Iodine Intake and Uptake in Populations at Risk for Iodine Deficiency

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Gothenburg 2021

Cover illustration: "Iodine Vapour" by Perennou Nuridsany

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ISBN 978-91-8009-286-9 (PRINT)
ISBN 978-91-8009-287-6 (PDF)

http://hdl.handle.net/2077/67337

Printed in Borås, Sweden 2021
Printed by Stema Specialtryck AB

Doubt is not a pleasant condition, but certainty is an absurd one

Voltaire, 1694–1778

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ABSTRACT

Background: Iodine is essential for the production of thyroid hormones. Both iodine deficiency (ID) and iodine excess may be harmful. Iodine intake in Sweden is considered adequate for the general population due to iodization of table salt since 1936 but data on pregnant and breastfeeding women (i.e. groups with increased need for iodine) is scarce in Sweden. Moreover, bariatric surgery is increasingly popular and it is unknown whether it causes ID by decreased iodine intake and/or uptake.

Aims: To investigate iodine status and thyroid function in populations that are known to be at risk for ID (i.e. pregnant and breastfeeding women) and in populations that are assumed to be at risk for ID (i.e. patients who have undergone bariatric surgery).

Methods: **PAPER I** is a cross-sectional, observational study on a representative population in Sweden of 743 pregnant women. **PAPER II** is a pilot, randomized, controlled trial comprising 200 women who received a daily multivitamin either with iodine 150 μg or without iodine followed until delivery. **PAPER III** is an observational prospective study of 84 women followed from the third trimester of pregnancy until 12 months postpartum with a focus on breastfeeding habits. **PAPER IV** is an interventional, non-randomized, controlled trial of patients undergoing gastric bypass or vertical-banded gastroplasty derived from the Swedish Obesity Subjects study. They were compared to obese non-operated subjects and to a population-based control group. The outcomes were urinary iodine, thyroid hormones,

thyroglobulin (Tg), breastmilk iodine concentration (BMIC), and dietary iodine intake.

Results: Pregnant women in Sweden presented mild ID. A daily supplement containing iodine 150 μg increased iodine status from mild ID to borderline iodine sufficiency with a positive influence on maternal Tg. Breastfeeding women in a local population presented mild ID. A minority (~20%) took iodine supplementation and presented BMIC double that of non-supplement users. Exclusively breastfeeding women at 4 months postpartum presented lower urinary iodine and higher Tg compared to the rest of the study population. Obese subjects at baseline presented higher iodine status than the general population. After bariatric surgery, iodine status decreased but remained at an adequate level at 10 years post-operatively. Whether this decrease was due to altered iodine uptake and/or intake is unknown but the patients did not develop ID.

Conclusions: In Sweden, pregnant women present mild ID. Breastfeeding women may be mildly iodine deficient, especially those who exclusively breastfeed their children. The main action to be taken is to improve the coverage of the current iodine fortification program – the efficacy and the safety of iodine supplementation to pregnant and breastfeeding women with mild ID is still unclear. Bariatric surgery does not appear to be a risk factor for ID. Regular monitoring of the iodine fortification program for both the general population and risk groups is strongly required.

Keywords: iodine, pregnancy, lactation, bariatric surgery, urinary iodine concentration, thyroglobulin, breastmilk iodine concentration, iodine supplementation, Sweden

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SAMMANFATTNING PÅ SVENSKA

Bakgrund: Jod är viktigt för produktionen av sköldkörtelhormon. Jodintaget i Sverige anses generellt vara adekvat, men kunskapen om jodintaget hos gravida och ammande kvinnor och patienter som har genomgått övervikts-kirurgi är begränsad.
Syfte: Att studera jodstatus och sköldkörtelfunktion i riskpopulationer för jodbrist – dvs gravida och ammande kvinnor – och i populationer som antas löpa risk för jodbrist – dvs patienter som har genomgått övervikts-kirurgi.
Metoder: Manuskript I är en tvärsnittsstudie på en representativ population av 743 gravida i Sverige. **Manuskript II** är en randomiserad, kontrollerad pilotstudie på 200 gravida kvinnor som har dagligen fått multivitamin antingen med eller utan 150 µg jod. **Manuskript III** är en prospektiv observationell studie på 84 kvinnor, som följdes från slutet av graviditeten upp till 12 månader postpartum. **Manuskript IV** är en icke-randomiserad, kontrollerad interventionsstudie på överviktiga patienter som har genomgått gastric bypass eller gastroplastik med band. De opererade grupperna jämfördes med två kontrollgrupper: en med överviktiga icke-opererade patienter och en befolkningsbaserad kontrollgrupp. **Effektmåtten:** urinjod, tyreoglobulin, sköldkörtelhormoner, bröstmjölksjodkoncentration (BMIC), och dietärt jodintag.
Resultat: Gravida kvinnor i Sverige uppvisade mild jodbrist. Ett dagligt jodtillskott på 150 µg förbättrade jodstatus från mild jodbrist till jodsufficiens. Ammande kvinnor uppvisade mild jodbrist. Full-ammande kvinnor 4 månader postpartum presenterade lägre urinjod och högre tyreoglobulin jämfört med resten av studiepopulationen. Dem som tog jodtillskott (~ 20%) uppvisade dubbelt så högt BMIC som de övriga. Överviktiga personer hade vid baseline högre jodstatus än den allmänna befolkningen. Tio år efter övervikts-kirurgi låg urinjodnivån lägre än vid baseline, men var fortfarande inom referensområdet.
Slutsatser: Gravida kvinnor i Sverige har mild jodbrist och ammande kvinnor kan vara lätt jodbristiga, särskilt om de full-ammar. Det är angeläget med bättre täckning av joderingsprogrammet. En allmän rekommendation av jodtillskott till gravida och ammande med mild jodbrist har inte klart bevisad effektivitet och säkerhet. Övervikts-kirurgi verkar inte vara en riskfaktor för jodbrist.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Iodine deficiency in pregnant women in Sweden: a national cross-sectional study

Manousou S, Andersson M, Eggertsen R, Hunziker S, Hulthén L, Nyström HF

European Journal of Nutrition. 2020;59(6):2535–45

II. A randomized, double-blind study of iodine supplementation during pregnancy in Sweden: pilot evaluation of maternal iodine status and thyroid function

Manousou S, Eggertsen R, Hulthén L, Nyström HF

European Journal of Nutrition. 2021(online ahead of print)

III. Inadequate iodine intake in lactating women in Sweden: a pilot 1 year, prospective, observational study

Manousou S, Augustin H, Eggertsen R, Hulthén L, Nyström HF

Acta Obstetrica et Gynecologica Scandinavica. 2021;100(1):48–57

IV. Iodine status after bariatric surgery: a prospective 10-year report from the Swedish Obese Subjects (SOS) Study

Manousou S, Carlsson LMS, Eggertsen R, Hulthén L, Jacobson P, Landin-Wilhelmsen K, Trimpou P, Svensson PA, Nyström HF

Obesity Surgery. 2018;28(2):349–57

CONTENTS

ABBREVIATIONS

24-UIC	24-Hour urinary iodine concentration
24-UIE	24-Hour urinary iodine excretion
BMIC	Breastmilk iodine concentration
BS	Bariatric surgery
CI	Confidence interval
DBS	Dried blood spot
eBMIE	Estimated breastmilk iodine excretion
eUIE	Estimated 24-hour urinary iodine excretion
FT4	Free thyroxine
GBP	Gastric bypass
ID	Iodine deficiency
MHC	Maternal healthcare center
NIS	Sodium/iodine symporter
OB-controls	Obese non-operated controls
RCT	Randomized controlled trial
SOS	Swedish obese subjects
Tg	Thyroglobulin
TgAb	Thyroglobulin antibody
TPOab	Thyreoperoxidase antibody
TSH	Thyroid-stimulating hormone
T3	Triiodothyronine
T4	Thyroxine
UIC	Urinary iodine concentration
U-creatinine	Urinary creatinine concentration
U-Na	Urinary sodium
VBG	Vertical-banded gastroplasty
WRA	Women of reproductive age
WHO MONICA	World Health Organization MONItoring of trends and determinants for Cardiovascular disease

1 INTRODUCTION

Bernard Courtois (1777-1838) discovered the element iodine in 1811, when he added sulfuric acid to the ashes of seaweed. A cloud of beautiful violet vapor rose. Iodine had been discovered. Its name was chosen after the Greek word "ιώδες" (iodes), which means "violet".

1.1 PHYSIOLOGY

Iodine: from nature to the human body and back

Iodine in nature is mainly located in the oceans; large amounts of iodine were leached from soil by glaciations, rain, and snow, and were carried by rivers to the seas. The main dietary sources of iodine for humans are saltwater fish and seafood (high native iodine content), dairy products (due to iodine-fortified cattle feed or iodine-containing disinfecting agents), and iodized salt used either as household salt or in food production (in countries with iodine fortification programs).[1] Raw (non-iodized) sea salt or mountain salt are, by contrast, poor in iodine.[2]

Ingested iodine (I_2) and iodate (IO_3^-) are reduced to iodide (I^-) in the gastrointestinal tract. Iodide is nearly completely (>90%) **absorbed** in the duodenum-jejunum[3] to be transported to the thyroid gland for storage.[4] Iodide uptake in the gastrointestinal tract is mainly mediated by the transmembrane sodium/iodine symporter (NIS), which transports two Na^+ ions along the electrochemical gradient and one I^- ion against the electrochemical gradient.[3,5] Another interesting observation is the transport (partly NIS-mediated) of iodide from circulating blood to the

gastric lumen, which does not occur in the opposite direction.[6] The physiological role of this transport is as yet unknown but could contribute to the recycling of iodide. NIS is present in the gastrointestinal tract but is mainly present and active in the thyroid gland, transporting iodine into thyroid cells.[7] NIS activity is increased by thyroid-stimulating hormone (TSH) levels and/or low iodine content in the thyroid gland, and is blocked by thiocyanate, a product of smoking.[4,8] Iodine gets transported to the thyroid cell by NIS and then diffuses to the colloid of the thyroid gland.[4] Iodide in the colloid is oxidized by thyreoperoxidase and combines with thyroglobulin (Tg), a storage protein, from which the thyroid hormones are produced.[4] The thyroid hormones, thyroxine (T4) and triiodothyronine (T3), are named after the number of atoms of iodine in their molecule, i.e. four and three atoms, respectively. After the production of the thyroid hormones, Tg and thyroid residuals are endocytosed in the follicular cells, whereas T4 and T3 are secreted into circulating blood. In extrathyroidal tissues, type 2 iodothyronine deiodinase metabolizes T4 to the active hormone T3, while iodide is released to the blood.[5]

Approximately 90% of ingested iodine is **eliminated** in 24–48 hours through the kidneys in iodine-sufficient individuals.[1,9] Just small amounts of iodide are excreted via respiration, sweat, and feces.[5] Iodine content in the thyroid gland and urinary iodine concentration (UIC) are highly associated with recent iodine intake,[4] which lays the ground for the clinical assessment of iodine status by UIC (Tables 1 and 2). The World Health Organization (WHO) bases the assessment of a country's iodine status on the level of median UIC in a representative population

of school children.[10,11] Median UIC 100–299 μg/L in schoolchildren corresponds to adequate iodine intake.[11]

Table 1. *Daily iodine intake and median urinary iodine concentration recommended by the World Health Organization (WHO).*[10]

	Recommended iodine intake (μg/day)	Recommended median UIC (μg/L)
Children 0–5 years	90	100–199
Children 6–12 years	120	100–199
Children >12 years and adults	150[a]	100–199[b]
Pregnancy	250[a]	150–249
Breastfeeding	250[a]	100–199

Abbreviation: UIC, urinary iodine concentration.

[a]Institute of Medicine (United States) 2001, European Food Safety Authority (EFSA) 2014, and Nordic Nutrition Recommendations 2012 agree with WHO on the recommended level of daily iodine intake of 150 μg for adults but give different recommendations to pregnant women (220, 200, and 175 μg/day respectively) and to breastfeeding women (290, 200, and 200 μg/day respectively).[12–14]

[b]Due to the differing hydration status of adults, the recommended UIC range for adults is uncertain and remains to be defined.[1]

Table 2. *Criteria for assessing iodine nutrition and status of a population through median urinary iodine concentration.*[10,11]

	Median UIC (µg/L)	Assessment of iodine intake	Assessment of iodine status
School children	<20	Insufficient	Severe ID
	20–49	Insufficient	Moderate ID
	50–99	Insufficient	Mild ID
	100–299[a]	Adequate	Adequate iodine status
	≥300	Excessive	Risk of adverse health consequences
Pregnancy[b]	<150	Insufficient	–
	150–249	Adequate	–
	250–499	More than adequate	–
	≥500	No added health benefit expected	–
Breastfeeding	<100	Insufficient	–
	≥100	Adequate	–

Abbreviations: ID, iodine deficiency; UIC, urinary iodine concentration; WHO, World Health Organization.

[a]In populations with recent long-standing ID or when iodine intake rises rapidly, median UIC ≥200 µg/L is considered as excessive.[15]

[b]Even though not defined by WHO, assessment of ID grade during pregnancy is usually based on median UIC in µg/L (severe ID <50 µg/L, moderate ID 50–100 µg/L, and mild ID 100–149 µg/L).[16]

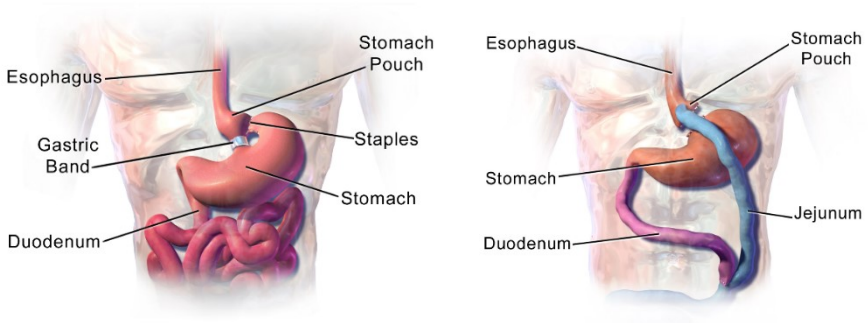
Iodine metabolism during pregnancy and breastfeeding

Pregnant women have higher iodine needs due to: (a) higher maternal thyroid hormone production; (b) transfer of iodine through the placenta for fetal production of thyroid hormone during the second half of pregnancy; and (c) increased glomerular filtration of iodine.[17] The increased renal clearance of iodine during pregnancy leads to a different recommended UIC range for pregnant women (Tables 1 and 2). NIS-mediated transport of iodine to the fetus through the placenta has been proposed, as observed *in vitro* and in rats.[5]

Higher iodine needs are also observed during breastfeeding. The hormone prolactin stimulates NIS to transport iodine to breast cells.[4,17] The fractional excretion of iodine into breastmilk increases during days of lower iodine intake,[18] which makes breastmilk iodine concentration (BMIC) a more reliable indicator of iodine status during breastfeeding compared to UIC.[18] Thiocyanate, a product of smoking, may compete with iodine in its active transport to breastmilk, which explains the association between smoking during breastfeeding and lower BMIC.[19]

Iodine metabolism in subjects who have undergone bariatric surgery

Bariatric surgery (BS) as therapy against morbid obesity is associated with multiple nutritional deficiencies due to reduced intake and/or malabsorption of micronutrients.[20] There are three BS types: the restrictive methods (where the transit of food is partially blocked); the malabsorptive methods (where the uptake of ingested food is decreased); and the combined methods (where both mechanisms are present). In this thesis, iodine metabolism is studied in relation to a restrictive method, i.e. vertical-banded gastroplasty (VBG), and a combined restrictive/malabsorptive method, i.e. gastric bypass (GBP). VBG is no longer performed and has been replaced by another restrictive method, sleeve gastrectomy, whereas GBP is still a frequently used method.[3]



Vertical-banded Gastroplasty Gastric Bypass

Figure 1. *Methods of bariatric surgery studied in relation to iodine status in this thesis.*

In VBG, a stomach pouch is created, with the rest of the intestinal tract remaining intact (Figure 1).[21] In GBP a stomach pouch is created, while the rest of stomach and the upper jejunum are bypassed (Figure 1).[21] Both restrictive and malabsorptive methods of BS are associated with alterations in nutritional preferences, probably secondary to increased gut hormone release and physiologically driven avoidance due to vomiting or dumping.[20,22] When it comes to GBP, not only intake, but also uptake of nutrients is affected, due to its malabsorptive component.[20,22]

The hypothesis of iodine malabsorption after BS was first generated in Scotland in 1964,[23] when six of the eight patients who had undergone total gastrectomy presented lower iodine status than controls despite estimated iodine intake that corresponded to 200% of the recommended daily intake. This hypothesis is based on several factors that may be present in some or all types of BS: (a) limited capacity of the body to reduce ingested iodine and iodate to absorbable iodide; (b) altered digestion and absorption of food; (c) reduced recycling of iodide through gastric mucosa; and (d) rapid passage through the gut. Beyond the hypothesis of iodine malabsorption after BS, another hypothesis may be added of reduced iodine intake due to reduced caloric intake or altered dietary preferences.[22]

1.2 IODINE-RELATED DISEASES

When iodine intake is not enough

In case of moderate or severe iodine deficiency (ID), TSH increases (even though usually remaining within the normal range) to maximize NIS activity and thereby the utilization of available iodine.[15] The TSH increase also leads to thyroid hypertrophy and hyperplasia (goiter), which may be detected by ultrasound or palpation and indirectly by elevated Tg in circulation.[15] When compensatory mechanisms fail, ID leads to ID-induced hypothyroidism. In the enlarged thyroid gland (goiter), thyroid nodules may develop (multinodular goiter), from which some may become autonomous (multinodular toxic goiter) with hyperthyroidism as a result.[19] When it comes to mild ID, goiter (homogenous or multinodular) may be present with elevated Tg but without TSH elevation.[15]

During pregnancy, it is well established that severe ID increases the risk of fetal hypothyroidism, and thereby abortion, stillbirth, or irreversible congenital abnormalities (mental retardation, short stature, spasticity, deaf-mutism, and anatomical facial abnormalities).[19] However, mild-to-moderate ID is a more common entity in pregnancy nowadays, being observed in about two-thirds of the European pregnant population.[24] The role of mild-to-moderate ID in fetal life and the benefit/harm of iodine supplementation to pregnant women with mild-to-moderate ID are unclear. Some observational studies suggest an association between mild-to-moderate fetal ID and worse child neurodevelopmental outcomes, whereas others have shown a neutral or even inverse

relationship.[25] Experts have different opinions regarding iodine supplementation in pregnancy: the European Thyroid Association,[26] the Medical Research Council in Australia and New Zealand,[27] and the American Thyroid Association[28] recommend iodine supplementation (although acknowledging the lack of strong evidence), while WHO does not recommend it in countries with iodine fortification programs and iodine sufficiency in the general population, such as Sweden.[10]

When iodine intake increases or is too much

Iodine intake and thyroid morbidity have a U-shaped relation, which is reflected by the U-shaped relationship between UIC and Tg, but not TSH or total T4.[15,29,30,84] In settings of iodine overload, the acute Wolff-Chaikoff effect is in place to protect from overproduction of thyroid hormones. Normally, a healthy individual will escape from the Wolff-Chaikoff effect within a few days and will remain euthyroid by down-regulating iodine transporters.[31] When the Wolff-Chaikoff effect fails, iodine excess may lead to iodine-induced hyperthyroidism (due to supply of iodine to autonomous thyroid nodules).[32] Iodine excess is also associated with an increased frequency of hypo- and hyperthyroiditis, probably on an autoimmune basis[32] but also as a failure to escape from the Wolff-Chaikoff effect.[31] The increased risk for thyroid diseases described above is not only observed with absolute excessive iodine intake but also when iodized salt is introduced to populations previously exposed to long-standing ID.[15]

During pregnancy or breastfeeding, increased/excessive iodine intake may block the thyroid gland of the mother/fetus/newborn, leading to

maternal/fetal/neonatal hypothyroidism.[15] The ability to escape from the Wolff-Chaikoff effect develops at around gestational week 36 and the fetus may therefore be exposed to iodine-induced hypothyroidism despite normal thyroid production in the mother. A negative association between iodine supplementation in pregnancy and psychomotor development in childhood has been observed in some studies.[16] Nevertheless, the small risks of physiologically excessive iodine are judged to be outweighed by the risks from ID.

Daily iodine intake above 600–1100 μg in adults is considered as non-tolerable[19] and excessive iodine intake for school children is defined as median UIC ≥200 μg/mL or ≥300 μg/mL depending on previous iodine status (Table 2).

Optimal iodine intake – a narrow interval

The different levels of iodine intake in a population are associated with differences in the panorama of thyroid diseases.[33] Optimal iodine intake, that is associated with the lowest prevalence of thyroid morbidity, is relatively narrow and specific for a population and time point, depending on previous iodine status. The challenge for authorities is to monitor and adjust iodine levels continuously to protect the population from preventable thyroid diseases.

1.3 IODINE STATUS IN SWEDEN AND OTHER NORDIC COUNTRIES

Historical iodine situation in Sweden

Endemic goiter was reported by Carl von Linné in the 18th century in Sweden and its prevalence increased during the 19th century, which was probably due to a switch of dietary habits going from consumption of iodine-rich herring to iodine-poor potatoes.[34] Another possible cause for the increasing goiter prevalence in the 19th century may have been the increasing population size in Sweden, which demanded the extension of farming land. Not only the most fertile land (which was previously seabed) but also less fertile and more iodine-poor land was then used for farming.[35] In 1929, Carl Axel Höjer observed goiter and cretinism in up to 60% of the population in the inland parts of Sweden, the so called "goiter belt".[34] In 1936, the Medical Board of Health introduced voluntary iodization of table salt with 10 μg/g and, in 1966, goiter prevalence had decreased but had not disappeared, which led to the increase of the level of voluntary iodization to 50 μg/g table salt.[34] A national survey on school children in 2007 confirmed iodine sufficiency in the general population (median UIC 125 μg/L)[36] without endemic goiter in former goitrous areas.[37] Since then, the situation may have changed, as there are reports of decreasing iodine content in Swedish milk from 16 μg/100 g (in 2001) to 11.7 μg/100 g (in 2009),[38] approximately 75% of consumed salt is non-iodized (personal communication with the salt industry, 2017), and the National Food Agency recommends reduction of salt consumption to prevent

hypertension.[39] The most recent assessment of iodine status in school children was performed by the National Food Agency in 2016–2017 and reported a median UIC 117–118 µg/L.[40]

In addition, reports on subgroups of the Swedish population raise concerns. Women of reproductive age (*n*=63) presented median UIC 74 µg/L (WHO recommendation: 100–200 µg/L) in a national mapping conducted 2010–2011 by the National Food Agency[41] and pregnant women (*n*=459) in two local cohorts presented median UIC 98 µg/L during the 3rd trimester of pregnancy (WHO recommendation: 150–250 µg/L).[42] Two small local studies on breastfeeding women from the 1980s reported median BMIC 92 µg/L (*n*=60)[43] and 90 µg/L (*n*=16).[44] The iodine status of individuals who have undergone BS had not been studied in Sweden when this thesis was prepared.

Iodine situation in the other Nordic countries: similarities with and differences from Sweden

The Nordic countries, although all covered with ice during the last Ice Age, present **different geological conditions** that have contributed to their differences in historical and current iodine status.[35] The northwestern part of Sweden is mountainous like Norway. Parts of south Sweden have been a seabed, like Denmark and some parts of Finland. Iceland is located on the transatlantic rift. The parts of Nordic countries that were seabed present higher iodine content in water compared to the

remainder, even though large intercountry variations are observed. Different geological conditions are also related to differences in agriculture, livestock, and dietary habits, which in their turn have led to differences in the prevalence of ID-related thyroid morbidity among Nordic countries.

As in Sweden, goiter was also highly prevalent in Norway and Finland in the 19th century.[35] Although Denmark was first considered iodine sufficient and the sale of iodized products was forbidden from 1874 to 1997, studies in 1980s and 1990s that focused on population groups other than school children suggested ID (mild ID in eastern Denmark and moderate ID in western Denmark, mainly due to different iodine content in ground and drinking water).[35] On the contrary, Iceland has always been known for its high iodine intake, even though some young women have presented, even there, low intake of fish and milk, and therefore inadequate iodine intake.[35]

All Nordic countries have chosen **different strategies to deal with ID**. In contrast to Sweden, the iodine fortification program in Norway has only been 5 μg/g table salt but has been combined, since 1950, with the mandatory iodine fortification of animal feed in an effort to prevent goiter in cattle.[35] Finland has also combined the voluntary iodine fortification of table salt at 25 μg/g, since the 1950s, with iodine fortification of cow feed, leading to higher iodine human intake through dairy products.[35] In Denmark, the voluntary iodine fortification (8 μg/g) for household salt and salt used in bakeries was initiated in 1998, became mandatory in 2000 (13 μg/g), and was further increased in 2019

to 20 μg/g.[35,45] In Denmark, bread was used as a vehicle in the iodine fortification program because it was mainly produced domestically and was considered adequate for an even distribution of iodine to the population.[34] In Iceland, the iodine content of milk has decreased dramatically since 1962 due to less fishmeal in cow feed, which led to the decision to fortify cow feed with iodine in compensation.[35]

Currently, the general adult population in Norway presents inadequate iodine intake, especially among young, pregnant, or breastfeeding women.[46,47] Denmark is considered iodine sufficient in school children[46] but presents mild ID in the adult population inclusive of pregnant and breastfeeding women.[35] In 1980s, Finland presented the highest iodine intake in Europe but is now classified as iodine deficient, probably due to decreased consumption of milk and iodized salt.[35,46] Even though Iceland is classified as iodine sufficient,[46] their dietary habits are getting closer to other Nordic countries, highlighting the fragility of a country's iodine status and the need for monitoring of the general population and vulnerable groups such as pregnant and breastfeeding women.[35]

In conclusion, although all Nordic countries are similar in presenting ID in larger or smaller parts of their population, the causes of ID may differ widely between them and their authorities have chosen different strategies to combat ID. The current challenges on iodine status in Nordic countries therefore need to be addressed separately for each country.

1.4 GAPS IN KNOWLEDGE

According to WHO, each country should monitor the iodine status of the general population and its subgroups.[10] The national study of school children in 2007 declared Sweden's general population as iodine sufficient.[36] This thesis is focused on subpopulations at risk for ID, i.e. those with higher iodine needs (pregnant and breastfeeding women) and those who may present lower iodine intake/uptake than expected in the general population (subjects that have undergone BS). The knowledge gaps that laid the ground for planning this thesis are of both national and international interest.

National interest

The Swedish National Food Agency has considered the Swedish study[42] on iodine status of pregnant women as limited by methodological weaknesses: the study was local; it was not primarily designed to assess iodine status; and it only investigated the third trimester of pregnancy.[48] This knowledge gap is addressed by **PAPER I**. In the same report,[48] the Swedish National Food Agency identified the total lack of recent data on breastfeeding women in Sweden, a gap addressed by **PAPER III**.

International interest

The global effort to combat ID through iodine fortification programs has led to a shift from severe to mild-to-moderate ID in many countries[46] and it is also known that women living in countries classified as iodine sufficient may present ID during pregnancy.[49] The clinical importance of mild-to-moderate ID during pregnancy and the possible effects of iodine supplementation are debated.[25,50,51] The need for randomized controlled trials (RCTs) on iodine supplementation of pregnant women with mild-to-moderate ID is repeatedly acknowledged; however, such RCTs have been considered unethical in many countries because iodine supplementation is, despite weak evidence, recommended by some authorities.[26–28] Sweden is a country where it is currently possible to carry out such a study. **PAPER II** presents the pilot study of an RCT which attempts to address this important gap in knowledge. Finally, the iodine status after BS had been hardly investigated and information on how iodine metabolism changes after different BS procedures is sparse. **PAPER IV** addresses this knowledge gap in a controlled setting. The increasing incidence of BS worldwide[52] makes this issue of global clinical interest.

2 AIMS

The overall aim of this thesis was to investigate iodine status and thyroid function in populations that are known to be at risk for ID, i.e. pregnant and breastfeeding women (we hypothesized relatively low iodine intake due to the known increased need for iodine during pregnancy and lactation) and in populations that are assumed to be at risk for ID, i.e. patients who have undergone BS (we hypothesized lower iodine uptake and/or intake).

I. To assess the iodine status and thyroid function of a representative population of pregnant women in Sweden and to compare those on prenatal iodine supplementation with the rest of the study population.

II. To investigate the effect of daily supplementation with iodine 150 μg in pregnant women in a pilot RCT.

III. To explore iodine and thyroid status among breastfeeding women in two ways: (a) in a cross-sectional manner by comparing exclusively breastfeeding women with the rest of the study population; and (b) in a longitudinal manner during the first 12 months postpartum.

IV. To investigate: (a) if gastric bypass leads to ID due to impaired iodine uptake; and (b) if patients who have undergone GBP or VBG present a reduced iodine status compared to obese non-operated controls due to lower iodine intake.

3 METHODS OF EVALUATING IODINE STATUS HISTORICALLY

Historically, thyroid volume has been used to assess the iodine status of individuals and populations. Nowadays, exposure factors (dietary iodine intake and urinary iodine) and functional biomarkers (Tg, TSH, and free thyroxine [FT4]) are more commonly used. The most used are urinary iodine (short-term indicator) and Tg (intermediate-term indicator). Thyroid volume remains a long-term indicator in countries without an iodine fortification program.[1] When it comes to breastfeeding women, the additional indicator of BMIC is the gold standard.[18]

Thyroid volume (mL) and goiter rate: In severe ID, inspection or palpation of the thyroid gland may be an appropriate indicator for individual iodine status. Goiter prevalence <5% in school children has been indicative of iodine sufficiency in a population.[1] Mild-to-moderate ID is more common nowadays, which implies the use of ultrasonography to evaluate thyroid volume, a method that is costly and requires training. In addition, goiter rate is a long-term indicator that responds slowly (months to years) and in some cases just partially to iodine treatment.[1]

Assessment of dietary iodine intake: Information on intake of iodine-rich foods, through food frequency questionnaires or 24-hour food intake recalls, is useful in monitoring iodine status and when choosing a vehicle for iodine fortification programs. As an indicator of the iodine status of individuals or populations, however, it is largely limited by the difficulty in accurately estimating the amount of iodine-fortified salt

used at home and by food industry.[1] Another limitation is the varying iodine content of foods and the usually unknown iodine content of drinking water.[48] In Sweden, water iodine concentration still influences iodine nutrition even though it is no longer correlated to thyroid volume due to the long-term iodine fortification program.[53]

Urinary iodine is assessed in several ways:

- 24-Hour urinary iodine excretion (**24-UIE, µg/day**) is the actual amount of iodine measured in 24-hour urine samples. 24-Hour urinary iodine concentration (**24-UIC, µg/L**) is the amount of iodine per liter measured in 24-hour urine samples;
- Urinary iodine concentration (**UIC, µg/L) in spot (random) urine samples**;
- Urinary iodine concentration (**UIC, µg/L**) **in timed urine samples** is measured in samples collected specifically in the morning (either fasting or non-fasting), midmorning, afternoon, or evening;
- UIC related to urinary creatinine concentration (U-creatinine) in spot urine samples (**UIC:U-creatinine ratio, µg iodine/g creatinine**) is a method suggested to adjust for variation in hydration;
- Estimated 24-hour urinary iodine excretion (**eUIE, µg/day**) is another method suggested to adjust for variation in hydration.

All these ways of estimating urinary iodine are developed in more detail later in this thesis (see Section 5.1).

Tg, TSH, T4, and BMIC are biomarkers that are also presented later, as they are extensively used in the papers of this thesis (see Section 5).

4 STUDY DESIGNS AND PARTICIPANTS

An overview of the study designs, study participants, interventions, and outcomes of PAPERS I–IV is presented in Table 3.

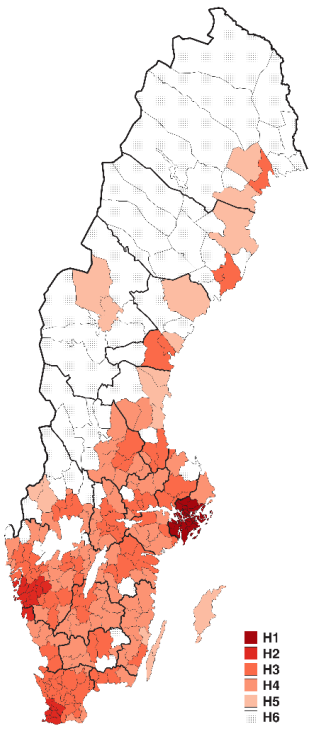
Table 3. *Overview of the characteristics of PAPERS I–IV in this thesis.*

	Paper I	Paper II	Paper III	Paper IV
Study design	Cross-sectional	Interventional Randomized Controlled	Observational Prospective	Interventional Non-randomized Controlled
Follow-up time points	–	Once in every trimester of pregnancy	Third trimester of pregnancy and 0.5, 4, and 12 months postpartum	Inclusion, 2 and 10 years
Study participants	Pregnant women (*n*=743)	Pregnant women (*n*=200) WRA (*n*=89)	Pregnant/ postpartum women (*n*=84)	GBP (*n*=188) VGB (*n*=188) OB-C(*n*=188) MON-C(*n*=412)
Intervention	–	Iodine supplement 150 µg/day	–	GBP or VGB
Outcomes	UIC UIC:U-crea Tg, TSH, T4 TPOabs	UIC eUIE Tg, TSH, FT4 TPOabs	UIC eUIE Tg, TSH, FT4 BMIC, eBMIE	24-UIC 24-UIE TSH, FT4 Dietary iodine intake
Study period	2015–2016	2012–2015	2008–2012	1987–2011

Abbreviations: 24-UIC, 24-hour urinary iodine concentration; 24-UIE, 24-hour urinary iodine excretion; BMIC, breastmilk iodine concentration; eBMIE, estimated breastmilk iodine excretion; eUIE, estimated 24-hour urinary iodine excretion; FT4, free thyroxine; GBP, gastric bypass; MON-C, controls from a random population-based sample; OB-C, obese non-operated controls; Tg, thyroglobulin; TPOabs, thyreoperoxidase antibodies; TSH, thyroid-stimulating hormone; T4, thyroxine; UIC, urinary iodine concentration; UIE, urinary iodine excretion; U-crea, urinary creatinine concentration; VGB, vertical-banded gastroplasty; WRA, women of reproductive age.

4.1 PAPER I

Study design: This was a national cross-sectional study. We applied a stratified, two-stage probability proportionate to size cluster design as recommended by WHO.[10,54]



Explanation: The number of clusters per H region was proportionate to the population size of each region. H regions in Sweden are defined as groups of municipalities with a specified population density. Region H1 has the highest population density and region H6 the lowest. During the first stage of the study, 25 clusters (maternal healthcare centers [MHCs]) from Sweden were selected. The higher population density an H region had, the higher number of MHCs from this H region that were planned to be included in the study. This model is described as a "probability proportionate to size cluster design", meaning that a cluster (i.e. MHC) is more likely to be selected for participation in the study if it is located in an area of high population density. During the second stage of the study, pregnant women were consecutively asked to participate and 30 were selected from each MHC. This random selection of pregnant women was stratified by pregnancy trimester, i.e. the selection was completed for each participating MHC when ten pregnant women from each trimester were enrolled. The first, second, and third trimesters were

defined as pregnancy weeks 1–12, 13–28, and ≥29, respectively. It is important to note that the design was applied to form a representative population of pregnant women on a national basis and not on an MHC or H region basis.

Considerations

There were difficulties in obtaining a positive response from MHCs due to their heavy workload. Only 18% of all MHCs that were asked accepted participation. MHCs from highly populated areas were particularly hard to involve, probably due to the heavy workload in areas with high nativity and tiredness of participating in different studies – these areas are equivalent with the location of the largest university hospitals. In Region H1 (the Stockholm area), only two of the planned six MHCs were recruited; the remaining four MHCs were recruited from Region H2.

- *Does this deviation from the study design influence the representativeness of the study population?* The question is highly relevant and an influence cannot be ruled out. However, we consider that the study population is fairly representative of the general Swedish pregnant population because the remaining four MHCs were recruited from the H region that was closest to H1 in population density and because the reason for declining participation – the heavy workload of midwives – should not be associated with different iodine intake in the pregnant population of the areas covered.

- *Should the study design take into account possible seasonal variation in iodine status?* A seasonal variation of urinary iodine has been observed in some studies but not all.[55–58] Seasonal variation is more pronounced when the main dietary iodine source has a seasonally varying iodine content (e.g. varying iodine content of dairy products due to varying animal feed). This is not the case in Sweden where the iodine content of milk does not present seasonal variation[38] and the main iodine source is iodized salt.[34]

Samples collected: The pregnant women left single samples on one occasion of the following:
(a) spot urine sample for analysis of UIC and U-creatinine in order to analyze UIC:U-creatinine ratio;
(b) dry blood spots (DBSs) for analysis of Tg, TSH, and T4;
(c) serum sample for analysis of TPOabs – applied to 13 of the 25 MHCs which had a capacity to collect and store serum samples.

Participants: As no exclusion criteria were applied, all pregnant women were eligible. We included 743 pregnant women between August 2015 and April 2016.

Considerations

How was the sample size chosen? The interindividual variability of UIC is high, ~30–55%.[1,58,59] To assess the median UIC of a population with 5% precision and 95% confidence interval (CI), ~490 individuals are needed.[60] To account for the cluster design, we planned to include 750 pregnant women.

4.2 PAPER II

Study design: This was a pilot RCT on daily supplementation with iodine 150 µg to pregnant women conducted at Skövde MHC in an area of southwestern Sweden. Subjects were randomized 1:1 to a daily multivitamin tablet either with or without iodine (Table 4) from inclusion in the first trimester until delivery. Samples were collected at baseline (before start of intervention) and in the second and third trimester.

Table 4. *Content of multivitamin tablets with iodine (intervention group) and without iodine (control group).*

Quantity per tablet (percent of recommended daily intake during pregnancy)	
Multivitamin with iodine	**Multivitamin without iodine**
Iodine 150 µg (85%)	Niacin 19 mg (111%)
Selenium 50 µg (71%)	Folic acid 200 µg (50%)
Calcium 250 mg (28%)	Vitamin A 400 µg (50%)
Iron 12 mg (30%)	Vitamin B_1 1.4 mg (93%)
Zinc 12 mg (133%)	Vitamin B_2 1.7 mg (106%)
Vitamin B_2 1.4 mg (87%)	Vitamin B_6 1.8 mg (128%)
Vitamin B_{12} 15 µg (750%)	Vitamin B_{12} 3 µg (150%)
	Vitamin C 60 mg (70%)
	Vitamin D 5 µg (50%)
	Vitamin E 10 mg (100%)

A control group of women of reproductive age (WRA group) of similar age and from the same area was identified using the Tax Registry. They provided urine and blood samples once.

This trial was a pilot study for the expanded, ongoing SWIDDICH study,[61] which is planned to include 1275 pregnant women with follow-up of their children up to 14 years of age using the children's mental development as a primary endpoint (ClinicalTrials. gov identifier: NCT02378246. See www.swiddich.se).

Considerations on the content of the study tablets

- *Why did we choose multivitamins instead of pure iodine and placebo?*

During the planning of the study, we contacted pharmaceutical companies, who were not interested in providing pure iodine tablets. It was not considered as profitable as iodine is a trace element. Therefore, we chose tablets that already existed on the market to ensure the feasibility of the study and its possible future implementation.

- *Do other components in the multivitamins used in the study induce bias by confounding?* Possibly yes.

– **Iron**, which is included in the intervention tablet, is part of thyreoperoxidase that oxidizes iodide in the thyroid cell and couples Tg to iodide. It could therefore lead to false confirmation of the hypothesis that iodine supplementation ameliorates iodine and thyroid status during pregnancy. However, the amount of iron in the tablet is only 12 mg and many pregnant women are anyway recommended 100-mg iron supplementation by their midwives related to a regular screening with capillary hemoglobin testing throughout pregnancy. So, we considered the possible confounding by iron in PAPER II to be unlikely.

- **Selenium** acts as antioxidant in the thyroid gland and is part of the deiodinases, which are active in converting T4 to active T3.[62] As selenium is included in the intervention tablet, confounding by selenium could lead to false confirmation of the hypothesis that iodine supplementation ameliorates iodine and thyroid status during pregnancy. Sweden is a selenium-deficient country[63] and the possible confounding by selenium in PAPER II was considered problematic.

- When it comes to the extended SWIDDICH study, the main outcome is the cognitive development of the children, where both iron and **vitamin B_{12}** may act as confounders.[64] Vitamin B_{12} is present in both tablets at an amount that exceeds the recommended daily intake for pregnant women. Even though the amount of vitamin B_{12} in the intervention tablet is higher, none of the groups is expected to present vitamin B_{12} deficiency and the possible confounding by vitamin B_{12} is considered unlikely.

- *How are we planning to handle the risk of bias by confounding in the extended SWIDDICH study?* In PAPER II, extra blood and serum samples were collected for additional analyses. In these, ferritin (as marker of iron status) and selenium are measured. The intervention and control groups presented no difference in their ferritin levels.[65] The statistical analysis is in progress and in case of difference in the levels of selenium between the studied groups, we plan to analyze the three variables: selenium, group affiliation, and Tg. The purpose is to investigate if the difference in Tg between the groups can be totally or partially explained by the difference in selenium. It should be noted that

we plan to analyze only Tg as an outcome and not UIC, because UIC cannot be considered as indicator of iodine status at an individual level. Vitamin B_{12} will also be measured and analyzed in the same way before the publication of the SWIDDICH study.

Other considerations on the study design

- *Wouldn't it be better if the intervention was already started before conception?* Yes, especially as the first trimester is important for brain development. Pre-conceptional inclusion, however, would have been logistically very difficult, as pregnant women in Sweden first contact an MHC after a positive pregnancy test and the first visit may be scheduled at the end of the first trimester. Inclusion through advertisements could have been a solution but would risk the introduction of selection bias (only 50–75% of pregnancies are planned in Sweden[66] and not all women would respond to advertisements). We therefore chose to include women at the earliest possible stage, which is the same as in similar RCTs.[67–69]

Samples collected:

The pregnant women provided:

(a) spot urine samples at all three visits for analysis of UIC and U-creatinine in order to analyze eUIE;

(b) blood samples at all three visits for analysis of Tg, thyroglobulin antibodies (TgAbs), TSH, and FT4, and at baseline and third trimester for analysis of TPOabs.

We obtained neonatal TSH from the neonatal screening program. The WRA group left spot urine and blood samples on one occasion for analysis of UIC, U-creatinine, Tg, TgAbs, TSH, and FT4.

Participants: We included 200 pregnant women between November 2012 and September 2014. Full inclusion and exclusion criteria are described in PAPER II. We wanted to include thyroid healthy women and avoid women at high risk of ID, such as women with a recent pregnancy or lactation and vegans. Vegans were excluded because they lack both fish and dairy products in their diet and they thereby have a medical indication for iodine supplementation during pregnancy.

For the WRA group, we included 89 women between February 2013 and March 2016. They were matched for smoking habits and age with the first 90 pregnant women included in the study.

Considerations

- *Can the results of this RCT be generalized to the Swedish population or to other countries with similar iodine status?* Although the RCT design provides higher internal validity and reduces bias by confounding, it poses challenges in the generalization of the results. In PAPER II, the women were recruited from an inland area of southwestern Sweden where the distance from the sea could be associated with lower iodine status. On the other hand, recent data no longer suggests ID in previously goitrous areas, probably due to a more even distribution of iodine intake since salt iodization began in 1936.[37] We therefore anticipated that the study population in PAPER II would

not deviate severely from the Swedish pregnant population. This was later confirmed as median UIC at baseline in PAPER II proved to be similar to median UIC during the first trimester of pregnancy in the national cross-sectional study of PAPER I.

- *What was the role of the WRA group in this trial?* The purpose of creating a WRA group was to investigate if the background population in the studied area was iodine sufficient, as anticipated. While interpreting the results, we were obliged to take into account the fact that national mapping from 2007[36] was based on the UIC of school children, which cannot directly be extrapolated to adults (see Section 5.1). In addition, the recruiting period would ideally be identical for the group of pregnant women and the WRA group.

- *How was the sample size chosen?* We did not perform power calculation for this pilot study due to the lack of comparable RCTs at the time the study was planned. Nonetheless, we planned for a sample size of 200 pregnant women, which was larger than that of previously published RCTs (n=25–120).[70] Despite the risk for type 2 error, the results did confirm the hypothesis. On the contrary, the planned sample size for the expanded ongoing version of this trial (the SWIDDICH study) with children's mental development as primary outcome is defined after cautious power calculation.[61]

4.3 PAPER III

Study design: This was a pilot, observational, prospective study of pregnant/breastfeeding women recruited between July 2008 and July 2011. It is a sub-study deriving from a study planned to investigate changes in skeletal health during breastfeeding.[71] The women were included at pregnancy week 35–37 and followed up at 0.5, 4, and 12 months postpartum (Figure 2). This sub-study served as pilot for an ongoing RCT on iodine supplementation to breastfeeding women (ClinicalTrials.gov ID: NCT02378233).

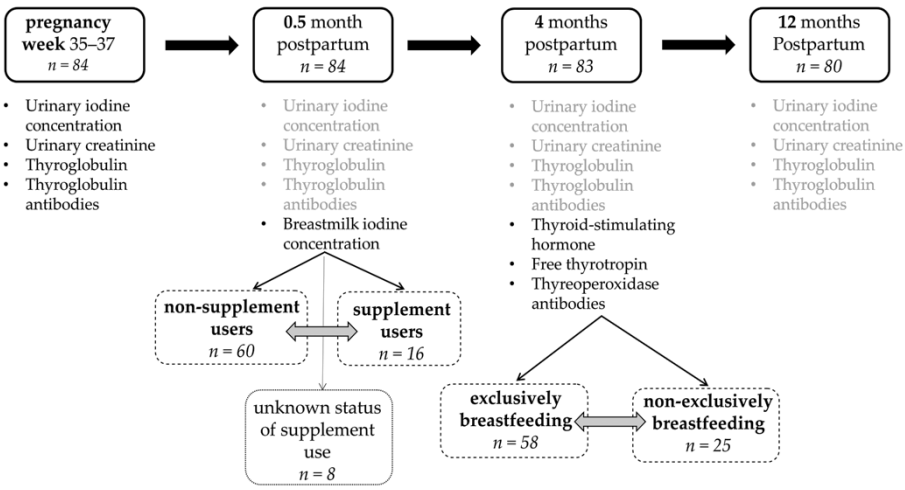


Figure 2. *Study design and illustration of the grouping in PAPER III. The groups compared cross-sectionally are marked with a gray arrow [©Wiley Blackwell. Reproduced with permission after Manousou et al.[72]].*

Consideration

How does it influence the study's validity that it was not initially planned to test this paper's hypothesis? It was practical to use urine, blood, and breastmilk samples that were already collected, especially as the

enrolment of breastfeeding women is challenging due to the high daily workload during the postpartum period. The price to pay is lower internal validity. Some of the exclusion criteria (e.g. gestational diabetes, pre-eclampsia, etc.) were not relevant to the current study. The primary endpoint of this study was BMIC and we would, ideally, have planned for breastmilk collection at several time points postpartum for a longitudinal analysis.

Samples collected: The women left:
(a) breastmilk samples for analysis of BMIC;
(b) spot urine samples for analysis of UIC and U-creatinine in order to calculate eUIE;
(c) blood samples for analysis of Tg and TgAbs.

Participants: Full inclusion and exclusion criteria are described in PAPER III. Of the total 95 women recruited for the study related to skeletal health, 84 had left breastmilk sample at 0.5 month and/or a urine sample at least once postpartum and were selected for the purpose of this pilot study.

Consideration on the sample size

Power calculation was not performed in this study due to the lack of published studies on iodine supplementation to breastfeeding women in a context of iodine sufficiency. We enrolled as many women as possible from the study population available. The goal of an RCT on breastfeeding women in 2004–2005 from New Zealand,[73] an iodine deficient country at that time,[74] was to enroll at least 24 individuals in

each study group. This was also possible for us to achieve. In addition, we studied more stable variables apart from BMIC and UIC. Tg presents less day-to-day variation and the sample size needed for assessing a median eUIE of a non-pregnant, non-breastfeeding population with a precision of 10% and 95% CI is ~96 individuals compared to ~125 needed for median UIC.[60]

4.4 PAPER IV

Study design: This was an interventional, non-randomized, prospective, controlled sub-study that included subjects from two studies: the obese patients derived from the Swedish Obese Subjects (SOS) study[75] and the random population-based samples derived from the World Health Organization MONItoring of trends and determinants for CArdiovascular disease (WHO MONICA) project.[76]

The SOS study was an interventional, non-randomized, prospective, controlled study of obese patients (*n*=4047) included from 1987 to 2001. In the SOS study, obese patients willing to undergo BS constituted the surgery group (*n*=2010) and a matched control group (*n*=2037) was formed in parallel. The WHO MONICA study, Gothenburg, examined a population-based cohort, which was recruited in 2008 and of which a subpopulation had left 24-hour urine samples (*n*=412).

We collected data from the SOS study at baseline (0 years), and 2 and/or 10 years after inclusion, and from the WHO MONICA study on one occasion.

Consideration

What are the challenges in this design compared to an RCT? This was a non-randomized study where the allocation to the intervention/control groups was based on individual treatment preferences. Those who chose BS may have differed from the rest with respect to factors that may also be associated with iodine status and that we could not match for. This study could not be randomized because, at that time, most ethical review

boards in Sweden considered the mortality rate after BS unacceptably high for randomization (based on data from the 1970s and 1980s).[75]

Samples/data collected:

(a) 24-hour urine samples were used for analysis of 24-UIC. 24-UIE was calculated based on 24-UIC and data on urinary volume;

(b) data was collected on already analyzed urinary sodium (U-Na) – a proxy for salt intake –, FT4 and TSH, and dietary iodine intake was assessed based on data already collected through food-frequency questionnaires.

Consideration

How did U-Na and food-frequency questionnaires work as markers for dietary iodine intake? They are both quite weak markers. U-Na is a proxy for salt intake but the amount of ingested salt that was iodized is still unknown. Recall bias and the fact that they were not primarily created to investigate iodine are other limitations related to the food-frequency questionnaires.

Participants: We identified those patients from SOS study who had undergone GBP (*n*=265), had left 24-hour urine samples at baseline and at least once more (at 2 and/or 10 years follow-up), and could be matched to VBG patients and obese non-operated controls (OB controls) from the SOS cohort (*n*=188) (Figure 3). The subjects from the GBP group were then matched for age, sex, body mass index, and smoking habits to VBG patients (*n*=188) and to OB controls (*n*=188), both deriving from the SOS cohort. The GBP group was also matched for age, sex, and smoking habits to MONICA controls (*n*=123), a group which derived from a cohort (*n*=412) of the WHO MONICA study. At 2 and 10 years after inclusion, a new MONICA control group was created from the same WHO MONICA cohort after matching with the corresponding GBP group: the MONICA control group at 2 years (*n*=137) and the MONICA control group at 10 years (*n*=119).

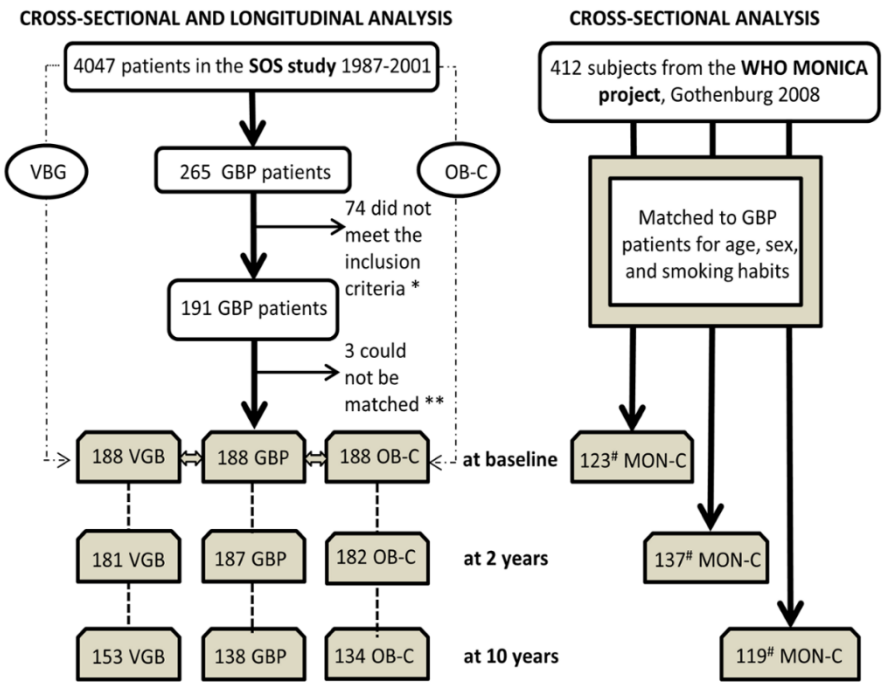


Figure 3. *Flow chart for the recruitment of 564 patients from the Swedish Obese Subjects (SOS) study where equal groups of patients with vertical-banded gastroplasty (VBG, n=188) and obese non-operated controls (OB-C, n=188) were matched with patients that had undergone gastric bypass (GBP, n=188) surgery. The three SOS groups have been followed for 10 years. Patients with GBP were also matched with controls from a random population-based sample, the World Health Organization MONItoring of trends and determinants for CArdiovascular disease, Gothenburg, Sweden, the WHO MONICA project (MON-C, n=412) [reproduced with permission after Manousou et al [77]].*

**24-Urine samples at 0, 2, and/or 10 years after inclusion.*
***GBP patients matched to VGB and OB controls for age, sex, body mass index, and smoking habits.*
[#]Numbers of subjects used on each matching occasion (baseline, and 2 and 10 years) from the whole MONICA population (n=412).

Considerations

- *Why did we use two control groups: one with obese non-operated controls (OB controls) and one population-based control group (MONICA controls)?* The comparison with OB controls answers the question: "Does iodine status change after BS?" We could have answered it by longitudinal analysis within the groups of operated patients but there are confounding factors that change over time, such as dietary trends and iodine content of usual foods. The comparison with the population-based MONICA controls answers the question: "How does the iodine status of the studied groups compare in relation to a random population sample?" One of the hypotheses of this paper was that patients after BS comprise a risk group for ID. To test this hypothesis, the BS groups needed to be compared to a background population. Ideally, the MONICA controls would have left samples at the same year as the SOS groups; however, the available samples were from 2008. Population-based 24-hour urinary sampling is cumbersome and rare.

- *Why did we study VGB, a method no longer clinically used?* The main focus of this paper was GBP, a method widely used nowadays. We hypothesized ID in this group. If the hypothesis was confirmed, the remaining question would be if this was due to low uptake of iodine or to dietary changes after BS. To investigate this question, we needed a comparative group with similar dietary restrictions but intact iodine uptake capacity. Luckily, we had access to data from the VBG patients

from the SOS study. VBG is a BS method associated with restrictions in dietary intake, even though not identical to those after GBP.[78]

- *How was the sample size chosen?* Power calculation was not performed because of the lack of published studies on the effect of BS on iodine status. We aimed to enroll at least 125 individuals in each group to be able to determine 24-UIC with an accuracy of 10% with 95% CI.[58,60] Even though 24-UIC presents the same interindividual variation as UIC, 24-UIE is a more robust marker, so the precision was expected to be higher for this variable.[58] We planned to enroll as many GBP patients as possible. If we had demanded urine samples at both 2 and 10 years after inclusion for eligibility, the sample size would have been seriously reduced. For the same reason, we were obliged to accept minor differences in the matching factors between the different SOS groups, i.e. GBP group, VBG group, and OB controls.

- *Did the differences in some matching factors, the use of iodine supplements or the lack of urine samples from all follow-up time points induce bias?* These are limitations which we tried to handle in several ways. Adjustment for the matching factors that presented small but significant differences between the compared groups led to the same effect size. Sensitivity analyses after exclusion of iodine supplement users or of those who had not left urine samples at all three time points provided similar results.

5 METHODS AND PROCEDURES

5.1 IODINE STATUS

As illustrated in Table 3, **UIC** was measured in all papers with the difference that it was measured in **spot urine samples** in PAPERS I, II, and III but in **24-hour urine samples** in PAPER IV. In PAPER III the participants collected urine in the morning whereas the urine collections were at random time points in PAPERS I and II.

Considerations

- *How does UIC work as a marker of iodine status?* As ~90% of ingested iodine is eliminated through the kidneys in 24–48 hours, urinary iodine mirrors recent (hours to days) iodine intake.[1] At the individual level, day-to-day variation is very high (30–40%)[1,58] and at least ten urine samples are required to determine an individual's iodine status.[60] A common mistake is considering all individuals with UIC <100 μg/L at a single evaluation as iodine deficient, whereas this may simply reflect that the individuals had an iodine-poor diet the day before the random sampling.[1] At the population level, however, day-to-day variation evens out when the sample size is large enough and a single evaluation is indicative of the population's iodine status.
- *Is it better to analyze UIC in spot or 24-hour urine samples?* UIC in 24-hour samples is considered a better proxy for urinary iodine but is logistically difficult when it comes to a large study population and is also limited by the difficulty in objectively confirming the completeness

of sampling. In PAPER IV, the participants reported the exact duration of sampling and we adjusted mathematically for deviations >4 hours.

- *Should spot urine samples be collected at a specified time point or randomly?* UIC in timed urine samples (meaning fasting or non-fasting morning, mid-morning, afternoon, or evening urine samples) have been measured in some studies. One study has suggested better correlation between UIC and 24-UIE when urine samples were collected in the afternoon, whereas others have not been able to identify a time that would be most appropriate.[79]
- *Why did we use different methods in our four papers?* In PAPERS III and IV, we analyzed urine samples that were already collected. In PAPERS I and II, we chose the method that was most widely used and most feasible.
- *Was the method used for analysis of UIC validated?* The laboratory participated in the EQUIP network (US Centers for Disease Control and Prevention, Atlanta, GA) and was successfully evaluated for analytical accuracy every 3 months. As an external quality control, urine samples were added to the daily sample measurements. The UIC analysis in PAPER I was validated against inductively coupled plasma mass spectrometry at the Genomics and Biomarkers Unit in Finland, a gold-standard laboratory, as part of the Euthyroid Project (Helsinki, Finland):[24] the correlation found by linear regression was high (R^2=0.875, p=0.023).
- *What are the limitations of UIC as a proxy of iodine intake regardless the way of urine is sampled?* UIC is limited by day-to-day variation of hydration and iodine intake. In large study populations, day-to-day

variation of UIC evens out.[1] Another limitation is related to the extrapolation of recommended intervals for children to other population groups. WHO classifies a population's iodine status according to the median UIC of school children as mentioned previously (Table 1).[10] Median UIC levels are calculated assuming that children produce ~1 L urine/day and UIC is therefore interchangeable with 24-UIE. The extrapolation of these cut-offs to adult populations is problematic as the average intake of fluid in adults is considered to be ~1.5 L. For the same (adequate) intake of iodine, a population of school children may present median UIC 100 μg/L, whereas a population of adults may present median UIC 60–70 μg/L. [1]

In an attempt to adjust for daily variation of hydration, **UIC:U-creatinine ratio** was used in PAPER I and **eUIE** was calculated in PAPERS II and III based on UIC, measured U-creatinine, and a constant. The constant used was the expected daily U-creatinine excretion for non-pregnant non-breastfeeding women 25–49 years of age, i.e. 1.23 g/day.[80] In PAPER IV, **24-UIE** was calculated as 24-UIC multiplied by urinary volume.

Considerations

Are these three methods equivalent as proxies of iodine excretion at the random day of sampling? **24-UIE** is superior with the prerequisite that the urine sampling is performed over 24 hours, which is logistically challenging. **UIC:U-creatinine ratio** is a method based on the parallel measurement of urinary creatinine. Creatinine is the product of muscle metabolism and is eliminated by the kidneys at a relatively stable rate,

i.e. ~1 g/day in well-nourished adults.[1] This method is, however, limited by the fact that urinary creatinine is influenced by age, gender, and body composition.[5] It can, however, be used in special cases such as when studying pregnant women, i.e. populations of the same gender and limited age range.[16] **eUIE** is based on age- and gender-specific estimates of mean 24-hour urinary excretion of creatinine,[81] but does not adjust for variations in urinary creatinine due to varying body composition. The formula used is eUIE (µg/day) = UIC (µg/L)/(U-creatinine g/L/*k* g/day), where constant "*k*" stands for the age- and gender-specific expected 24-hour urinary excretion of creatinine. It should be noted, though, that well-defined estimates of 24-hour urinary excretion of creatinine for pregnant and breastfeeding women are lacking. In a small study (*n*=20), pregnant women had similar 24-hour urinary creatinine excretion to non-pregnant controls.[82] This may not be the case in breastfeeding women as creatinine is also excreted in breastmilk.[83]

5.2 THYROGLOBULIN

Tg in PAPER I was analyzed using DBSs whereas in PAPERS II and III, it was analyzed using serum samples accompanied by analysis of TgAbs. In the case of elevated TgAbs in an individual, the Tg value was excluded from the analysis due to risk for analytical interference.

Considerations

- *How does Tg work as a biomarker for iodine status?* Tg is the functional biomarker of the thyroid gland which is most sensitive (over a period of weeks to months) to iodine intervention.[1,30] Strong evidence on appropriate cut-offs for Tg is lacking even though serum Tg <10–13 μg/L or a prevalence of serum Tg >40 μg/L in <3% of the study population has been proposed as indicative of iodine sufficiency in school children.[1,30] When it comes to pregnant women, a median DBS Tg <10 μg/L is suggested as indicative of iodine sufficiency in a population.[84]

- *Are DBS Tg and serum Tg equivalent?* DBS Tg is a field-friendly method recommended by WHO for assessing the iodine status of school children[10] and presents a U-shaped association with median UIC.[84] The correlation between DBS Tg and serum Tg is high.[84]

- *Is the lack of data on TgAbs in PAPER I problematic?* This is a limitation in PAPER I. However, the grade of analytical interference between Tg and TgAbs is largely dependent on the method used and it has been suggested as unimportant in studies of pregnant women.[84,85]

- *What are the limitations of Tg as a proxy of iodine intake regardless of the sampling method?* Tg is limited by the fact that it may be influenced by factors not necessarily related to iodine metabolism, such as thyroid activity (e.g. in hyperthyroidism) or volume (non-iodine related goiter). The major challenge in interpreting Tg, though, is the high intermethod variability. Instead of using cut-offs, it is suggested

that Tg is used for between-groups comparisons, ideally in comparison with an iodine-sufficient reference population for each study.[86] We did not have such a reference population in any of the papers, but Tg was mainly used in comparisons between groups or longitudinally in the same group.

5.3 THYROID AXIS HORMONES AND THYREOPEROXIDASE ANTIBODIES

In PAPER I, TSH and T4 were analyzed using DBSs. In PAPERS II, III, and IV, TSH and FT4 were analyzed in serum samples. Neonatal TSH screening was performed in heel prick blood. In PAPER IV, the methodology for TSH and FT4 changed three times during the study.

Considerations

- *How do TSH and FT4 work as biomarkers for iodine status?* In ID, TSH may increase and FT4 decrease, but usually within the normal range, which makes them insensitive indicators of iodine status.[1] An exception is newborns, whose TSH is sensitive for ID because the thyroid gland has higher iodine turnover that requires increased TSH stimulation.[1]

- *Is T4 equivalent to FT4 as a biomarker of iodine status?* No, FT4 is superior as a biomarker because FT4 is the form of thyroxine that can enter and affect the tissues. T4 includes both FT4 and thyroxine attached to thyroxin-binding globulin. The latter has no clinical importance and varies depending on the amount of binding proteins available. In

PAPER I, we measured T4 as a proxy for FT4 because FT4 could not be measured in DBS samples.

- *How did we handle the change of analytical method for TSH and FT4 in PAPER IV?* To minimize bias by measurement error, TSH and FT4 were only analyzed in a cross-sectional way at baseline and at 10 years. The dominant method on each occasion (75% dominance at baseline and 52% at 10 years) was used as reference and the conversion was performed through conversion factors obtained by the laboratory.

5.4 BREASTMILK IODINE CONTENT

In PAPER III, breastfeeding women were asked to collect 5–10 mL breastmilk just before a breastfeeding session, in the morning, and to report details on breastmilk collection. **BMIC** was analyzed and estimated breastmilk iodine excretion (**eBMIE**) was calculated as BMIC multiplied by the expected daily fluid intake at the age of 0.5 months, i.e. 150 mL/kg/day for children with weight <5 kg or 100 mL/kg/day for children with weight 5–10 kg.[87]

Considerations

- *Is UIC or BMIC a better biomarker for iodine status in breastfeeding women?* During breastfeeding, BMIC is a more reliable indicator of iodine status than UIC as the fractional excretion of iodine into breastmilk increases on days of lower dietary iodine intake.[18]

- *How does BMIC work as a biomarker for iodine status?* The cut-off for BMIC is not well-defined as consensus is lacking regarding infant

requirements.[88] Several BMIC cut-offs have been proposed, i.e. 50, 75, 92, and 100 μg/L, the latter being recently recommended and used based on the fact that a full-term infant in considered to need iodine 15 μg/kg/day.[4,73,89]

- *When should breastmilk samples be collected during the day and in relation to a breastfeeding period?* BMIC does not seem to vary depending on the sampling method (time of day, before or after the lactating session, and left or right breast).[90,91]

5.5 DEFINITIONS

Iodine supplement user: An individual who takes a daily supplement containing iodine ≥150 μg (PAPER I) or ≥75 μg (PAPERS III and IV).

The cut-off of 150 μg was chosen for the pregnant women in PAPER I as this is the level of iodine supplementation that international associations recommend during pregnancy.[26–28]

An iodine level of 75 μg was chosen for breastfeeding women in PAPER III as this is half the level of iodine supplementation that international associations recommend during lactation.[26–28] Women on daily iodine supplement >150 μg were few (n=3–6) at all follow-up time points, which explains why the cut-off chosen in PAPER III is lower than in PAPER I.

An iodine level of 75 μg was chosen for the obese participants of PAPER IV because it corresponds to 50% of the recommended daily intake for non-pregnant, non-breastfeeding adults (Table 1).

Definition of reference ranges for serum analyses: Kit- and laboratory-specific reference ranges were used. During pregnancy, trimester-specific reference ranges were used for TSH, T4, and FT4.

Consideration

Are trimester-specific reference ranges for thyroid hormones well defined? No. The need for local population-based trimester-specific reference ranges is acknowledged but they are still lacking.[28] In PAPER II, we used the reference ranges currently used clinically in Sweden. The risk for misclassification was even larger in PAPER I because reference ranges specifically for DBS TSH and DBS T4 are lacking. We applied the reference ranges recommended for serum T4.[28] Furthermore, we analyzed thyroid axis hormones not only in a binary (normal/abnormal) manner but also as continuous variables.

Hypothyroidism: Abnormally high TSH with normal (subclinical) or abnormally low (clinical) T4 or FT4.

Hyperthyroidism: Abnormally low TSH with normal (subclinical) or abnormally high (clinical) T4 or FT4.

Isolated hyper- or hypothyroxinemia: High or low T4 or FT4 with normal TSH.

Exclusively breastfeeding women: Those whose infants obtained ≥90% of their daily energy intake from breastmilk.

Non-exclusively breastfeeding women: Those who either did not breastfeed at all or whose infants obtained <90% of their daily energy intake from breastmilk.

BMIC indicating adequate iodine intake: BMIC ≥100 μg/L. This cut-off was chosen as it was recently recommended and is used (see Section 5.4).[4,73,89]

5.6 STATISTICAL METHODS

The statistical methods used are presented in the papers. UIC is a variable that is usually non-normally distributed but, in PAPER I, it presented normal distribution after log-transformation. Even though the analyses were then performed using parametric methods, the UIC is presented as a median for consistency with the literature. In other cases of normal distribution after log-transformation, the variable is presented as a geometric mean.

5.7 ETHICAL CONSIDERATIONS

All study participants gave their informed consent prior to their inclusion in the studies. All studies in this thesis were conducted in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The Dnr for the studies were as follows: 095-15 and T666-15 for PAPER I; 431-12 for PAPER II; 129-08 for PAPER III; and S604-01, 088-06, and T282-11 for PAPER IV.

Ethical considerations on the RCT (PAPER II)

- *Iodine affects autoimmunity[33] and may increase the risk for postpartum thyroiditis,* even though this hypothesis was not confirmed in a Danish RCT.[92] Postpartum thyroiditis is an underdiagnosed, common (6–50%)[15,93] condition that is considered as harmless and often resolves spontaneously.

- *Iodine supplementation may lead to iodine excess and thereby block thyroid function in the fetus with a risk for fetal hypothyroidism, especially in the case of pre-conceptional ID*.[15] There is only scarce data on the iodine status in women of reproductive age in Sweden[41,95] and the recommended UIC range for adults is uncertain,[1] as elaborated in Section 5.1. School children in Sweden were iodine sufficient in 2007, which defines the current iodine status of the general population in Sweden as sufficient.[36] Furthermore, iodine supplementation with 150 μg/day is recommended by several international thyroid associations[26,28] to pregnant women as the fear for the adverse effect of possible fetal ID overrides the fear for the consequences of iodine excess.

- *Could the other contents in the multivitamin preparations be harmful for the woman or the child?* The two kinds of multivitamins used in the study were carefully selected by co-workers from the Department of Clinical Nutrition and were judged as safe for use during pregnancy at the given dose.

- *Was it ethical to allow the pregnant women in the control group to refrain from iodine supplementation?* There is no current

recommendation on iodine supplementation during pregnancy in Sweden so the control group followed usual management. This is also in accordance with WHO, which does not recommend iodine supplementation to pregnant women living in countries with an iodine fortification program and iodine sufficiency in the general population.[10] However, vegans were excluded from the study because they are prone to ID from the exclusion of two important iodine sources by their diet (milk, and fish and seafood).

6 RESULTS

6.1 PAPER I

➢ The women were recruited at median pregnancy week 23. Of **the whole study population**, 50% took some kind of supplement and 35% took a daily supplement with iodine ≥150 μg. The overall median UIC was 101 μg/L (*n*=737) and the overall geometric mean DBS Tg was 22.1 μg/L (*n*=675). UIC, UIC:U-creatinine, and DBS Tg did not present differences across trimesters (*p*=0.381, *p*=0.153, and *p*=0.579, respectively). Elevated DBS Tg was noted in 19% of the study population.

➢ The study population **was divided into supplement users and non-supplement users**: the two subgroups were compared regarding UIC and DBS Tg (Figure 4). The prevalence of elevated DBS Tg was 13.1% among supplement users and 22.5% of among non-supplement users (*p*=0.004). The two groups did not differ in prevalence of TPOab positivity (*p*=0.107).

➢ The only kind of **thyroid dysfunction** that was considerably frequent was isolated hypothyroxinemia (24.8% of the study population) and was present in a similar proportion of supplement and non-supplement users (*p*=0.633, *n*=631).

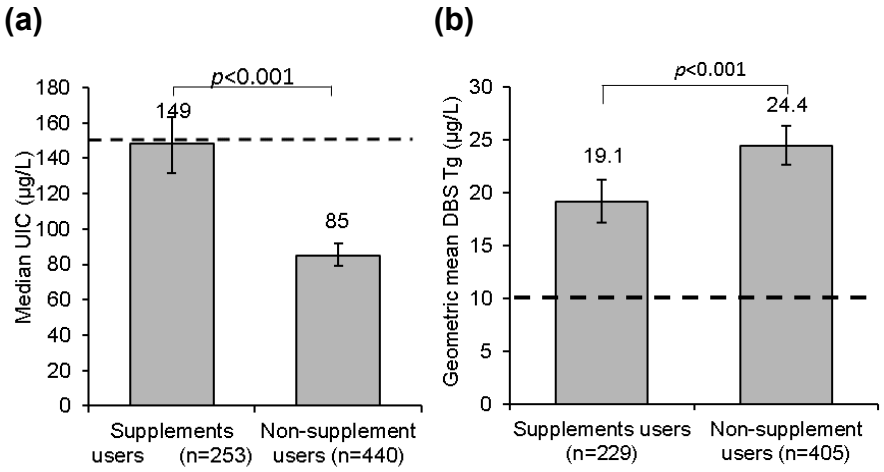


Figure 4. *(**a**) Median (bootstrapped 95% CI) UIC and (**b**) geometric mean (95% CI) DBS Tg in pregnant women who consumed a daily supplement of iodine ≥150 µg/day (supplement users) versus those who consumed no supplements or supplements containing iodine <150 µg/day (non-supplement users). Dashed lines represent the lower recommended median UIC during pregnancy in panel (**a**) and the highest Tg level expected in an iodine-sufficient population in panel (**b**).*[10,84]

Abbreviations: CI, confidence interval; DBS Tg, dried blood spot thyroglobulin concentration; UIC, urinary iodine concentration [reproduced with permission after Manousou et al.[94]*].*

Main conclusions of PAPER I

A representative population of pregnant women in Sweden presented mild ID in all trimesters. Those on daily supplementation with iodine ≥150 µg (35%) presented iodine sufficiency and a Tg concentration that was lower than in the rest of the study population. This implies mild ID during pregnancy in Sweden.

6.2 PAPER II

➢ **Median UIC** in the intervention group was below the recommended limit of 150 μg/L at baseline (p=0.032) and did not differ from this limit in the second and third trimester (p=0.42 and p=0.87, respectively). On the contrary, the control group presented ID on all three occasions (p=0.002, p<0.001, and p<0.0001).

➢ **UIC, eUIE, and Tg** were analyzed in longitudinal and cross-sectional manners (Figure 5a–c). Median UIC at baseline was similar between the intervention and placebo groups but differed in the second (139 *vs* 90 μg/L, respectively) and third trimester (136 *vs* 65 μg/L, respectively). Maternal TSH, FT4, TPOab positivity, and neonatal TSH were similar between the two groups.

➢ **The WRA group** presented median UIC 65 μg/L (i.e. lower [p<0.001] than the recommended limit of 100 μg/L for non-pregnant women),[10] eUIE 61 μg/day, and Tg 18 μg/L.

Main conclusion of PAPER II

A daily supplement containing iodine 150 μg increased the iodine status of pregnant women from mild ID to borderline iodine sufficiency with a positive influence on maternal Tg.

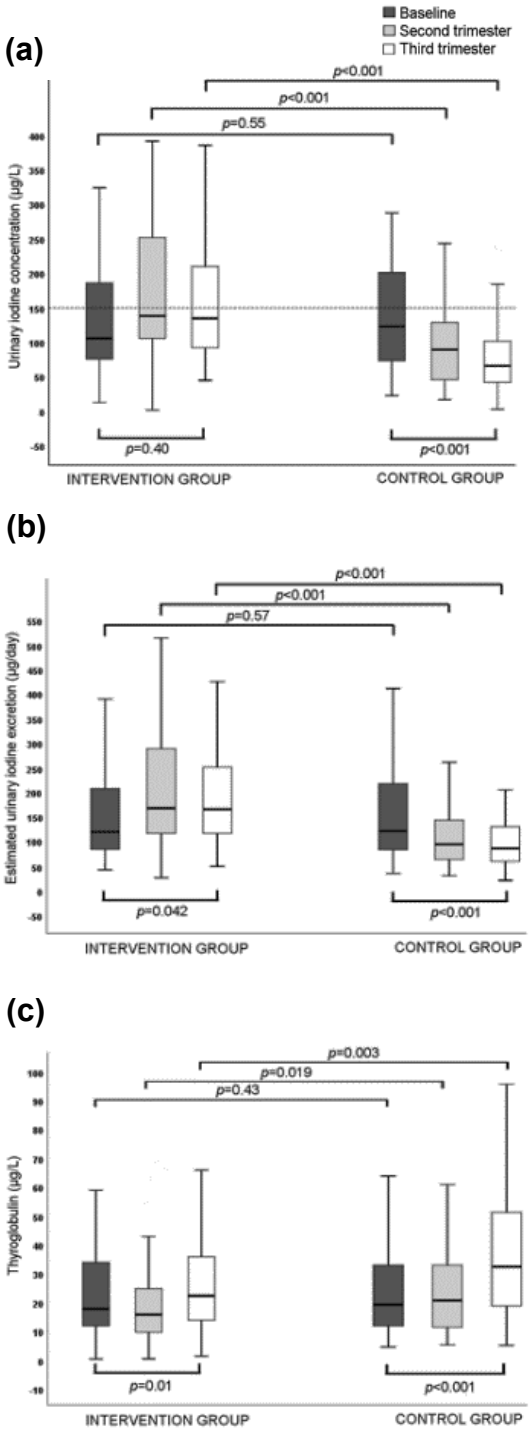


Figure 5. *Box plots comparing (**a**) urinary iodine concentration, (**b**) estimated urinary iodine excretion, and (**c**) serum thyroglobulin at baseline, and during the second and third trimesters for the intervention and control groups. Dotted line in panel (**a**) represents the lower recommended median urinary iodine concentration during pregnancy[10] [reproduced with permission after Manousou et al.[95]].*

6.3 PAPER III

The study population was investigated in three ways:

a) As a whole. The baseline characteristics are presented in Table 5.

b) Divided by breastfeeding habits at 4 months postpartum. The two groups were similar in their background characteristics except for the fact that those exclusively breastfeeding at 4 months had a higher education level than non-exclusively breastfeeding women (p=0.002).

c) Divided by supplement use. Supplement users at 4 months postpartum presented a lower education level than non-supplement users (p=0.045). The background characteristics were otherwise similar in the two groups on all three occasions.

- Median BMIC and eBMIE at **0.5 months postpartum in the whole group** were 90 μg/L (*n*=70) and 53 μg/day *(n*=69), respectively. Fifty-eight percent of the women had BMIC <100 μg/L and 9% had BMIC <50 μg/L. BMIC and eBMIE for the population **according to supplement** use at 0.5 months are presented in Figure 6.

- The main results at **4 months postpartum after division of the study population by breastfeeding habits** are presented in Figure 7. The two groups did not differ with respect to eUIE, TPOab positivity, TSH, or FT4. Thyroid morbidity was not observed in any study participant.

- We studied **the subgroup of women who were exclusively breastfeeding at 4 months and had ceased lactation before 10 months (*n*=34) in a longitudinal way**. Median UIC and eUIE at 4

months were lower than at 12 months postpartum (p=0.005 and p=0.014, respectively) whereas Tg was unchanged.

Table 5. *Background data for the population as a whole during the postpartum period (N=84) and at 0.5 months specifically for the subgroup of mothers who provided a breastmilk sample at 0.5 months (n=70). [© Wiley Blackwell. Reproduced with permission after Manousou et al.[72]].*

	0.5 months (*N*=84)	**4 months (*N*=84)**	**12 months (*N*=84)**	**0.5 month (*n*=70)**
Breastfeeding (%)				
Exclusive[a]	86.9	69.0	0	87.1
Partial	10.7	21.4	15.5	12.9
None	2.4	8.3	81.0	–
Unknown[b]	–	1.2	3.6	–
Iodine supplementation[c] (%)				
Yes	19.0	11.9	9.5	21.0
No	71.4	81.0	81.0	79.0
Unknown[d]	9.5	7.1	9.5	–
Smoking (%)	–	1.2	3.6	–

[a]At least 90% of the infants' daily energy intake coming from breastmilk.
[b]Missing data is due to drop-out, move to other town, or new pregnancy.
[c]At least 75 µg/day.
[d]Missing data is due to incomplete report from the study participant or to unknown iodine content of the reported supplement.

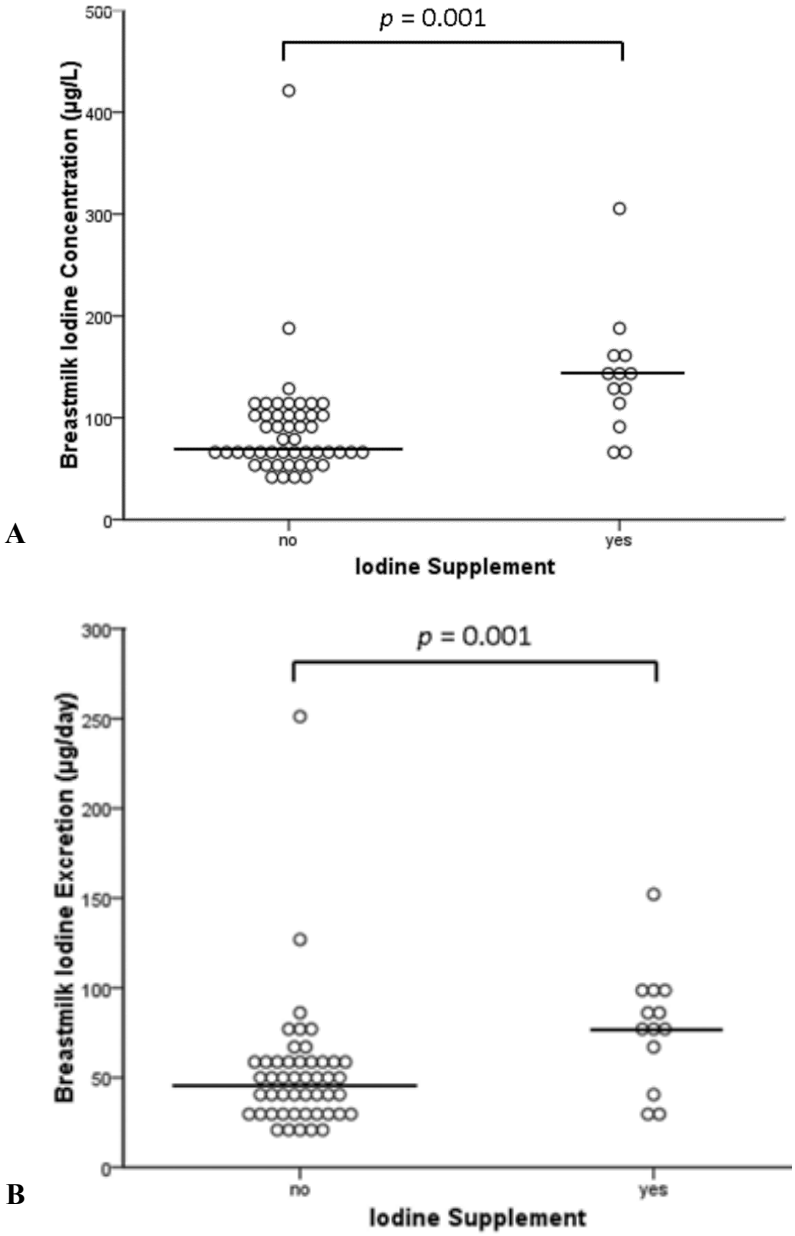


Figure 6. *Dot plot illustrating (**A**) breastmilk iodine concentration and (**B**) breastmilk iodine excretion of non-supplement users (n=49 in panel A and n=48 in panel B) and supplement users (n=13) at 0.5 months postpartum. The median is presented as a line [©Wiley Blackwell. Reproduced with permission after Manousou et al.[72]].*

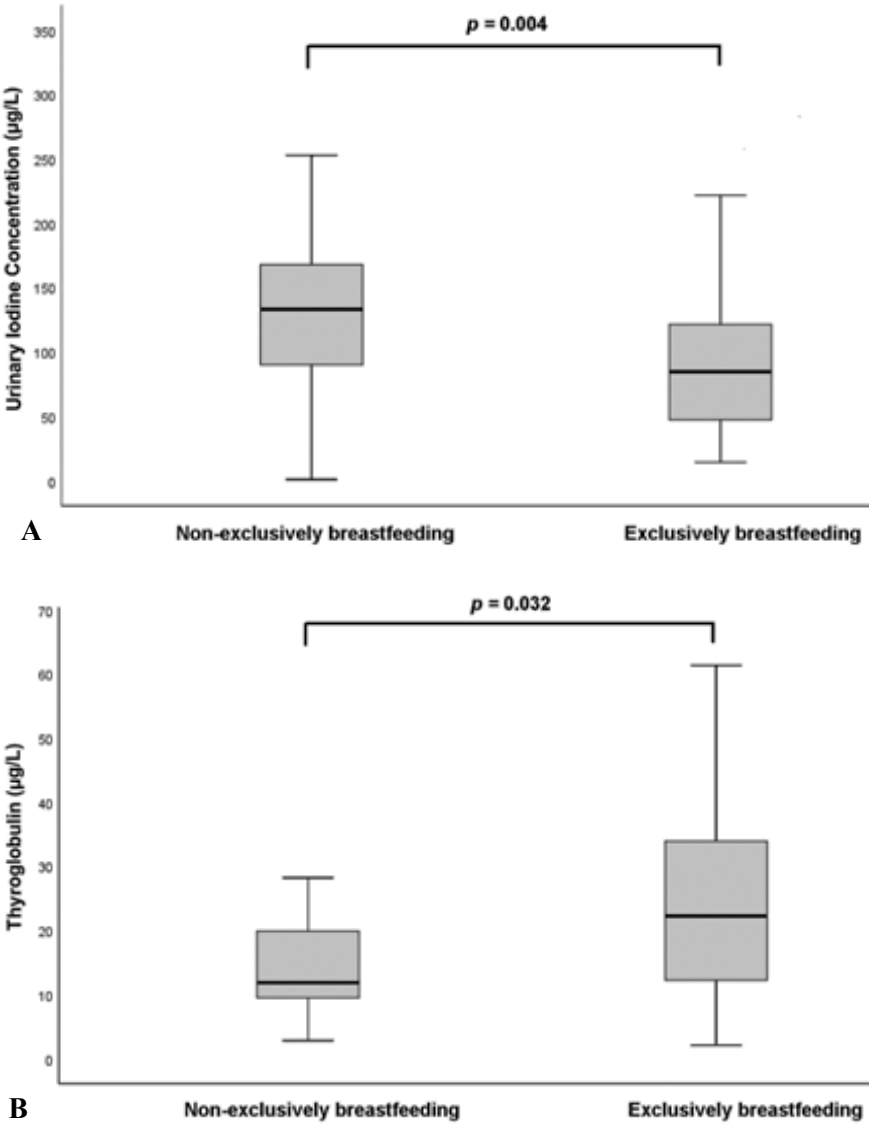


Figure 7. *Box plot illustrating* ***(A)*** *urinary iodine concentration and* ***(B)*** *thyroglobulin of non-exclusively breastfeeding women versus exclusively breastfeeding women at 4 months postpartum [©Wiley Blackwell. Reproduced with permission after Manousou et al.*[72]*].*

Main conclusions of PAPER III

Breastfeeding women in this local population presented mild ID. A minority (~20%) took an iodine supplement and presented BMIC double that of non-supplement users. Exclusively breastfeeding women at 4 months postpartum presented lower UIC and higher Tg compared to the rest of the study population. This local study suggests mild ID during breastfeeding in Sweden but the results should be interpreted with caution because of the lack of iodine data for a representative population of breastfeeding women in the country.

6.4 PAPER IV

➢ **24-UIC and 24-UIE** analyses are presented in (Figure 8).

➢ **Comparison with the recommended range** of UIC 100–200 μg/L:[10] although 24-UIC decreased in both the GBP and VBG groups after BS, median 24-UIC was within the recommended range throughout the study for all SOS groups and MONICA controls.

➢ **Comparison with MONICA controls**: at baseline, all SOS groups presented higher 24-UIC than MONICA controls (p<0.001). At 10 years after BS, the GBP group reached a 24-UIC similar to that of MONICA controls whereas the VBG group had still higher 24-UIC (p=0.002) compared to MONICA controls.

➢ Proxies of dietary iodine intake (**U-Na** and **food frequency questionnaires data**) are presented in Table 6. Assessed iodine intake through food frequency questionnaires decreased significantly (p<0.001) in the GBP group from baseline to 10 years, but minimally (from 770 to 660 μg/week).

Main conclusions of PAPER IV

The hypothesis of ID after GBP was not confirmed. Obese subjects at baseline presented higher iodine status than the general population. After BS (i.e. either GBP or VBG), iodine status decreased but remained at an adequate level at 10 years postoperatively. Whether this decrease was due to altered iodine uptake and/or intake is unknown.

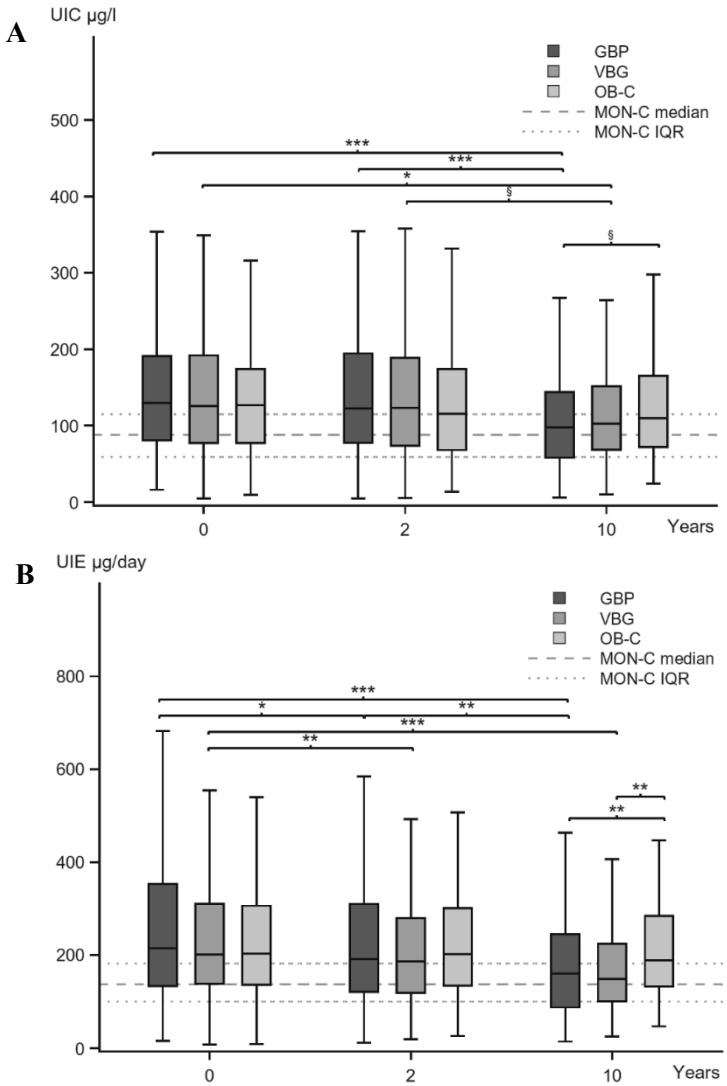


Figure 8. *Box plots comparing (**A**) 24-hour UIC and (**B**) 24-hour UIE of the GBP, VBG, and OB-C groups at baseline, and 2 and 10 years after inclusion, and median and IQR range of the three subgroups from the WHO MONICA project (MONICA controls). Abbreviations: UIC, urinary iodine concentration; UIE, urinary iodine excretion; GBP, gastric bypass; IQR, interquartile range; MON-C, WHO MONICA project controls; OB-C, obese non-operated controls; VBG, vertical-banded gastroplasty. ***p<0.001, **p<0.01, *p<0.05, §p<0.10 [reproduced with permission after Manousou et al.[77]].*

Table 6. *Proxies of dietary iodine intake for GBP, VBG, OB-C, and MON-C groups at baseline (0), 2, and 10 years after inclusion [reproduced with permission after Manousou et al.[77]].*

	Year	Group				Between group comparison (*p*-value)					
		GBP	VBG	OB-C	MON-C	GBP/ VBG	GBP/ OB-C	GBP/ MON-C	VBG/ OB-C	VBG/ MON-C	OB-C/ MON-C
Sample size (*n*)	0	188	188	188	123	–	–	–	–	–	–
	2	187	181	182	137	–	–	–	–	–	–
	10	138	153	134	119	–	–	–	–	–	–
Mean (SD) U-Na (mmol/day)	0	127 (44.3)	121 (44.0)	118 (46.9)	98 (47.9)	NS	0.051[a]	<0.001	NS	<0.001	<0.001
	2	112 (42.4)	106 (44.5)	112 (45.6)	97 (46.9)	NS	NS	<0.001	NS	0.034	0.001
	10	110 (44.3)	107 (42.4)	113 (40.7)	94 (43.1)	NS	NS	0.002	NS	0.004	<0.001
Median (IQR) dietary iodine intake (µg/wk)	0	770 (515,1270)	870 (570,1290)	730 (480,980)	–	NS	NS	–	0.012	–	–
	2	685 (450,940)	890 (570,1250)	735 (520,1040)	–	<0.001	NS	–	0.006	–	–
	10	660 (390,870)	900 (540,1230)	700 (450,960)	–	<0.001	NS	–	0.002	–	–
Median (IQR) iodine intake change (µg/wk)	0 *vs* 10	–135.0 (–490,120)	0.00 (–210,290)	–95.0 (–230,180)	–	<0.001	0.036	–	NS	–	–

Abbreviations: GBP, gastric bypass; IQR, interquartile range; MON-C, WHO MONICA project controls; NS, not significant ($p \geq 0.10$); OB-C, obese non-operated controls; SD, standard deviation; VBG, vertical-banded gastroplasty; wk, week.

[a]Marginally significant ($0.05 < p < 0.10$).

[b]Assessment based on ingestion of milk, yoghurt, and fish from patient questionnaires.

7 DISCUSSION

The goal of the iodine fortification program initiated in Sweden in 1936 was to improve iodine nutrition in order to prevent diseases related to ID. The goal of this thesis was to evaluate the Swedish iodine fortification program in populations with known risk factors for ID (pregnancy and lactation) and to explore BS as a possible risk factor for ID. An additional aim of international interest was to set the basis (through the pilot study in PAPER II) for a large RCT (the SWIDDICH study) planned to investigate whether iodine supplementation to pregnant women with mild ID influences the child's neuropsychological development. The main four questions that this thesis intends to answer are discussed below.

A. Pregnant and breastfeeding women in Sweden: are they iodine deficient?

PAPER I confirms the hypothesis of mild ID in pregnant women and PAPER III suggests that breastfeeding women may be at risk of ID.

Although the general population in Sweden is considered iodine sufficient,[36] the median UIC during pregnancy in PAPER I was 101 μg/L compared to the recommended WHO range of 150–250 μg/L.[10] Mild ID before the start of intervention was also observed in PAPER II. ID in pregnant women in otherwise iodine-sufficient countries is common – for instance in Denmark[35,46] and Switzerland[96] – and may be due to different dietary patterns in this subgroup and/or higher iodine needs.

In PAPER I, pregnant women presented similar UIC, UIC:U-creatinine ratio, and Tg in all trimesters, which is in line with data from some pregnant populations, although the pattern of median UIC and UIE by trimester of pregnancy varies in the literature.[5] This finding in PAPER I is in contrast with the decreased UIC and eUIE in the placebo group in PAPER II, which probably depends on the fact that the placebo group in PAPER II was not allowed to take iodine supplementation during the second and third trimester (median UIC 90 μg/L in the second and 65 μg/L in the third trimester) and is therefore more similar to the group of non-supplement users of PAPER I (median UIC 85 μg/L). In addition, PAPER I was designed to map iodine status during pregnancy in Sweden and therefore has generalizability.

Mild ID is also suggested during breastfeeding according to the results in PAPER III, the pilot observational study. The studied population presented median BMIC 90 μg/L at 0.5 months postpartum compared to the proposed cut-off of 100 μg/L.[4] Median eBMIE was low at 53 μg/day compared to the recommended daily iodine intake for newborns at 90 μg.[10] These results are in line with previous Swedish data.[43,44] Low BMIC levels are also reported in several European countries, Thailand, Zaire, and Australia.[90] It should be noted, however, that the time point of this evaluation, at 0.5 month postpartum, may have led to an overestimation of iodine status during lactation as BMIC tends to decrease during the postpartum period.[97]

As expected, exclusively breastfeeding women were more prone to ID than non-exclusively breastfeeding women. This conclusion is based on

cross-sectional data at 4 months postpartum and on longitudinal data at 4 and 12 months postpartum. A limitation of PAPER III is that breastmilk was only available from 0.5 months postpartum. The conclusions from the rest of the postpartum period are based on urinary iodine and Tg, i.e. less accurate iodine markers than BMIC. PAPER III reveals the need for a cross-sectional study on the iodine status of a representative population of breastfeeding women in Sweden.

B. How does iodine supplementation of pregnant and breastfeeding women affect maternal iodine and thyroid status?

Daily iodine supplementation of 150 μg during pregnancy led to borderline iodine sufficiency with a positive effect on thyroid metabolism in PAPER II, which is supported by the secondary analyses in PAPER I. The intervention group in PAPER II reached a median UIC in the second and third trimester, which was similar (p=0.42 and p=0.87, respectively) to the recommended lowest WHO limit of 150 μg/L,[10] whereas the placebo group remained iodine deficient. Although TSH and FT4 were similar between the study groups, the intervention group presented lower Tg than the placebo group in the third trimester. The results of PAPER II suggest mild ID in the placebo group and borderline iodine sufficiency in the intervention group. The positive effect of daily iodine supplementation (containing iodine ≥150 μg) is also suggested by PAPER I where supplement users presented median UIC at the lowest of the recommended WHO limit and higher than the median UIC of non-supplement users. Similar to PAPER II, this difference in median

UIC was mirrored in Tg in PAPER I (lower in iodine supplement users) but not in TSH or FT4.

When it comes to breastfeeding women, 20% of the studied population in PAPER III took iodine supplements and presented iodine sufficiency at 0.5 month postpartum (median BMIC 140 μg/L), which was double that in non-supplement users (median BMIC 70 μg/L). The low number of supplement users in the subgroups, which were created according to breastfeeding habits, did not allow further investigation of the effect of iodine supplementation in these subgroups.

C. Is iodine supplementation to pregnant and breastfeeding women who present mild ID to be recommended?

The answer is no. The results from PAPERS I, II, and III do not provide enough evidence to support such a recommendation but they lay the ground for further investigations.

Even though PAPERS I and II indicate improved maternal iodine status and Tg after iodine supplementation, the issues that divide the experts[10,26–28] on this question are still unclear:

- Is the effect of iodine supplementation to pregnant women with mild ID of clinical importance? Maternal iodine status is a surrogate endpoint whereas the main clinical interest is children's neuropsychological development. The three published RCTs[67–69] with child outcome as an endpoint failed to confirm a positive effect from iodine supplementation. It is, however, important to be aware of the methodological weaknesses of these previous studies: limited sample

size in two[67,68] and the unexpected finding of adequate iodine status in the placebo group in the other.[69] A sufficiently powered RCT with child outcome is still lacking.

➢ Is iodine supplementation safe? Even though TPOab positivity and thyroid morbidity did not differ between supplement and non-supplement users in PAPERS I and II, a possible side effect with respect to children's neuropsychological development cannot be ruled out. As elaborated in the introduction of this thesis, it is possible that not only iodine excess but also a sudden increase of iodine intake may be harmful. The risk is higher in populations with previously long-standing ID.[15] It is therefore important to be aware of the pre-conceptional iodine status of women in Sweden. The WRA group in PAPER II presented median UIC 60 μg/L, which is in line with the national mapping of women of reproductive age from 2010 to 2011 where median UIC was 74 μg/L.[41] This level is defined by WHO as mild ID but, as noticed in the introduction of this thesis, the recommended WHO range for UIC in adults is extrapolated from school-aged children and is uncertain. It is argued that a median UIC of 60–70 μg/L could be normal for adult populations.[1]

This reasoning on iodine supplementation to pregnant women with mild ID is supported by a recent review and meta-analysis[25] that acknowledges the weak evidence on its safety and efficacy. This knowledge gap motivated the ongoing SWIDDICH study, i.e. the extended version of PAPER II, which is planned to follow up the children's neuropsychological development up to 14 years of age.

When it comes to breastfeeding women, PAPER III suggests a positive effect of daily supplementation (with iodine ≥75 μg) on BMIC. However, the efficacy and safety of iodine supplementation during breastfeeding is even less studied than during pregnancy.[28,92] An ongoing trial on iodine supplementation during breastfeeding (ClinicalTrials.gov ID: NCT02378233) is planned to investigate surrogate outcomes (BMIC, maternal UIC, and Tg) but an RCT with child outcome would provide a higher evidence grade for such a recommendation.

PAPERS I, II, and III do not support iodine supplementation to pregnant and breastfeeding women with mild ID but they do reveal the need for better coverage of the iodine fortification program in Sweden. This opinion is also supported by the Swedish Food Agency, the authority with the mandate to regulate such issues. The level of salt iodization in Sweden (50 μg/g) is higher than that recommended by WHO 20–40 μg/g.[10] However, ~75% of the consumed salt in Sweden is non-iodized while most household salt appears to be iodized (personal communication with the salt industry, 2017). Therefore, the food industry should be encouraged to use iodized salt and the iodine fortification program needs regular monitoring both for the general population and for known risk groups such as pregnant and breastfeeding women. If efforts to improve the coverage of the iodine fortification program fail, it could be argued to apply an iodine fortification program on a mandatory basis.

D. Is BS a risk factor for ID?

The answer is no. PAPER IV does not confirm this hypothesis. Neither obesity (both GBP and VBG groups at baseline and OB controls at all studied time points) nor BS (GBP and VBG groups at follow-up) were associated with lower iodine status than the random population-based sample (MONICA controls). In this study, BS led to decreased urinary iodine but it remained within the WHO recommended range. The mechanism behind this decrease remains unclear.

A normal iodine status after BS has also been observed in two studies that were published after the planning of our study: a Greek study[98] that followed 35 patients before and 6 months after BS and a cross-sectional Spanish study[99] that compared 90 patients 1.5–5 years after BS with a group of obese non-operated patients and a group of non-obese subjects. A decrease in urinary iodine was not observed in the Greek study, probably due to the smaller sample size and shorter follow-up duration. In contrast to PAPER IV, the Spanish study suggested that obesity confers ID and iodine status normalizes after BS, and reported higher urinary iodine in non-obese subjects than in obese or post-bariatric patients. The contradictory results may be due to methodological differences between PAPER IV and the Spanish study: the Spanish study was cross-sectional, the obese non-operated group followed a weight-reducing diet (that may have induced ID), and was not matched with post-bariatric patients for body mass index. Nonetheless, all three studies agree in the main conclusion that BS does not confer ID in contrast to the hypothesis-generating study from 1964.[22]

The observed decrease in urinary iodine after BS in PAPER IV was an interesting finding that could not be explained by the available data. The hypothesis of less iodine intake after BS could not be confirmed as assessed iodine intake through foods did not decrease from baseline to 10 years to a clinically relevant degree. In addition, neither U-Na nor assessed iodine intake through foods was lower in the BS groups compared to OB controls. The hypothesis of worse iodine uptake in the GBP group compared to the VBG group was also not confirmed as these groups had similar iodine status at 10 years. Some possible remaining explanations are that: (a) the food frequency questionnaires did not cover iodine-rich foods that differed in consumption between the study groups; or (b) both the GBP and VBG groups suffered from a decreased iodine uptake (due to rapid stomach emptying for example) which was masked by high iodine intake. The physiology of iodine uptake and its alterations after BS is an area to be explored.

8 MAIN POINTS

- Pregnant women in Sweden present mild ID in all trimesters despite adequate iodine status in school children.
- Breastfeeding women in Sweden may present mild ID, especially exclusively breastfeeding women and those who do not take iodine-containing supplements. This finding is of clinical importance as the prevalence of breastfeeding in Sweden is high (85%, 74%, and 63% at 2, 4, and 6 months postpartum, respectively) and a considerable proportion of mothers (64%, 51%, and 15% respectively) choose to exclusively breastfeed their children.[100]
- The main action to undertake with the data available seems to be to improve the coverage of the iodine fortification program rather than to increase the concentration of iodine in table salt. Regular monitoring of the iodine fortification program in both the general population and risk groups is warranted.
- The international literature is inconclusive regarding the clinical importance of mild ID during fetal and neonatal life. This thesis presents positive effects of iodine supplementation on maternal surrogate endpoints and serves as pilot for a large RCT during pregnancy with assessment of child outcome (the SWIDDICH study).
- BS appears not to be a risk factor for ID. This thesis neither suggests iodine supplementation to bariatric-operated patients nor points to a need for special monitoring of the iodine status of this patient group. Nevertheless, the evaluation of nutritional status regarding other minerals or vitamins after BS is still warranted.

9 FUTURE PERSPECTIVES

Iodine fortification programs, globally, have succeeded in reducing the prevalence of severe ID. The current challenges are:

- To maintain the goals achieved since the iodine content of foods and beverages as well as dietary habits vary over time. Hence, regular monitoring is warranted within the framework of the national nutritional policies supported by the National Food Agencies globally.
- To establish a biomarker that can be used for evaluation of iodine status at the individual level and to determine specific cut-offs for median UIC in adult populations.
- To eliminate mild-to-moderate ID that is nowadays common in many countries or in subpopulations in otherwise iodine-sufficient countries.
- To investigate the role of mild-to-moderate ID in fetal/neonatal life and the benefit/harm of iodine supplementation for pregnant/breastfeeding women with mild-to-moderate ID.

The current thesis confirms mild ID during pregnancy in Sweden and suggests mild ID during breastfeeding as well as a positive effect of iodine supplementation during pregnancy/breastfeeding on maternal surrogate endpoints. In Sweden, the first actions to undertake should aim at better coverage of the current iodine fortification program.

If the iodine fortification program in Sweden fails to guarantee adequate iodine nutrition during pregnancy and breastfeeding, and if mild-to-moderate ID proves harmful for the offspring, the authorities should

consider recommending iodine supplementation during pregnancy/breastfeeding. The expansion of PAPER II (the SWIDDICH study) is an ongoing RCT on pregnant women in Sweden that investigates the effect of iodine supplementation on their offspring's development up to 14 years of age and intends to provide authorities with more data in this direction (see www.swiddich.se).

ACKNOWLEDGEMENTS

The study participants. Thank you for taking the time and effort to support these projects.

Helena Filipsson Nyström, my main supervisor. No, I would not register as a PhD student; I would just do the one obligatory project for my specialist training in endocrinology. That was for sure! Well, the journey with you was too exciting to be abandoned at the very beginning! Thank you for letting me test my boat in the waves, for waving from a distance with a smile, and for ALWAYS sailing near me when you felt I needed it. I know it would have been easier for you to take over but you really wanted to teach me the art and you did it in a respectful way. I appreciate not only your competence but also your honesty and thirst to contribute to knowledge. Thank you, Helena, for being such an honest, dedicated, curious, caring supervisor.

Robert Eggertsen, my co-supervisor. Thank you for your encouraging attitude, your calm presence, and your ability to laugh when both at ease and during difficulty.

Lena Hulthén, my co-supervisor. I remember quite clearly how I felt coming into a team with experienced researchers. At that time, I could understand very little of what was going on. There was always someone who came at the end of a meeting and whispered words of encouragement regardless of how little or how much I had done. This continued throughout the entire journey of my PhD studies. Thank you, Lena, for having been that person.

Kerstin Landin-Wilhelmsen, my co-supervisor. Thank you for your inspiring excitement, your positivity, and for believing in me.

Maria Andersson, co-author in PAPER I and an expert sounding board in discussing study design. I am grateful for the scientific excitement that your answers have offered me. I vividly remember the deep enthusiasm I felt when I presented my very first statistical analyses for you. How did you manage to make me feel so proud of my painfully naive first steps?

Hanna Augustin, co-author in PAPER III and a supportive consultant in nutritional issues. Thank you, Hanna, for your kind support and availability.

Your support has been valuable after getting review comments when deadlines were pressing, after receiving nutritional questions by study participants, or when I simply needed a key to get access to study material in the middle of a sunny summer.

Helge Malmgren, member of the SWIDDICH research group. Thank you, Helge, for your kind availability and your enthusiasm whenever discussing scientific dilemmas or uncertainties. Your enthusiasm is contagious!

The teachers from the Research School of Clinical Epidemiological Methodology. You helped me obtain structure in how to read a scientific publication critically, something that I felt I needed for a long time. You led me to the unpleasant – but still somehow satisfying – world of uncertainty.

Elisasabeth Gramatkovski, laboratory engineer and study coordinator of the papers of this thesis. Thank you for all the iodine analyses and for your fantastic work in recruiting MHCs for PAPER I. I will never forget that you saved me more than once at critical moments. You helped me process study materials and answer reviewers' methodological questions a long time after your retirement, even during summer vacation or Christmas holidays. Thank you, Elisabeth, for your generous support!

Åsa Sikter, the enthusiastic recruiter of the pilot RCT on pregnant women. I do admire your extraordinary ability to make the difficult seem easy and to inspire colleagues and study participants. Thank you Åsa for supporting us – I am happy our journey has not ended yet!

Stella Nakate, biomedical scientist. Thank you, for coordinating the WHO MONICA cohort.

Agneta Lindo, **our SWIDDICH coordinator.** Thank you for your great contribution to our study. Your competence, work capacity, and professionalism are admired.

The rest of **the SWIDDICH research group.** Thank you for creating a multidisciplinary group of committed, supporting, funny, competent researchers, pushing our ambitious project forward.

The Diabetes Team at Frölunda Specialist Hospital. Thank you for your deep engagement with our diabetes patients and the excitement in your eyes when discussing scientific questions. You have inspired me to use the knowledge from my PhD studies in everyday clinical practice. I enjoy my clinical days with you so much!

Maria and Georgios Manousos, my parents. You taught me that "the eye is often afraid but the hand dares" (μάτι δειλό, χέρι τολμηρό). You taught me to take on challenges and control my fears or hesitations. You supported my studies practically and ethically even when that meant I had to leave our homeland for another country. Your love has been and still is precious for me.

Christos Ladopoulos and Kostas Manousos, my big brothers. Thank you for teaching and teasing me when I was little, for always believing in me, and caring for me so deeply. Thank you for spending the summer vacations with me, all three beloved families, because the whole clinical and research journey would have been quite tough without this family warmth.

Maria Panteliou, my cousin and friend. I exercised my Swedish with you the day before my first work interview although you could not speak one Swedish word. We have discussed fears and hopes, possible bias in publications, and the need for future studies. It could have been any subject in the world and any feeling or thought. Thank you for that and a lot more.

Andreas and Danai Meintanis, my children. Not easy at 5–7 years of age to understand what mummy does at work when she is doing research. I once answered that I am looking for the truth. Thank you for your curiosity on the truths of life. It will be a challenge for me to serve the principles I have learned in my PhD studies while I continue answering to your questions throughout your teens and even later. The most wonderful journey of my life!

Dimitris Meintanis, my partner. It could not have been any easy task to support all these crazy goals. Still, you have seen the importance in every one of my ambitions or needs, you have in some cases become almost more enthusiastic than myself! You have supported me with your endless confidence in me, your optimism, and your pragmatism. Thank you for saving me when I had lost myself in the mysteries of Excel or other highly advanced software programs. The main supporter in my efforts has been the silent smile of the

man who does believe in his wife and who knows she will soon discover the same confidence in herself. Thank you for that smile!

Finally, a thank you to the funders contributing to this thesis (The Swedish state under the agreement between the Swedish government and the county councils, the ALF Agreement, the Research and Development (FOU) Fund, the Queen's Fund, General BB's Memorial Fund, the Majblomman Fund, and the Iris scholarship) and to the sources of the images that have illustrated this thesis (*Vertical Gastric Gastroplasty*: by BruceBlaus, CC BY 3.0 via Wikimedia commons; *Gastric Bypass*: by BruceBlaus, CC BY-SA 4.0 via Wikimedia commons; *puzzle*: Freepik.com and *H-regions*: Statistics Sweden).

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