour urinary creatinine excretion previously measured in Danish pregnant women: mean 1.09 g/24 h [11]. [d] Estimated from the 24-hour urinary creatinine excretion previously measured in a Belgian population of men aged 25–49 years: mean 1.74 g/24 h [12]. [e] Estimated from the 24-hour urinary creatinine excretion previously measured in a German population of children [13].

Rotkreuz, Switzerland). Equipment was calibrated according to the manufacturer's instructions and external standards were included.

Statistical Analyses

Urinary iodine excretion was expressed as spot urine concentration (µg iodine/l) and as estimated 24-hour iodine excretion (µg iodine/24 h) calculated from the reported mean 24-hour urinary creatinine excretion in a group of Danish pregnant women (1.09 g creatinine/24 h) [11], Belgian men aged 25–49 years (1.74 g creatinine/24 h) [12], and German children [13] (mean values by gender and height; missing height (n = 4) was substituted by the average height for age in Danish children [14]).

UIC showed skewed distribution, and results were expressed as medians with 25th and 75th percentiles (interquartile range, IQR) or 95% confidence interval (95% CI). The Mann-Whitney U test was used to compare median urinary measurements by independent groups (iodine supplement vs. no iodine supplement and hospital vs. at home), and the Kruskal-Wallis test was used for comparison by time of the most recent iodine supplement intake. For paired analyses (two samples from the same pregnant woman and pregnant woman vs. male partner), the Wilcoxon signed-rank test was applied. For linear regression, logarithmically transformed urinary measurements were used.

Statistical analyses were performed using Stata 11 (StataCorp, College Station, Tex., USA) and a 5% level of significance was chosen.

Results

Study Population

Among the pregnant women enrolled in the household study (fig. 1), the median gestational week at the initial inclusion in the hospital was week 20 (range 11–37),

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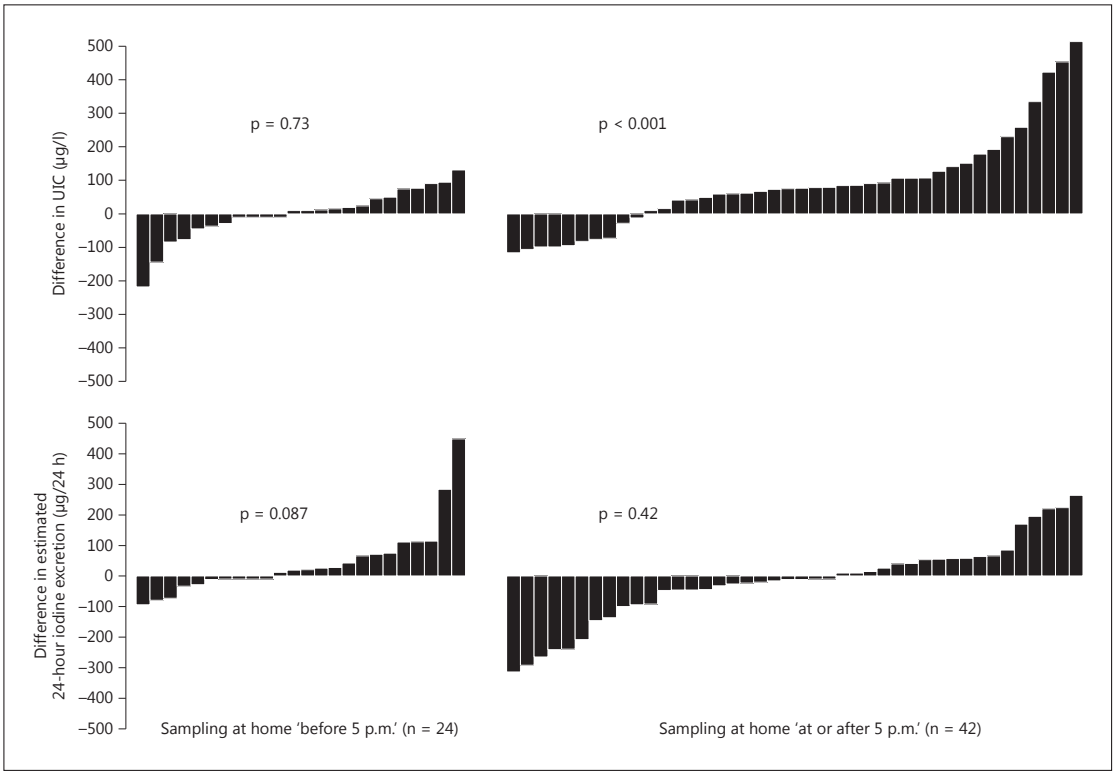

Fig. 2. Bar charts illustrating the value at home minus the value in the hospital in UIC (upper row) and estimated 24-hour urinary iodine excretion (lower row) for the 66 pregnant women who delivered a spot urine sample both in the hospital at inclusion and later at home. Results are stratified according to the time of sampling at home: left column was before 5 p.m. and right column was at or after 5 p.m. Differences in the range from –1 to –9 (n = 6) were set to –10 and differences in the range from 0 to 9 (n = 5) were set to 10 for illustration. p values are results of the Wilcoxon signed-rank test: at home versus in the hospital.

and 62% were expecting their first child. Table 1 describes characteristics of the pregnant women and their household members. Current smoking was more frequent among the male partners, and also the frequency of dietary supplement intake differed. Intake of iodine supplement in pregnancy was common, and the iodine supplements most frequently used contained 175 µg iodine/day. On the other hand, iodine supplement intake was less common among both male partners and children, and the iodine supplements used contained less iodine.

Urinary Measurements

Overall, the median UIC among adults (pregnant women and male partners, n = 315) was 104 µg/l (IQR 61–150) and the estimated 24-hour iodine excretion was 129 µg/24 h (IQR 95–193). In general, both median UIC and estimated 24-hour urinary iodine excretion were higher when iodine supplement was used (table 2).

Urine Sampling in the Hospital versus at Home

In the pregnant women and in the male partners, both UIC and urinary creatinine concentration (table 2) tended to be higher at home than in the hospital (at home vs. hospital: median UIC all pregnant women p = 0.17, all male partners p < 0.001; median urinary creatinine concentration: all pregnant women p = 0.060, all male partners p = 0.006). On the other hand, when urinary creatinine concentration was used to estimated 24-hour uri-

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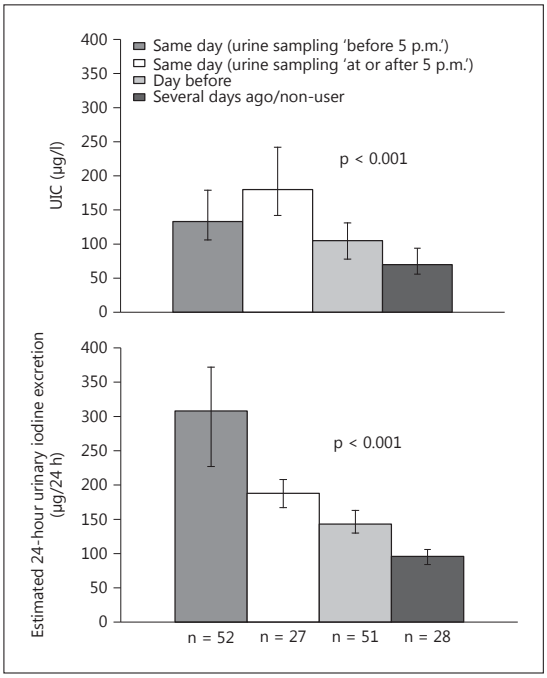

Fig. 3. Median (95% CI) UIC (upper figure) and estimated 24-hour urinary iodine excretion (lower figure) in 158 pregnant women stratified by time of the most recent iodine supplement intake prior to urine sampling. p values are results of the Kruskal-Wallis test: the same day with sampling before 5 p.m. versus the same day with sampling at or after 5 p.m. versus the day before versus several days ago/non-user.

nary iodine excretion, the differences in results diminished (all pregnant women p = 0.25, all male partners p = 0.10).

Individual comparison in the 66 pregnant women who delivered a urine sample both in the hospital and at home also showed that UIC and urinary creatinine concentration were higher at home (median UIC 133 µg/l vs. in the hospital 84 µg/l, p = 0.002; median urinary creatinine concentration 9.2 vs. 5.7 mmol/l in the hospital, p = 0.042), whereas no difference in estimated 24-hour urinary iodine excretion was observed (159 vs. 154 µg/24 h at home, p = 0.79).

In a post hoc analysis, we evaluated whether the time of urine sampling could be involved in our findings. All children delivered the urine sample at home, and their time of sampling was at median 5 p.m. (IQR 4–6 p.m.). The pregnant women and male partners participated either in the hospital or at home, and the time of sampling differed between the two locations: hospital: median 10 a.m. (IQR 9–11 a.m.), home: median 6 p.m. (IQR 3–8 p.m.). Thus, the majority of families had performed the sampling at home in the afternoon or evening, whereas sampling in the hospital was in the morning.

We focused our post hoc analysis on the 66 pregnant women who delivered a urine sample both in the hospital and at home. Differences in UIC and estimated 24-hour iodine excretion in the 66 pregnant women who delivered a urine sample both in the hospital and at home are depicted in figure 2. The women were stratified according to the time of sampling at home: 'before 5 p.m.' (median 2 p.m., IQR 10 a.m. to 4 p.m.) and 'at or after 5 p.m.' (median 7 p.m., IQR 6–9 p.m.). No difference was observed in UIC and estimated 24-hour iodine excretion between hospital and home samples when sampling at home was performed 'before 5 p.m.'. On the other hand, when sampling at home was performed 'at or after 5 p.m.', UIC was significantly higher at home, whereas no difference was observed in estimated 24-hour iodine excretion.

Pregnant Women versus Members of Their Household

We performed a pairwise comparison of UIC in female/male couples stratified according to iodine supplement intake. In the largest group, where only the pregnant woman took iodine supplement (n = 109 couples), UIC was significantly higher in the pregnant woman than in the male partner as might be expected (median UIC 130 vs. 91 µg/l in the male partners, p < 0.001). On the other hand, no significant difference in UIC between the pregnant woman and her male partner was observed, when both (n = 24 couples; median UIC 122 vs. 91 µg/l in the male partners, p = 0.90) or none (n = 17 couples; median UIC 70 vs. 95 µg/l in the male partners, p = 0.20) took iodine supplement, with similar results for estimated 24-hour urinary iodine excretion (data not shown).

Median UIC in the children (table 2) was not significantly different from the median UIC in the pregnant women (p = 0.89) and in the male partners (p = 0.074) sampling at home and results were similar when stratified by iodine supplement intake (data not shown). In multivariate linear regression including age of the child (grouped by quartiles), gender of the child (male/female) and iodine supplement intake (yes/no), higher age was a significant predictor of higher urinary creatinine concentration (p < 0.001), but not UIC (p = 0.45) and 24-hour estimated iodine excretion (p = 0.66). Gender of the child was not a significant predictor of UIC (p = 0.55).

Altogether 29 families delivered a urine sample from the pregnant woman, the male partner and 1–3 children.

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Multivariate linear regression including iodine supplement intake and type of family member did not indicate that UIC was significantly different between family members [pregnant woman (reference), male partner (p = 0.81), child (p = 0.50)].

Time from Iodine Supplement Intake to Urine Sampling

Both UIC and estimated 24-hour iodine excretion were influenced by the timing of the most recent iodine supplement intake prior to urine sampling (fig. 3). Median values were higher when iodine supplement intake was the same day prior to urine sampling (first and second bar). Looking only at groups with iodine supplement intake the same day as urine sampling (first and second bar), results were different for UIC and estimated 24-hour iodine excretion (fig. 3). Considering this in relation to figure 2, UIC was influenced by both the time of sampling and the time of the most recent iodine supplement intake, whereas mainly the time of the most recent iodine supplement intake influenced estimated 24-hour iodine excretion.

Among the male partners, the trend was similar to that observed in pregnancy [iodine supplement intake the same day (n = 10): median UIC 152 μg/l (95% CI 94–296 μg/l), the day before (n = 11): 124 μg/l (34–175 μg/l), several days ago/non-user (n = 134): 90 μg/l (77–101 μg/l), p = 0.004].

Sensitivity Analyses

For the 66 pregnant women who delivered two urine samples, the interval between the two samples was median 16 days (IQR 6–29). When analyses were stratified by the median time between samplings, results were the same as depicted in figure 2 (data not shown). None of the 66 women had made any change in iodine supplement intake between the two samples, and 57 women took iodine supplement. Considering the time of the most recent iodine supplement intake prior to urine sampling, results (fig. 2) were similar when analyses were restricted to the 44 women with iodine supplement the same time prior to both the first and the second sampling (data not shown).

Discussion

Principle Findings

This study evaluates urinary iodine status in Danish pregnant women according to the location and time of spot urine sampling, the time of the most recent iodine supplement intake prior to spot urine sampling and in comparison to members of the household. Differences in urinary measurements between the hospital and at home sampling were largely explained by differences in time of sampling during the day. UIC and urinary creatinine concentration were higher when sampling was performed in the evening, but when UIC was adjusted by urinary creatinine, the time-dependent differences leveled out. The time of the most recent iodine supplement intake prior to sampling was a predictor of UIC and in general, no systematic differences were observed between the pregnant woman and her male partner except that iodine supplement use was much more frequent in pregnancy.

Iodine Metabolism in the Pregnant versus the Non-Pregnant State

Inorganic iodine is almost completely absorbed from the gastrointestinal tract [15], and plasma inorganic iodine is primarily balanced by iodine intake, transfer of iodine into the thyroid gland and renal excretion of iodine. UIC reflects iodine intake as >90% of ingested iodine is excreted into the urine [1]. Physiologically, the pregnant state differs from the non-pregnant state by: iodine transport to the fetus across the placenta [16], increased use of iodine in the thyroid gland [17], and increased renal excretion of iodine (the glomerular filtration is 50% higher by the end of first trimester which is maintained throughout pregnancy [18]). Following this, the recommended range for median UIC in a population of pregnant women is 150–249 μg/l, whereas in the non-pregnant state, median UIC in the range from 100 to 199 μg/l indicates adequate iodine intake [1].

In many populations, the use of iodine supplements in pregnancy is important to ensure adequate iodine intake [7, 19, 20], and it is of particular importance in women with a low intake of iodine-containing foods. Severe iodine deficiency in pregnancy can cause fetal brain damage [1], but recent studies have shown that also less severe iodine deficiency in pregnant women may associate with lower cognitive scores in the child [3, 4].

UIC and Time of Sampling

Controversies in urinary iodine determinations have been discussed for years [21]. One aspect is the time of sampling during the day. In a Danish study of 22 individuals [22], estimated 24-hour iodine excretion from a fasting morning spot urine sample was significantly lower than the actual iodine excretion measured in a 24-hour sample. In a study from Switzerland [23], a total of 3,023 spot urine samples were collected from 42 indi-

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viduals, and a circadian rhythm in UIC was observed with the lowest levels in the morning and increasing levels in the afternoon and evening. UIC peaks occurred 4–5 h after main meals, and the time of the peaks was different for children and adults. As discussed by the authors, this may reflect different intake of iodine-containing food and a close relationship between recent iodine intake and the UIC profile. In our study, both UIC and urinary creatinine concentration were higher in samples obtained at or after 5 p.m. We speculate whether the evening meal may have influenced UIC and urinary creatinine concentration in samples obtained in the evening and/or if the urine production was smaller at that time of the day.

The 'golden standard' to measure urinary iodine excretion is to collect one or preferably several full 24-hour urine samples [1]. This would alleviate problems related to the time span between meals, supplement intake and spot urine sampling. However, a 24-hour full urine collection is often unrealistic in field studies and optimally an objective marker such as *p*-aminobenzoic acid should be used to verify the completeness [24].

UIC and Recent Iodine Supplement Intake

In a study of 16 lactating women, Leung et al. [25] showed that an acute, high intake of iodine gave rise to an increase in breast milk iodine concentration with a peak median 6 h after ingestion, but median UIC remained stable over the study period (8 h). We recently showed [26] that breast milk iodine concentration was dependent on the time of the most recent iodine supplement intake (not acute high dose) in a similar pattern to what we now illustrate for median UIC in both the pregnant women and their male partners. However, more studies are needed to corroborate results both in pregnant and non-pregnant populations.

UIC in Pregnant Women versus Male Partners

The mandatory iodine fortification of salt was introduced in Denmark in the year 2000, and the Danish investigation of iodine intake and thyroid disease (DanThyr) has monitored the iodine excretion in two regions of Denmark with a different iodine intake before the iodine fortification (moderate vs. mild iodine deficiency) [10]. The most recent data after the iodine fortification (2008–2010) have shown an overall increase in iodine excretion in both regions, but with a median UIC just below the level recommended [10]. The DanThyr study initially included women aged 18–65 years (mainly non-pregnant) and men aged 60–65 years, urine samples were obtained between 8.00 a.m. and 5.30 p.m., and 35% of participants used iodine supplements [10]. We examined only pregnant women, men in younger age groups, and part of the urine samples were obtained after 5.30 p.m. Median UIC (women and men combined) was higher in our study (104 μg/l vs. DanThyr 83 μg/l [10]), which could be explained by the higher frequency of iodine supplement use, the later time of sampling, or both. When looking at the estimated 24-hour iodine excretion (129 μg/24 h vs. DanThyr 125 μg/24 h [10]) or at median UIC among no iodine supplement users only (89 μg/l vs. DanThyr 74 μg/l [27]), the difference in study results was less pronounced.

The only disparity between the pregnant women and their male partners that we observed was the more frequent use of iodine supplements in pregnancy. The couples appeared similar in terms of differences in urinary concentrations according to the time of sampling and the time of the most recent iodine supplement intake, and no significant differences were observed in the pairwise comparison of UIC when they both used or did not use iodine supplement. UIC reflects recent iodine intake, and our results indicate that the pregnant women and their male partners had comparable intake of iodine. Living in the same household implies the same drinking water and also to some extent the same diet. In our Danish study population, it did not seem as if the pregnant woman had changed her diet to a considerably different iodine intake from the male partner.

UIC in Pregnant Women versus Children

Traditionally, the median UIC in schoolchildren has been the recommended method for the assessment of iodine status in populations, including pregnant women [15]. A few studies have evaluated median UIC in schoolchildren as a proxy for iodine status in pregnancy in a household design [8, 28]. In these studies, median UIC was higher in the children than in the pregnant women suggesting that median UIC in school-aged children should not be used for monitoring iodine status in pregnancy. In general, the median UIC in children is often higher than that of pregnant women, although it may be the other way around [29]. Notably, none of the studies reported on the time of urine sampling. In one study [8], urine from the pregnant woman was sampled in the antenatal clinic or factory, whereas the child sampled at home. Considering our results, this disparity may itself lead to differences in results when looking at UIC alone and not adjusted by urinary creatinine.

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Our study is the first study to report data on urinary iodine excretion in Danish children. The median UIC was within the level recommended in both iodine supplement users and non-users [1]. We were able to compare household members who sampled at home around the same time, and we found no significant difference in UIC when comparing children to pregnant (and non-pregnant) adults. However, the number of children was limited and an examination of schoolchildren in Denmark is needed. More studies in different populations should be performed to clarify the use of non-pregnant populations as a proxy for iodine status in pregnancy.

Perspective

This study was designed to evaluate some of the challenges in the interpretation of urinary iodine status in pregnancy. Although the number of pregnant women and household members recruited was sufficient for the evaluation of median UIC in a population [30], the disparity in use of iodine supplements among pregnant women and male partners made the numbers small in some of the stratified analyses.

The family members were instructed to perform the urine sampling at home as close in time as possible, and to facilitate this, the time of sampling was not specified except that they were instructed to make the urine samples non-fasting. Following this design, the majority of families had sampled the urine in the afternoon or evening. In our post hoc analyses it appeared that the time of sampling did influence the urinary measurements obtained, and to evaluate the possible role of time and location of sampling in more detail, it is preferable to design a study where the time of sampling is fixed and similar in both locations.

More studies are needed to corroborate results, but the findings advocate that studies evaluating iodine status in pregnant women (as well as in non-pregnant populations) from spot urine samples optimally should report the time of urine sampling and the time of the most recent iodine supplement intake prior to urine sampling and measure urinary creatinine concentration.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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Appendix G. Paper 4

Correction page 769, column 2, line 20-22: Among women included, one woman had a low *MIC* (23 µg/L) in comparison to *UIC* (94 µg/L).

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Iodine Concentrations in Milk and in Urine During Breastfeeding Are Differently Affected by Maternal Fluid Intake

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Background: Breastfed infants are dependent on iodine transport into breast milk for production of thyroid hormones. Thyroid hormones are important regulators of brain development. It has been considered whether breast milk iodine concentration (MIC) could be predicted by maternal urinary iodine concentration (UIC), but reports on correlations have been inconsistent. We used urinary creatinine concentration as a proxy for maternal fluid intake and speculated if this might differently influence UIC and MIC.
Methods: We examined 127 breastfeeding women after the introduction of the mandatory iodine fortification of salt in Denmark. Maternal spot urine and a breast milk sample were obtained at a median of 31 days after delivery (interquartile range: 25–42 days), and the women were asked about intake of iodine containing supplements postpartum.
Results: Median UIC was 72 μg/L (46–107 μg/L) and higher in iodine-supplemented mothers (47.2% of participants); 83 μg/L (63–127 μg/L) versus 65 μg/L (40–91 μg/L), $p=0.004$. Median MIC was 83 μg/L (61–125 μg/L) and also higher in iodine-supplemented mothers; 112 μg/L (80–154 μg/L) versus 72 μg/L (47–87 μg/L), $p<0.001$. There was a weak correlation between UIC and MIC ($r=0.28$, $p=0.015$). A strong correlation was present between UIC and urinary creatinine concentration ($r=0.76$, $p<0.001$), whereas urinary creatinine concentration was not correlated to MIC ($r=-0.049$, $p=0.58$). When UIC and urinary creatinine were used to estimate 24-h urinary iodine excretion, the correlation between this estimate and breast milk iodine excretion was stronger ($r=0.48$, $p<0.001$).
Conclusions: Intake of an iodine supplement should be recommended in Danish breastfeeding women. Our results indicate that UIC, but not MIC, depends on maternal fluid intake and that maternal estimated 24-h iodine excretion may be a better indicator of iodine supply to the breastfed infant than UIC.

Introduction

Iodine is necessary for thyroid hormone production, and lack of thyroid hormones during infant brain development may lead to permanent brain damage (1). Thus, maternal iodine requirements are increased during the period of breastfeeding to ensure an adequate supply of iodine both to the developing infant via breast milk and to the mother. Consequently, the World Health Organization (WHO), United Nations Children's Fund (UNICEF), and the International Council for the Control of Iodine Deficiency Disorders (ICCIDD) define an adequate maternal intake of iodine as 250 μg/day during breastfeeding (2).

Population median urinary iodine concentration (UIC) is the recommended method to assess population iodine intake (2). In nonlactating women, around 90% of ingested iodine is excreted into urine, whereas in lactating women, part of plasma inorganic iodine is transported into breast milk by the sodium iodide transporter (NIS) (3). Urinary iodine excretion is consequently lower, and the median UIC indicating sufficient iodine intake in breastfeeding women is similar to the nonpregnant state ($\geq$100 μg/L), although maternal iodine requirements are increased (2).

WHO, UNICEF, and ICCIDD define an adequate intake of iodine in children younger than two years of age as 90 μg/day (2). As previously reviewed in detail (3–5), a number of studies have measured breast milk iodine concentration (MIC) and found a wide range of median or mean concentrations. As recently highlighted by Leung *et al.* (6), physiological mechanisms may challenge the interpretation of studies of breast milk iodine measurements. These authors illustrated a rise in MIC following acute maternal dietary iodine intake.

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Another aspect previously considered is how reliably the iodine supply to the breastfed infant can be evaluated by maternal UIC (7,8). It has been shown that UIC, adjusted by urinary creatinine as a proxy for fluid intake, might more precisely estimate iodine intake in nonlactating women and in men (9–11), and in a study from Australia of 50 breastfeeding women, there was no significant correlation between UIC and MIC, but MIC was significantly correlated with the urinary iodine/creatinine ratio (12).

In Denmark, information on maternal iodine nutrition during breastfeeding and infant iodine nutrition only exists in studies (13,14) performed before the mandatory iodine fortification of salt introduced in the year 2000 (15). In the present study, we examined whether Danish women living in an area with previously moderate iodine deficiency took iodine-containing supplements postpartum, and we evaluated iodine status in breastfeeding women by measurement of maternal urinary iodine excretion. In addition, we studied iodine content of infant feeding by measuring breast milk iodine excretion and iodine content of infant formulas. Data were used to investigate the relationship between urinary iodine excretion and breast milk iodine excretion in breastfeeding women and to study the feasibility of using urinary creatinine excretion to adjust for differences in maternal fluid intake.

Materials and Methods

Study population and design

This study is a postpartum follow-up of women initially included in the Obstetrics Department, Aalborg University Hospital in pregnancy. From June to August 2012, we consecutively recruited 245 healthy pregnant women referred to the Obstetric Department for ultrasound as part of the prenatal investigation program. The initial examination aimed at evaluating whether Danish pregnant women living in an area of Denmark with previously moderate iodine deficiency took iodine-containing supplements and to evaluate iodine intake by measurement of urinary iodine concentration in a spot urine sample (16).

In the present follow-up study, the women were contacted by phone in the postpartum period after birth of a live-born child. If willing to participate, a telephone interview was performed, and the women were asked about intake of iodine-containing supplements in the postpartum period, smoking habits, breastfeeding, and/or use of infant formulas. The same interviewer performed all interviews.

Additionally, the women were asked if it would be possible to bring a spot urine and a breast milk sample to the hospital within the coming weeks. If infant formulas were used, they were also asked to bring a sample of prepared infant formulas. Vials for sampling were mailed to the women willing to participate, and they were instructed to collect the urine and breast milk sample nonfasting and as close together in time as possible. The women were asked about the timing of sampling in relation to breastfeeding of the child, timing of last iodine supplement intake prior to sampling, and whether milk was collected from one or both breasts. A small group of women ($n = 13$) were instructed to collect breast milk samples from the same breast both immediately before and after breastfeeding of the child.

The study protocol was approved by the local ethical committee, and informed consent was obtained from each participant.

Laboratory procedures

Urine and raw breast milk samples were stored at −20°C from time of sampling until collection at home and until measurement of UIC and MIC in batches during the time of the study inclusion.

After thawing and brief mixing of the samples, UIC and MIC were determined by the cerium/arsenite method after alkaline ashing to dryness (combusting organic material), as previously described (17). The analytical sensitivity of the assay was 2 μg/L and the lowest standard above the zero blank contained 10 μg iodine/L. For urine samples, the recovery of iodine was 95.5% (SEM 2.4%) and the interassay CV was 2.7%, as previously described (15). For breast milk samples, the recovery of iodine was 93.6% (SEM 1.04%), when 75 μg iodine/L was added to 10 breast milk samples with a median iodine concentration of 78 μg/L (range 51–118 μg/L). Serial dilution of 10 breast milk samples containing 51–118 μg iodine/L gave curves parallel to the standard curve. The interassay CV was 3.6% when seven breast milk samples were measured in triplicate. The iodine laboratory was certified by the U.S. Centers for Disease Control and Prevention EQUIP program, which includes measurement of "blind" external controls three times a year.

Urinary creatinine concentrations were measured on a Cobas 8000 system (Roche, Switzerland). Equipment was calibrated according to the manufacturer's instructions, and external standards were included.

Statistical analyses

Characteristics of the women according to intake of iodine supplements were compared using the chi-square test or Fisher's exact test, and multivariate logistic regression was used to examine predictors of iodine supplement intake postpartum.

Urinary iodine excretion was expressed as spot urine concentration (μg iodine/L) and as estimated 24-h iodine excretion (μg iodine/24 h) calculated from the mean 24-h urinary creatinine excretion (1.09 g creatinine/24 h), previously measured in a group of Danish pregnant women (18). This calculation was used for both pregnancy and postpartum urinary iodine concentrations, as the 24-h urinary creatinine excretion was almost identical to that found in a general female population aged 25–34 years (19). For calculation of 24-h breast milk iodine excretion, a breast milk volume of 800 mL/24 h was used (20).

UIC, MIC, and urinary creatinine concentrations showed skewed distributions, and results were expressed as medians with 25th and 75th percentiles (interquartile range, IQR). The Mann–Whitney *U* test was used to compare urinary iodine excretion stratified by iodine supplement intake and time of sampling. The Wilcoxon signed rank test was used for comparison of related samples (pregnancy vs. postpartum and before vs. after breastfeeding the child). Cuzick's test for trend was used to compare median 24-h estimated breast milk iodine excretion by timing of last supplement intake prior to sampling. Urinary and breast milk iodine and urinary creatinine measurements were log-transformed for illustration of correlations and calculation of Pearson's correlation. The ratio between 24-h estimated breast milk iodine excretion and urinary iodine excretion was log-transformed for calculation of the geometric mean with 95% confidence interval (CI). The

Student's *t*-test was used to compare the geometric mean ratio by iodine supplement intake.

Statistical analyses were performed using Stata v11 (StataCorp, College Station, TX), and a 5% level of significance was chosen.

Results

Study population

Figure 1 illustrates the selection of the women included in the follow-up study postpartum. Among women included in pregnancy, 85.3% were interviewed by phone in the postpartum period (median day 22 postpartum; range 9–146), and in this group of women, 70.3% sampled breast milk and/or urine at home. A total of 130 women delivered both a urine sample in pregnancy and postpartum and a breast milk sample. Three women were excluded because they reported that they were smokers, thus leaving 127 women for urine and breast milk analyses (Fig. 1). The time interval between samplings (milk–urine) was a median of 0 minutes (IQR: −30 to 7 minutes). The women who delivered a urine and breast milk sample were slightly older (median age 30 years vs. 29 years, $p=0.02$) with a higher educational level ($p<0.001$), whereas no difference was observed in the use of iodine supplements in pregnancy ($p=0.78$) and postpartum ($p=0.90$).

Iodine supplement intake

At the time of the postpartum interview, 117 of 209 women (56.0%) reported intake of dietary supplements, and 98 of the 209 women (46.9%) took a vitamin and/or mineral supplement containing iodine. In pregnancy, 174 of these 209 women (83.3%) were iodine supplement users.

Table 1 presents characteristics of the women interviewed according to intake of iodine supplements postpartum. Only iodine supplement use in pregnancy significantly predicted iodine supplement intake postpartum with similar findings in multivariate analyses examining all variables in Table 1 as categoricals. In addition, maternal cohabitation, ethnicity, occupation, and prepregnancy BMI as reported in pregnancy were examined in univariate and multivariate analyses. None of these variables significantly predicted iodine supplement intake postpartum.

In pregnancy, almost all the iodine supplements used contained 150 or 175 μg iodine/day, and most women continued such a supplement postpartum. However, a subgroup of the iodine supplement users ($n=15$) took a multivitamin pill that was labeled by the manufacturer as "supplement for breastfeeding women" and contained only 45 μg iodine/day.

Urinary and breast milk iodine

Table 2 presents time of sampling and results of urinary iodine and creatinine excretion and breast milk iodine

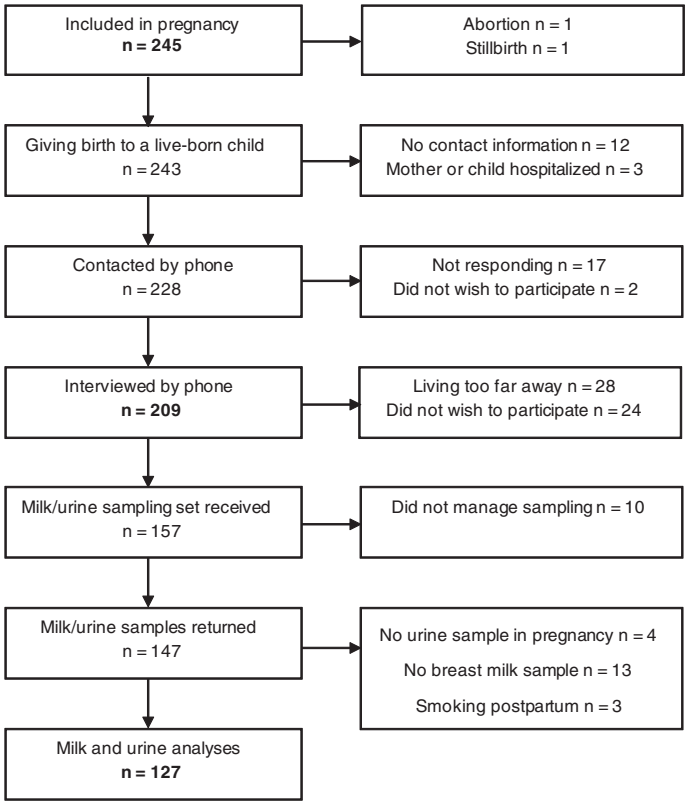

FIG. 1. Flowchart illustrating the selection of the women participating in the postpartum follow-up study.

Table 1. Characteristics of the Mothers Participating in the Postpartum Interview at Initial Inclusion in Pregnancy, at Delivery, and at the Time of the Postpartum Interview According to Maternal Intake of Iodine Supplements Postpartum

	Iodine supplement postpartum		*No iodine supplement postpartum*		
	n	%	n	%	p[a]
Total participants ($N=209$)	98	46.9	111	53.1	
At initial inclusion in pregnancy					
Gestational week					
10–15	38	38.8	33	29.7	0.38
19–21	49	50.0	65	58.6	
28–37	11	11.2	13	11.7	
Maternal age (years)					
<25	5	5.1	12	10.8	0.25
25–35	78	79.6	79	71.2	
>35	15	15.3	20	18.0	
Maternal parity[b]					
1	50	51.0	59	53.1	0.95
2	38	38.8	41	37.0	
≥3	10	10.2	11	9.9	
Maternal education[c]					
Basic	4	4.1	11	9.9	0.11
Low	25	25.5	32	28.8	
Middle	48	49.0	38	34.2	
High	21	21.4	30	27.1	
Iodine supplement intake in pregnancy					
Yes	90	91.8	84	75.7	0.002
No	8	8.2	27	24.3	
At delivery					
Gestational week					
<37	3	3.1	5	4.5	0.73
37–41	83	84.7	89	80.2	
>41	12	12.2	17	15.3	
Method of delivery					
Vaginal	80	81.6	89	80.2	0.79
Cesarean section	18	18.4	22	19.8	
At the postpartum interview					
Time of interview (days after delivery)					
≤14	17	17.4	13	11.7	0.50
15–28	46	46.9	57	51.4	
>28	35	35.7	41	36.9	
Maternal smoking					
No	92	93.9	105	94.6	0.82
Yes	6	6.1	6	5.4	
Breastfeeding					
Full	73	74.5	75	67.6	0.54
Partly	14	14.3	21	18.9	
No	11	11.2	15	13.5	

[a]Chi-square test or Fisher's exact test: iodine supplement postpartum vs. no iodine supplement postpartum.
[b]Previous live- and stillbirths including index pregnancy.
[c]Highest educational level fulfilled or initiated. General education: "basic" (primary/secondary education only; 9–13 years). General education and education qualifying for a profession: "low" (vocational education and training: 9–13 years), "middle" (short- or medium-cycle higher education: 14–16 years), "high" (long-cycle higher education: ≥17 years).

excretion in pregnancy and postpartum stratified by maternal intake of iodine supplements.

UIC postpartum ranged from 8 to 422 μg/L. In comparison to women not taking iodine supplements, median UIC and estimated 24-h urinary iodine excretion were significantly higher in iodine supplement users both in pregnancy and postpartum. When comparing related urine samples in pregnancy and postpartum in individual mothers (Table 2), UIC was lower ($p<0.001$) and urinary creatinine higher ($p<0.001$) in the postpartum period than in pregnancy.

MIC ranged from 19 to 301 μg/L, as illustrated in Figure 2. Median MIC and estimated 24-h breast milk iodine excretion was higher in women taking iodine supplements postpartum in comparison to nonusers (Table 2). When evaluating related urine and breast milk samples postpartum in individual mothers (Table 2), MIC was higher than UIC ($p=0.013$), but when stratified by iodine supplement intake, only iodine supplement users had significantly higher MIC (iodine supplement intake: $p=0.016$; no iodine supplement intake: $p=0.33$). Estimated 24-h breast milk iodine excretion was not significantly different from estimated 24-h urinary iodine excretion ($p=0.29$), nor when stratified by iodine supplement intake.

Iodine content of the supplements differed, and urine and breast milk iodine excretion was considerably lower in women with the lowest iodine supplement intake (45 μg/ day; $n=9$) in comparison to women with a higher intake of iodine from supplements (150 or 175 μg/day; $n=49$); estimated 24-h urinary iodine excretion: 58 μg/24 h (IQR: 50–87 μg) versus 89 μg/24 h (61–147 μg), $p=0.030$; 24-h breast milk iodine excretion: 54 μg/24 h (51–85 μg) versus 98 μg/24 h (74–123 μg), $p=0.048$.

Among women with no iodine supplement intake postpartum ($n=67$), UIC and MIC were not significantly higher in the group of women who had used iodine supplements in pregnancy ($n=49$) in comparison to women with no iodine supplement intake either in pregnancy or postpartum ($n=18$; data not shown).

Correlations between urinary iodine, urinary creatinine and breast milk iodine

Figure 3 illustrates the relationship between maternal urinary iodine excretion postpartum, maternal urinary creatinine excretion postpartum, and breast milk iodine excretion. Figure 3A depicts that UIC and MIC were only modestly correlated. We hypothesized that this relatively weak correlation might be caused by differences in maternal fluid intake. As illustrated in Figure 3C, a strong correlation was observed between urinary creatinine (a proxy for fluid intake) and UIC, whereas no correlation was observed between MIC and urinary creatinine concentrations (Fig. 3D). Thus, fluid intake as evaluated by urinary creatinine influenced strongly on UIC, but had no influence on MIC (Fig. 3C vs. Fig. 3D). As a consequence of this disparity, the correlation between iodine excretion in urine and breast milk was stronger, when UIC was adjusted by urinary creatinine and expressed as 24-h urinary iodine excretion (Fig. 3B vs. Fig. 3A).

To evaluate at the individual level how breast milk iodine excretion could be determined from maternal urinary iodine excretion postpartum, we calculated the ratio between estimated 24-h breast milk iodine excretion and maternal 24-h

Table 2. Urinary Iodine and Breast Milk Iodine Excretion According to Maternal Iodine Supplement Intake in Women who Delivered a Urine Sample both in Pregnancy and Postpartum and a Breast Milk Sample

	All women	*Iodine supplement*	*No iodine supplement*	p[a]
In pregnancy				
Pregnant women	127 (100%)	106 (83.5%)	21 (16.5%)	
Gestational week at sampling	20 (13–20)	20 (13–20)	20 (15–20)	0.99
Urinary iodine (μg/L)	91 (61–140)	98 (66–150)	63 (53–92)	0.003
Urinary creatinine (mmol/L)[b]	6.2 (3.0–11.8)	5.8 (3.0–10.9)	10.6 (3.0–14.3)	0.28
Estimated 24-h urinary iodine (μg)[c]	147 (93–260)	163 (113–278)	83 (55–109)	<0.001
Postpartum				
Breastfeeding women	127 (100%)	60 (47.2%)	67 (52.8%)	
Days after delivery at sampling	31 (25–42)	34 (26–44)	29 (25–41)	0.14
Urinary iodine (μg/L)	72 (46–107)	83 (63–127)	65 (40–91)	0.004
Urinary creatinine (mmol/L)[b]	10.1 (5.0–15.9)	9.6 (4.9–15.9)	10.3 (5.0–16.0)	0.91
Estimated 24-h urinary iodine (μg)[c]	69 (50–100)	87 (55–144)	60 (44–83)	<0.001
Breast milk iodine (μg/L)	83 (61–125)	112 (80–154)	72 (47–87)	<0.001
Estimated 24-h breast milk iodine (μg)[d]	66 (49–100)	90 (64–123)	58 (38–70)	<0.001
Urinary iodine $<100\,\mu$g/L	92 (72.4%)	37 (61.7%)	55 (82.1%)	
Breast milk iodine $<90\,\mu$g/24 h	89 (70.1%)	30 (50.0%)	59 (88.1%)	

Values are expressed as *n* (%) or median (25th–75th percentile).
[a]Mann–Whitney *U* test: iodine supplement vs. no iodine supplement.
[b]Urinary creatinine: 1 mmol/L = 0.1131 μg/L.
[c]Calculated from mean 24-h urinary creatinine previously measured in a group of Danish pregnant women: 1.09 g creatinine/24 h (18).
[d]Calculated from previously estimated average daily breast milk intake: 800 mL/24 h (20).

urinary iodine excretion. The geometric mean ratio was 0.93 [CI: 0.85–1.03] and not significantly different when stratified by iodine supplement intake (iodine supplement intake: 1.02 [CI 0.90–1.15]; no iodine supplement intake: 0.86 [CI 0.75–0.99]; $p = 0.076$).

Sampling time

Time from delivery to sampling of urine and breast milk ranged from 14 to 135 days. UIC ($r = 0.20$, $p = 0.026$) and urinary creatinine concentration ($r = 0.24$, $p = 0.008$) correlated with time from delivery, but when UIC was adjusted by urinary creatinine to calculate 24-h urinary iodine excretion, no correlation with time from delivery was observed ($r = -0.044$, $p = 0.62$). Similarly, estimated 24-h breast milk iodine excretion did not correlate with time from delivery to sampling, neither in iodine supplement users ($r = -0.17$, $p = 0.19$), nor in iodine supplement nonusers ($r = -0.11$, $p = 0.37$).

Time of sampling during the day ranged from 00:20 to 23:50. Median estimated 24-h urinary iodine excretion was slightly higher when sampling was performed in the late afternoon/evening (4:01 p.m.–12:00 a.m., $n = 34$: 83 μg/24 h (65–122 μg); 8:01 a.m.–4:00 p.m. $n = 81$: 62 μg/24 h (48–94 μg); 12:01 a.m.–8:00 a.m., $n = 12$: 63 μg/24 h (49–93 μg); $p = 0.031$). Time of sampling did not influence on estimated 24- breast milk iodine excretion ($p = 0.42$). As illustrated in Figure 4, a trend was observed in 24-h breast milk iodine excretion according to timing of maternal most recent iodine supplement intake prior to sampling.

Breast milk sampling from one or both breasts did not influence median MIC, and no difference in median MIC was observed when sampling was performed before or after breastfeeding the child (data not shown). A small group of women sampled breast milk both immediately before and after breastfeeding the child ($n = 13$). The difference in MIC (before – after) was small (median 4 μg/L; IQR: 2–13 μg/L), but the concentration was significantly higher before breastfeeding, $p = 0.017$.

Infant formulas

A total of 35 infant formulas were obtained. The iodine concentrations ranged from 62 to 167 μg/L, and the overall median iodine concentration was 122 μg/L (IQR: 93–155 μg/L). Stratified by brand, the median iodine concentrations were: Nan-1, $n = 20$: 155 μg/L (137–158 μg/L); Allomin-1, $n = 9$: 87 μg/L (86–92 μg/L); Althera, $n = 5$: 102 μg/L (98–103 μg/L), and Hipp Organic, $n = 1$: 98 μg/L.

Discussion

Principal findings

In a follow-up study of women giving birth in an area of Denmark with previously moderate iodine deficiency, iodine supplement use postpartum was less frequent than in pregnancy. Urinary iodine and breast milk iodine concentrations were below the levels recommended but significantly higher with iodine supplement intake and higher than the levels reported before the iodine fortification of salt in Denmark. Among iodine supplement users, the subgroup of women with the lowest iodine supplement intake was in particular at risk of iodine deficiency.

Maternal UIC, but not MIC, was strongly dependent on urinary creatinine concentration as a proxy for maternal fluid intake, and results indicate that UIC adjusted for creatinine more precisely predicts the iodine content of breast milk.

Comparison with previous Danish studies

Iodine content of breast milk was previously evaluated in Denmark before the mandatory iodine fortification of salt

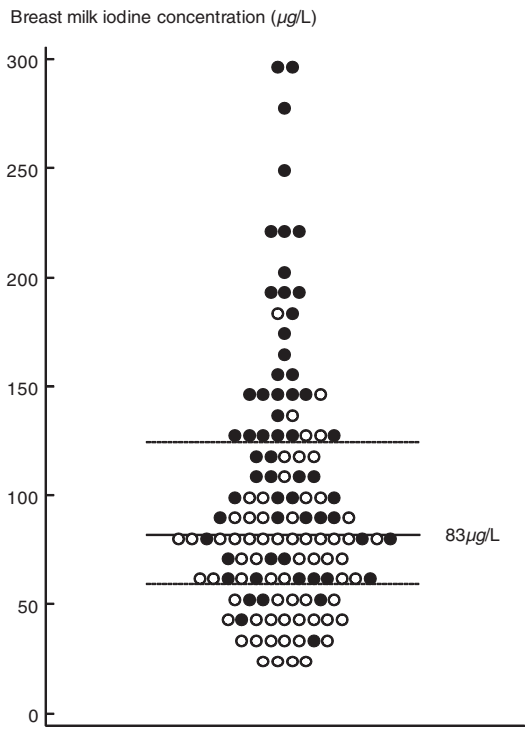

FIG. 2. Dotplot illustrating all breast milk iodine concentrations ($n = 127$). Black dots illustrate iodine supplement users; white dots illustrate iodine supplement nonusers. The solid line represents the median; dotted lines are the 25th and 75th percentiles. Values were stratified into 30 bands, in the range from 19 to 301 μg/L, each band corresponding to 9.4 μg/L.

introduced in the year 2000 (15). In a study by Pedersen *et al.* (13) performed in an area of Denmark with previously moderate iodine deficiency, 53 pregnant women (30% smokers) were randomized to 200 μg iodine/day ($n = 27$) or controls ($n = 26$) from gestational week 17–18 until 12 months after delivery. Median MIC day 5 postpartum was significantly higher in iodine-supplemented mothers (41 vs. 28 μg/L in controls). In another study by Nohr *et al.* (14), 148 mothers in five cities of Denmark were included (35% smokers). The women reported on intake of iodine supplements at arrival for delivery and were instructed to continue their current supplement intake in the postpartum period. Sampling of breast milk was also performed five days after delivery in this study. Median MIC was significantly higher in iodine supplemented mothers (57 vs. 34 μg/L) with regional differences (higher values in East Denmark with previously mild iodine deficiency). In addition to this, it was illustrated that smoking mothers had considerably lower MIC than nonsmoking mothers (26 vs. 54 μg/L) (21).

Median MIC was also higher in iodine supplemented mothers in our study (112 vs. 72 μg/L) and had increased in comparison to the previous studies. In our study, breast milk samples were collected later in the postpartum period, but our results were not dependent on time from delivery to sampling, neither in iodine supplement users nor in nonusers. Studies evaluating breast milk iodine content at different time points postpartum either reported a significant decrease or no difference according to time after delivery (22–24).

Mechanisms of iodine excretion in breast milk

Iodide is transported into breast milk by the sodium iodide transporter (NIS) (25). NIS is present in various tissues including the thyroid gland and the placenta (26). NIS is competitively inhibited by a number of chemicals, and in Denmark the most frequent NIS inhibitor is thiocyanate from smoking. In the thyroid gland (26), and presumably also the placenta (27), inhibition of NIS-mediated iodide transport is autoregulated by iodide. However, in the lactating mammary gland, no autoregulation of NIS seems to occur, as illustrated by lower breast milk iodine concentrations in smokers (21). The frequency of maternal smoking was much lower in our present study than in the previous Danish studies. Smokers ($n = 3$) were excluded from urine and breast milk analyses in our study. Among women included, one woman had a low UIC (23 μg/L) in comparison to MIC (94 μg/L). She reported smoking cessation a few weeks before the interview.

Maternal fluid balance

Iodine status should be assessed by measurement of urinary iodine concentration rather than dietary intake (28). Spot urine samples are the easiest and recommended method in population studies, and median UIC is considered a valid marker for population iodine intake (2). In general, it has been much discussed whether urinary iodine concentration should be adjusted for differences in fluid intake by urinary creatinine (11). Our study suggests that UICs are dependent on fluid intake in breastfeeding women, whereas MIC is not. Thus, MIC was not dependent on maternal fluid intake, an observation in line with studies indicating that breast milk volume is not influenced by maternal fluid intake (29,30). On the other hand, maternal fluid intake influenced UIC, as illustrated by the strong correlation with urinary creatinine concentration. Thus, the iodine status of breastfeeding women might be more precisely estimated when UIC is adjusted by differences in fluid intake, as previously suggested for nonlactating women and for men (9,10). Furthermore, the correlation with breast milk iodine excretion was stronger when urinary iodine content was adjusted by creatinine in accordance with a previous study (12).

Infant formulas

Iodine content of infant formulas used in Denmark was previously reported in 1999 (31). In that study, infant formulas in dry preparation were prepared using iodine-free demineralized water, whereas in our study the mothers prepared the infant formulas at home according to the manufacturers' instructions. The median iodine concentration was considerably higher in our study (122 vs. 57 μg/L), as well as for the individual brands that were measured in both studies, even taking the iodine content of tap water into account. The content of iodine in the infant formulas corresponded to the breast milk iodine concentrations measured in iodine supplement users.

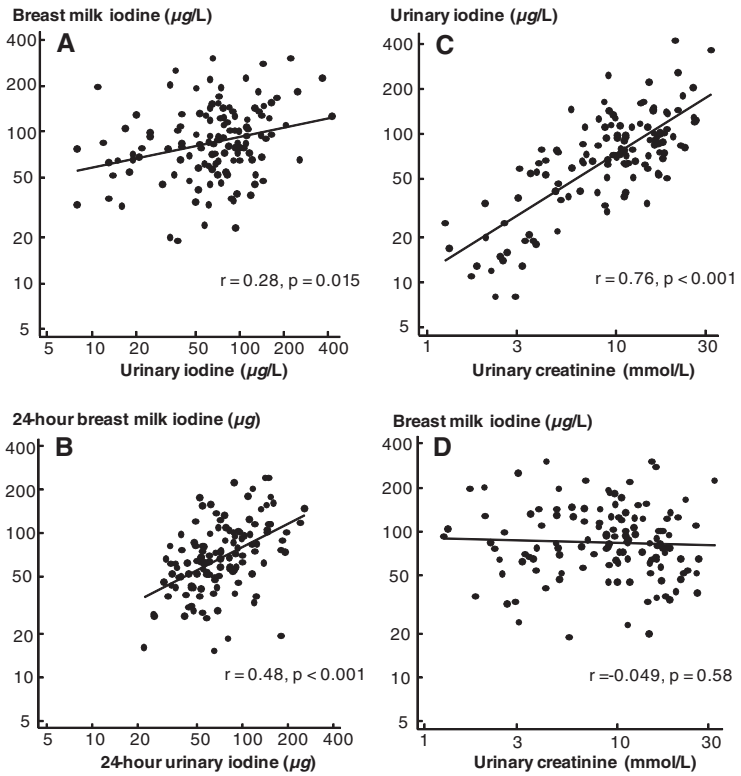

FIG. 3. Scatter plots of 127 samples illustrating the correlation between **(A)** maternal postpartum urinary iodine concentration and breast milk iodine concentration, **(B)** estimated 24-h breast milk iodine excretion and maternal postpartum 24-h urinary iodine excretion, **(C)** maternal postpartum urinary creatinine and urinary iodine concentrations, and **(D)** maternal postpartum urinary creatinine and breast milk iodine concentrations. All values were log-transformed for illustration and calculation of Pearson's correlation. Values on the axes are antilogged.

Strength and limitations

The strength of our study is the high rate of participation among invited women in the postpartum interview and the relatively large number of urine and breast milk samples obtained (10). However, participants differed slightly from nonparticipants in some characteristics, and numbers in subgroups after stratification were limited. We are aware that the study is a postpartum follow-up of women initially included in pregnancy and that, according to ethical requirements, the women were informed about the importance of iodine intake at the initial visit. This information was kept low grade, and data on urinary iodine concentrations in pregnancy and press releases were not reported until after the last inclusion postpartum. Our study only included women in one region of Denmark. However, we found no significant predictors of iodine supplement intake besides iodine supplement intake in pregnancy, and we find it unlikely that iodine supplement use in Danish women would considerably differ between regions (32). We did not have information on maternal dietary habits postpartum, and we do not know if differences in maternal fluid intake were caused by differences in intake of iodine containing drinks such as dairy products or water-based drinks such as tea or coffee. The level of iodine in drinking water in the region investigated is low and corresponds to the level in western Denmark (31).

FIG. 4. Median estimated 24-h breast milk iodine excretion with confidence intervals stratified by timing of maternal most recent iodine supplement intake prior to breast milk sampling, $p < 0.001$ for trend, excluding nonusers; $p = 0.05$.

National perspective

Intake of iodine supplements fell by nearly half comparing pregnancy to the postpartum period. Iodine was obtained in a multivitamin pill, and during pregnancy official recommendations exist on intake of folic acid, iron, and vitamin D often leading to intake of a multivitamin pill. On the other hand, no

official recommendations exist on intake of dietary supplements during breastfeeding in Denmark. Women with no intake of iodine supplements and women with an intake of iodine supplements with low iodine content (45 μg/day) were particularly at risk of being iodine deficient. Thus, iodine supplement intake in Danish breastfeeding women should be officially recommended. One of the concerns about iodine intake in pregnancy and postpartum has been the risk of exacerbating thyroid autoimmunity (33). A previous study in Denmark did, however, not detect an increased risk of postpartum thyroiditis from iodine supplement in thyroid peroxidase antibody-positive mothers (34). The 24-h iodine excretion in breast milk was below the level recommended in the women not using iodine supplements. In iodine supplements users, it was estimated to be 90 μg/24 h, which corresponds to the recommended iodine intake in children younger than two years of age (2). On the other hand, maternal UIC was below the level recommended both in iodine supplement users and nonusers. Together with the findings in Danish pregnant women (16), and findings from the general Danish population (35), these results may indicate a need for a modest increase in the level of iodine added to salt in Denmark.

Conclusion

Maternal estimated 24-hour urinary iodine excretion showed a better correlation to maternal MIC than did maternal UIC. This may suggest that estimated 24-h urinary iodine excretion is a useful indicator of maternal iodine status during breastfeeding and of iodine supply to the breastfed infant. However, more studies on the usefulness of this indicator are warranted in populations with different dietary or environmental habits.

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Author Disclosure Statement

The authors declare that they have no conflicts of interest.

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SUMMARY

Iodine is required for the synthesis of thyroid hormones, which are crucial regulator of early brain development. The source of iodine in the fetus and the breastfed infant is maternal iodine, and adequate iodine intake in pregnant and breastfeeding is of major concern. Severe iodine deficiency can cause irreversible brain damage, whereas the consequences of mild to moderate iodine deficiency are less clear. Denmark was previously iodine deficient with regional differences (mild iodine deficiency in East Denmark and moderate iodine deficiency in West Denmark), and also pregnant and breastfeeding women suffered from iodine deficiency. A mandatory iodine fortification of household salt and salt used for commercial production of bread was introduced in Denmark in the year 2000. The PhD thesis investigates intake of iodine supplements and urinary iodine status in Danish pregnant and breastfeeding women after the introduction of the mandatory iodine fortification of salt in a region of Denmark with previously moderate iodine deficiency. Additionally, the PhD thesis addresses mechanisms of iodide transport to the fetus across the placenta and methodological challenges in the evaluation of urinary iodine status in pregnant and breastfeeding women.

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