Iodine status in pregnant & breastfeeding women

A Danish regional investigation

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THE FOUR ORIGINAL PAPERS

I. Andersen SL, Nøhr SB, Wu CS, Olsen J, Pedersen KM, Laurberg P. Thyroglobulin in smoking mothers and their newborns at delivery suggests autoregulation of placental iodide transport overcoming thiocyanate inhibition. Eur J Endocrinol 2013;168:723-731.

II. Andersen SL, Sørensen LK, Krejbjerg A, Møller M, Laurberg P. Iodine deficiency in Danish pregnant women. Dan Med J 2013;60:A4657.

III. Andersen SL, Sørensen LK, Krejbjerg A, Møller M, Laurberg P. Challenges in the evaluation of urinary iodine status in pregnancy: The importance of iodine supplement intake and time of sampling. Eur Thyroid J 2014;3:179-188.

IV. Andersen SL, Møller M, Laurberg P. Iodine concentrations in milk and in urine during breastfeeding are differently affected by maternal fluid intake. Thyroid 2014;24:764-772.

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].

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].

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.

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].

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].

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.

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.

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.

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 (Figure 1-1).
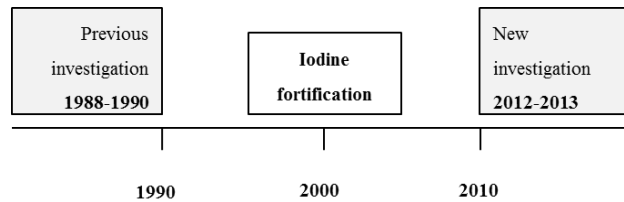

Figure 1-1 Illustration of the investigations before and after the Danish mandatory iodine fortification of salt which was introduced in the year 2000.

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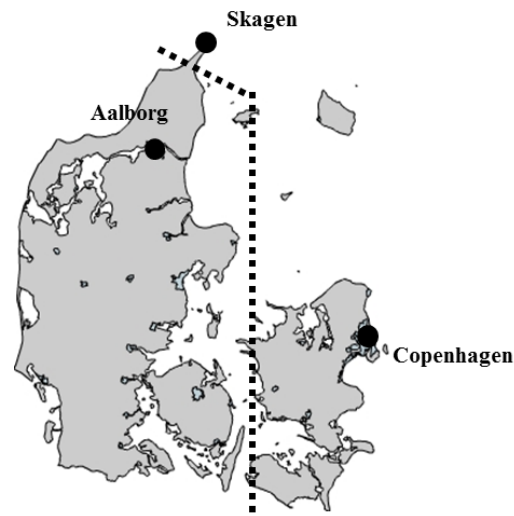
Figure 2-1 Map of Denmark. The dotted line illustrates the division by the Great Belt into East Denmark with a higher content of iodine in drinking water and West Denmark with a lower content of iodine in drinking water.

IODINE METABOLISM
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 (Figure 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].
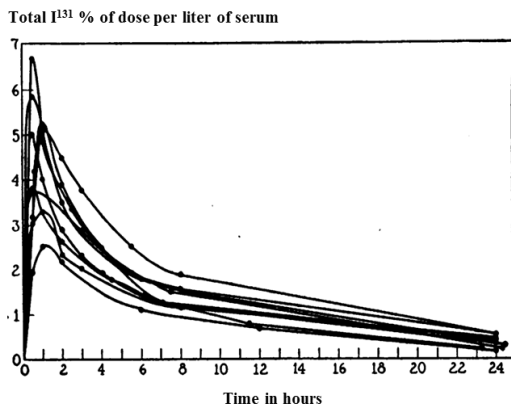

Figure 2-2 Oral administration of radioiodine in nine euthyroid subject. Reproduced from [48] with permission.

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 (Figure 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].
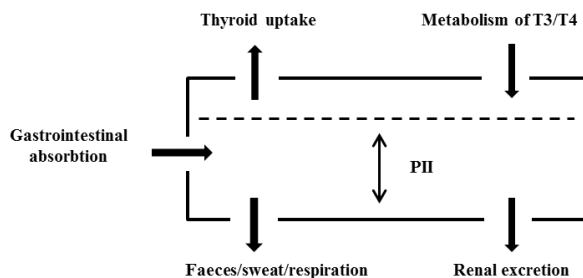

Figure 2-3 Inputs and outputs from the plasma inorganic iodide (PII) pool.

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 (Figure 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].

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).
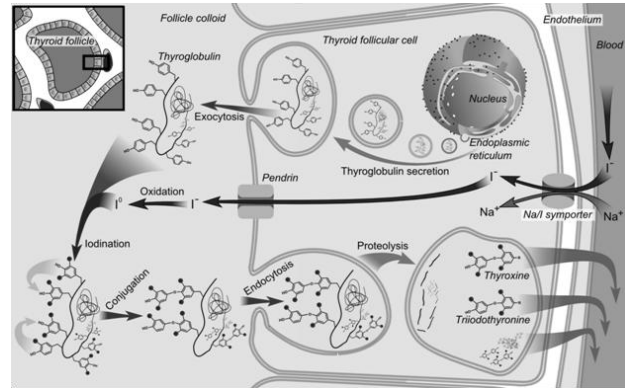

Figure 2-4 Uptake of iodide in the thyroid gland and synthesis of thyroid hormones.

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 (Figure 2-3) [54].

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 (Figure 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.
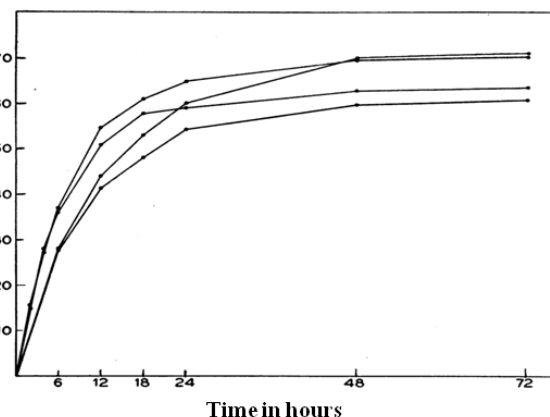

Figure 2-5 Urinary excretion of radioiodine in four euthyroid male subjects. Reproduced from [52] with permission.

EVALUATION OF IODINE STATUS

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

Table 2-1

Methods to assess iodine status in a population.

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

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].

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].

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].

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].

Table 2-2

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

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	-

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].

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.

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].

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].

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 (Figure 2-6) [79].
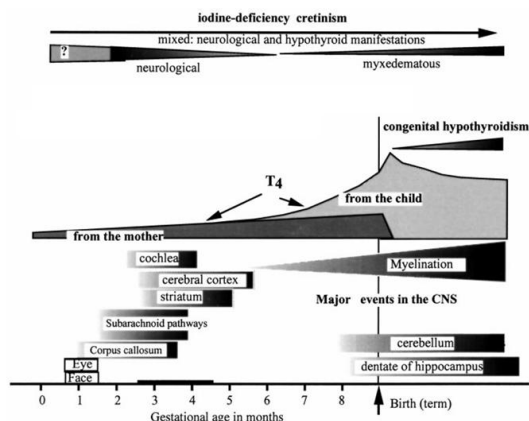

Figure 2-6 Illustration of events during early brain development. Reproduced from [79] with permission.

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 (Figure 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 (Figure 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 neurodevelopmental 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].

IODINE, PLACENTA & NIS

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]).

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 (Figure 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.

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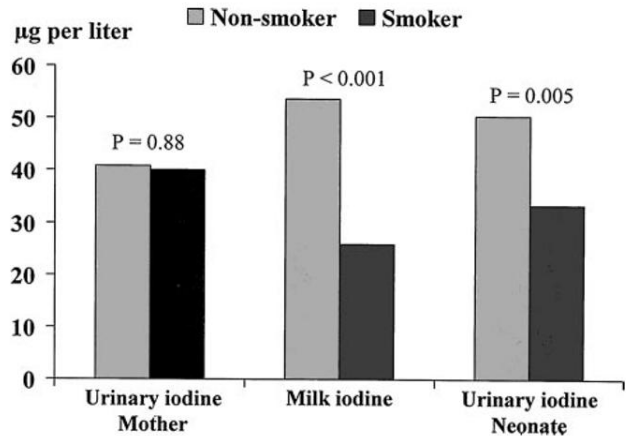
Figure 3-1 Median maternal urinary iodine concentration, breast milk iodine concentration and neonate urinary iodine concentration stratified by maternal smoking status. Samples were obtained on day five after delivery. Reproduced from [19] with permission.

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].

AUTOREGULATION OF PLACENTAL IODIDE TRANSPORT (PAPER I)
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.

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).

Table 3-1

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

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

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].

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.

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].

Table 3-2

Hill's viewpoints of causation [111].

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 repeatedly shown in different populations
Analogy	Alternative explanations
Plausibility	Plausible mechanism between exposure and outcome
Coherence	In line with the natural history of the outcome

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].

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.

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 (Figure 4-1). hCG is a glycoprotein increasingly secreted by the placenta in the first trimester of pregnancy (Figure 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 (Figure 4-1).
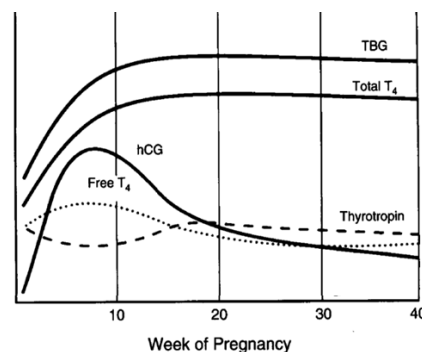
Figure 4-1 Relative changes in maternal thyroid function in pregnancy. Reproduced from [123] with permission, copyright Massachusetts Medical Society.
TBG; Thyroxine-binding globulin, T4; tetraiododthyronine, hCG; human chorionic gonadotropin, Thyrotropin; thyroid stimulating hormone.

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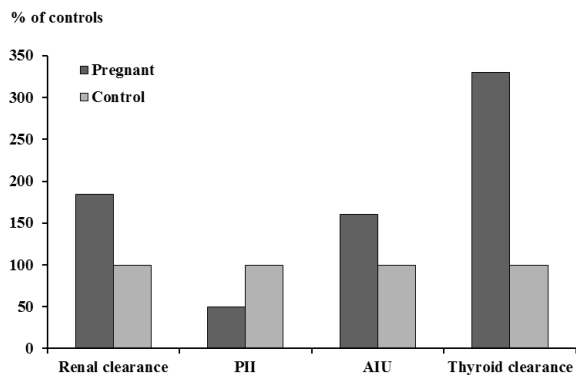
Figure 4-2 Relative changes in maternal renal iodine clearance, plasma inorganic iodide (PII), thyroid absolute iodine uptake (AIU) and thyroid clearance of iodine in pregnant women vs. controls. Data from [128].

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 (Figure 4-2). However, as illustrated in Figure 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 Figure 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].

RECOMMENDATIONS IN PREGNANCY
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). 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.

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.

Table 4-1

Recommended daily iodine intake in pregnancy by different authorities.

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 4-2

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

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

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.

Table 4-3

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

Author, year	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.

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.

IODINE STATUS IN DANISH PREGNANT WOMEN (PAPER II)
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.

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.

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 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 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 II and III.

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 II, 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).

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.

	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 h)	146	154	147	0.96

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).

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).

	Study participants		Random sample		
	n = 245		n = 107		p[1]
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.

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.

CHALLENGES IN EVALUATION OF IODINE STATUS IN PREGNANCY (PAPER III)

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.

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.

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).

Urine samples from home were sent by mail immediately after collection. All male partners and children completed a questionnaire 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.

Table 4-6

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

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 4-7

Illustration of urine samples collected in the hospital and at home.

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

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). 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.

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.

	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

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 III, 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 (Figure 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.

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.

IODINE METABOLISM DURING BREASTFEEDING

Breastfeeding is associated with changes in maternal iodine metabolism (Figure 5-1) [170]. During breastfeeding, around 40-45% of ingested iodine is excreted into breast milk (Figure 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].
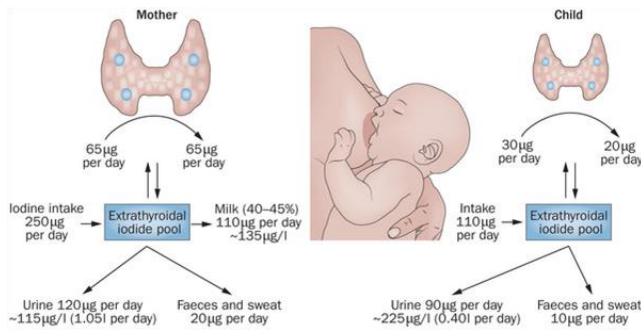

Figure 5-1 Iodine metabolism during breastfeeding. Reproduced from [170] with permission.

RECOMMENDATIONS DURING BREASTFEEDING
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.

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.

Table 5-1

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

	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 5-2

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

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

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.

Table 5-3

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

Author, year	Sampling	Median UIC	
	Time after delivery	+ Iodine	No iodine
Pedersen et al., 1993 [14]	1 week 6 months 10 months	45 μg/l 115 μg/l 85 μg/l	30 μg/l 55 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 5-4

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.

Author, year	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

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].

IODINE STATUS IN DANISH BREASTFEEDING WOMEN (PAPER IV)
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.

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.

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 IV.

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).

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.

CHALLENGES IN EVALUATION OF IODINE STATUS DURING BREASTFEEDING (PAPER IV)

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.

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 sam-

pling 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.

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.

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.

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 (Figure 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].

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.

Table 5-5

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

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

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.

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.

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].

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].

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 and a mandatory iodine fortification of salt was introduced in the year 2000. The PhD thesis investigated intake of iodine supplements and urinary iodine status in Danish pregnant and breastfeeding women after introduction of iodine fortification in a region of Denmark with previously moderate iodine deficiency. Additionally, the PhD thesis addressed 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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