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A 21-year population based investigation

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THE IMPACT OF THE DANISH IODINE FORTIFICATION PROGRAM ON THYROID DYSFUNCTION AND ITS NOSOLOGICAL SUBTYPES

A 21-YEAR POPULATION BASED INVESTIGATION

BY
MADS PETERSEN

DISSERTATION SUBMITTED 2019

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English summary

Background: Iodine deficiency (ID) has been and remains a global health issue of considerable importance. ID may lead to impaired neurological development among children and the development of toxic and non-toxic nodular goitre among adults. Salt iodization is considered a safe and effective way to alleviate ID within a population, yet adverse effects such as increased incidence of hypothyroidism and a transient increase in thyrotoxicosis incidence have been linked to iodine fortification (IF) programs. Voluntary salt iodization in Denmark was initiated in July 1998 (8 ppm) and replaced with mandatory salt iodization (13 ppm) in July 2000.

Purpose: The purpose of this dissertation has been to investigate the impact of salt iodization on the incidence rates of overt thyroid dysfunction and its nosological subtypes in Denmark.

Methods: All new cases of overt thyroid dysfunction were monitored in two areas of Denmark with different pre-existing levels of ID before and long after the introduction of salt iodization. These open cohorts encompassed around 10% of the Danish population at the initiation of the study. The first cohort was located in Northern Jutland (moderate ID prior to IF)) while the other cohort was located in the Danish capital Copenhagen (mild ID prior to IF). The identification of potential new cases of overt thyroid dysfunction was based on applying diagnostic algorithms to all thyroid function tests sampled within the cohort areas and subsequently contacting the current general practitioner of each case for verification. The monitoring program was initiated in the year 1997 and is still ongoing in Northern Jutland while the monitoring program in Copenhagen was concluded in the year 2008. Individual manual scrutiny of the medical history of all cases discovered in the cohort area with previous moderate ID during the years 1997-00 and 2014-16 was performed to determine the severity, duration, need for treatment and nosological subtype of each case of overt thyroid dysfunction.

Results: A highly significant 50% decrease in the incidence rate of overt thyrotoxicosis was observed in the cohort with previous moderate ID between 1997-00 and 2014-16. This was primarily the product of a substantial reduction in the incidence rate of multinodular toxic goitre (÷82%) and solitary toxic adenoma (÷74%). A smaller yet significant reduction in the incidence rate of Graves' disease (÷33%) was also observed. Our monitoring program for hypothyroidism incidence found significant increases in both cohort areas following IF (+30% in the mild ID cohort in 2004-05 and +50% in the moderate ID cohort in 2014-16). However,

manual scrutiny of the medical history of all overtly hypothyroid cases between 1997-00 and 2014-16 revealed that no increase in the incidence rate of hypothyroidism had occurred for the population as a whole when cases with spontaneous normalization of thyroid function, and no medical history suggesting a condition known to cause transitory thyroid dysfunction, were excluded from the analysis. An altered age distribution of new hypothyroid cases was however discovered in 2014-16 with more cases among the young and fewer cases among the elderly.

Conclusions: Mandatory salt iodization successfully reduced the incidence rate of overt thyrotoxicosis in an area of Denmark with moderate ID prior to IF without causing an increased incidence rate of sustained overt hypothyroidism requiring treatment.

Danish summary/Dansk résumé

Baggrund: Jodmangel har historisk været et betydeligt globalt sundhedsproblem. Jodmangel kan føre til hæmmet neurologisk udvikling blandt børn samt udviklingen af toksisk og non-toksisk multinodøs struma blandt voksne. Jodberigelse af salt bør betragtes som et relativt effektivt og sikkert tiltag for at mindske forekomsten af jodmangel blandt befolkningen, dog kan visse utilsigtede virkninger, såsom øget incidens af overt hypothyroidisme og en midlertidig stigning i incidensen af overt thyrotoksikose, følge implementeringen af jodberigelses programmer. Frivillig jodberigelse af salt blev introduceret i Denmark i juli 1998 (8 µg jod per gram salt) og efterfølgende erstattet med obligat jodberigelse af salt i juli 2000 (13 µg/g).

Formål: Formålet med denne afhandling har været at undersøge effekten af den danske jodberigelse af salt på incidensraterne af overt thyrotoksikose, hypothyreose og deres forskellige undertyper.

Metoder: Alle nye tilfælde af overt thyroidea-dysfunktion blev registreret i 2 områder af Danmark med forskelligt forudgående niveau af jodmangel før og længe efter introduktionen af jodberiget salt. Disse to åbne kohorter omfattede omkring 10 % af den danske befolkning da undersøgelsen startede. Den første kohorte lå placeret I Nordjylland (moderat jodmangel før jodberigelsen) og den anden kohorte var placeret i København (mild jodmangel før jodberigelsen). Identifikationen af potentielle nye tilfælde af overt thyroidea-dysfunktion beroede på anvendelsen af to diagnostiske algoritmer på alle thyroidea funktions prøver taget inden for kohorteområderne med efterfølgende verifikation af alle potentielle nye tilfælde hos patienternes almen praktiserende læger. Monitoreringsprogrammet blev påbegyndt i året 1997 og er stadig aktivt i Nordjylland, mens monitoreringsprogrammet blev afsluttet i København i år 2008. Manuel gennemgang af alt tilgængeligt journalmateriale blev udført for alle potentielle nye tilfælde af overt thyroidea dysfunktion i den nordjyske kohorte i perioderne 1997-00 og 2014-16 for at fastlægge sværhedsgrad, varighed, behandlingsbehov og undertype af thyroidea dysfunktion hos disse patienter.

Resultater: Et højsignifikant fald i incidens-raten af overt thyrotoksikose på 50% blev observeret i kohorten med forudgående moderat jodmangel mellem 1997-00 og 2014-16. Dette var primært forårsaget af substantielle reduktioner i incidens-raterne af multinodøs toksisk struma (÷82%) og solitært toksisk adenom (÷74%). En mindre reduktion i incidens-raten af Graves' sygdom blev også fundet at været signifikant (÷33%). Vores monitoreringsprogram for hypothyreose fandt signifikante stigninger

i begge kohorteområder efter introduktionen af jodberiget salt (+30% i kohorten med forudgående mild jodmangel i årene 2004-05 og +50% i kohorten med forudgående moderat jodmangel i årene 2014-16). Her var der tale om enkelte blodprøvesæt, og udførlig verifikation var ikke påkrævet for at blive identificeret som case. Manuel gennemgang af journalmateriale for alle de overt hypothyroide tilfælde fundet i perioderne 1997-00 og 2014-16 afslørede dog at incidens-raten af overt hypothyreose ikke havde ændret sig efter jodberigelsen, hvis man ekskluderede tilfælde med spontan normalisering af thyroidea funktion, som ikke havde indikatorer for tilstande der normalt forårsager midlertidig thyroidea dysfunktion. En ændret aldersfordeling for incidens-raterne af overt hypothyreose var dog tydelig med flere unge og færre ældre tilfælde.

Konklusioner: Obligat jodberigelse af salt fik succesfuldt reduceret incidens-raten af overt thyrotoksiskose i et område med forudgående moderat jodmangel uden at forårsage en stigning i incidens-raten af vedvarende og behandlingskrævende overt hypothyreose.

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This PhD thesis was completed under the careful supervision of Inge Bülow Pedersen and Allan Carlé whose prudent guidance was absolutely essential and on occasion sorely needed. Special thanks should also be extended to Lars Stig Andersen for his tireless quest to improve the quality of my writing. Peter Vestergaard was exceedingly helpful in navigating the bureaucracy of undertaking a PhD at Aalborg University.

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Global thyroid research and the DanThyr project in particular suffered a tremendous loss when Professor Peter Laurberg tragically passed away in the summer of 2016. Professor Laurberg introduced me to the exiting field of thyroid research and was crucial in my decision to enroll with the PhD program at Aalborg University. He was a true giant of his field and will forever be sorely missed.

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List of publications

1. Thyrotoxicosis after iodine fortification. A 21-year Danish population-based study.
   Petersen M, Knudsen N, Carlé A, Andersen S, Jørgensen T, Perrild H, Ovesen L, Rasmussen LB, Thuesen BH, Pedersen IB
   Clinical Endocrinology (Oxf). Volume 89, Issue 3, Pages 360-366, September 2018.
   Published: June 27, 2018.

2. Increased Incidence Rate of Hypothyroidism After Iodine Fortification in Denmark: A 20-Year Prospective Population-Based Study
   Petersen M, Knudsen N, Carlé A, Andersen S, Jørgensen T, Perrild H, Ovesen L, Rasmussen LB, BH Thuesen, Pedersen IB
   The Journal of Clinical Endocrinology & Metabolism. Volume 104, Issue 5, Pages 1833–1840, May 2019.
   Published: December 14, 2018

3. Changes in subtypes of overt thyrotoxicosis and hypothyroidism following iodine fortification
   Petersen M, Pedersen IB, Knudsen N, Andersen S, Jørgensen T, Perrild H, Ovesen L, Rasmussen LB, Thuesen BH, Carlé A
   Submitted to Clinical Endocrinology (Oxf) and tentatively accepted for publication July 26, 2019.

4. Biochemical severity of chronic autoimmune overt hypothyroidism upon diagnosis in Denmark.
   Petersen M, Pedersen IB, Carlé A,
   Submitted to the European Thyroid Journal on July 29, 2019.

Table of content

Abbreviations

ID	**Iodine deficiency**
IF	**Iodine fortification**
SIR	**Standardized incidence rate**
SIRR	**Standardized incidence rate ratio**
TRAb	**Thyrotropin receptor antibodies**
TPO-Ab	**Thyroidea peroxidase antibodies**
Tg-Ab	**Thyroglobulin antibodies**
TBG	**Thyroid hormone binding globulin**
Tg	**Thyroglobulin**
GP	**General practitioner**
WHO	**World Health Organization**
DanThyr	**The Danish Investigation on iodine Intake and Thyroid Disease**
CI	**Confidence interval**
HT	**Hashimoto's thyroiditis**
TFT	**Thyroid function test**
UIC	**Urinary iodine concentration**

Chapter 1: Introduction

1.1 Background

1.1.1 Iodine deficiency

Iodine deficiency (ID) is one of the most common micronutrient deficiencies in the world with more than two billion people still affected[1]. Insufficient iodine intake is the primary cause of iodine deficiency and remains extremely prevalent in certain geographical areas[1]. ID within a population is most commonly measured using spot urine concentrations of iodine[1]. Urinary iodine concentration (UIC) is a suitable estimate for iodine intake as 90% of ingested iodine is excreted in urine[2]. Spot urine concentrations of iodine are however largely dependent on an individual's fluid intake and current level of hydration[3,4], which is why the median UIC value for the entire sample population is often used when estimating iodine intake within an area. Alternatively, 24-hour urine collection or correction using creatinine concentration in a spot urine sample may be used to estimate iodine excretion more precisely at the level of the individual[5]. The World Health Organization recommends the median UIC among men, school age children and women who are not pregnant or lactating to be between 100 and 199 µg/l[1]. Median UIC between 50 and 99 µg/l is categorized at mild ID, between 20 and 49 µg/l as moderate ID and below 20 µg/l as severe ID[1]. Severe ID may lead to endemic cretinism resulting in substantially impaired neurological development in children[6], this is however currently a relatively rare phenomenon[1]. Mild and moderate ID during pregnancy may also lead to impaired cognitive development among children albeit to a lesser degree[7]. Mild and moderate ID within a population can furthermore lead to the development of thyroid nodules and multinodular goiter, some percentage of which will be autonomously functioning and lead to thyrotoxicosis[8–11]. Endemic goiter, toxic and non-toxic multinodular goiter, endemic cretinism and impaired neurological development among other conditions can collectively be termed iodine deficiency disorders (IDDs). Around 31% of the global population remain affected by some degree of ID in spite of substantial efforts to eradicate IDDs[1].

1.1.2 Iodine fortification

Universal salt iodization (USI) is often recognized as one of the most cost-effective, safe and sustainable methods to eradicate ID[1,12]. Globally, it is estimated that around 70 percent of households have access to iodized salt[1]. Though USI is effective in combatting IDDs, a sudden increase in iodine intake may also have some negative consequences with regard to thyroid function. A significant albeit temporary increase in the occurrence of thyrotoxicosis should be expected when initiating salt iodization among an iodine deficient population[13–15]. Some evidence suggests that this may both result from increased substrate availability for pre-existing autonomously functioning nodules as well as a temporary increase in the incidence rate of Graves' disease (GD) due to increased thyroid autoimmunity[16,17]. In addition to a transient increase in the occurrence of thyrotoxicosis, an elevated frequency of hypothyroidism has been linked to increased iodine intake[18–22]. This may at least partly be the product of increased thyroid autoimmunity as indicated by the increased prevalence of thyroid auto-antibodies observed after initiation of salt iodization[23,24]. Both the transient increase in thyrotoxicosis incidence and the increased occurrence of hypothyroidism following salt iodization seems to primarily affect the younger age groups[15,19,22]. Thus, a cautious approach to iodine fortification should be advised with particular focus on the young.

1.1.3 DanThyr

During the 1990s, significant focus was granted to the investigation and eradication of IDDs among the Danish population[25]. Distinct geographical differences in the severity of ID within Denmark were present, with moderate ID predominant in the Western parts of the country and mild ID in the Eastern parts[26]. These differences in iodine intake among the population were primarily the product of variation in the iodine content of tap water[27]. A tendency for increased serum thyroid stimulating hormone (TSH) in late pregnancy among Danish women were indicative of insufficient thyroid hormone production during fetal development[28–30]. Furthermore, toxic and non-toxic multinodular goiter was quite frequent, particularly in the Western parts of Denmark among the elderly[26,31,32]. Iodine fortification of salt was to be introduced in order to combat IDDs among the population while the DanThyr project were launched to monitor the effects of salt iodization and if necessary to adjust it[25]. Voluntary iodization of table salt and salt used for the commercial production of bread at a level of 8 µg/g were introduced in July 1998[33]. The IF

program was aiming for a 50 µg increase in average daily iodine intake but this voluntary program only achieved a 5-10 µg increase[25,34]. Thus, voluntary salt iodization was replaced with mandatory iodization at a level of 13 µg/g in July 2000. Manufactures were however allowed to sell their storages of non-iodized salt produced prior to July 2000 for the remainder of the year, thus mandatory salt iodization was not fully in effect before the end of the year 2000. The DanThyr project was designed to be multifaceted and monitored the impact of salt iodization on several different levels:

I. Two open cohorts with different levels of ID prior to initiation of IF (mild vs. moderate ID) were utilized for continuous registration of all new cases of overt thyroid dysfunction before and after introduction of iodized salt (figure 1). The cohorts encompassed around 10% of the Danish population at the initiation of the study in 1997[35]. Preliminary results from the cohort studies have been published previously[15,19]. Thorough individual scrutiny of the medical records of all cases with overt thyroid dysfunction discovered within the cohort areas between 1997 and 2000 was undertaken to compliment the monitoring program with data on: normalization of thyroid function, initiation of treatment and the specific nosological subtype of each case[10,36]. No such procedure had been performed for cases discovered after the introduction of mandatory salt iodization.

II. Two cross-sectional studies and one follow-up study were performed in two areas of Denmark with different pre-existing levels of ID (mild vs. moderate) before and after initiation of iodine fortification[37–39]. These cross-sectional studies were conducted in: 1997-98 (C1a, n=4649), 2004-05 (C2, n=3570), 2008-10 (C1b, n=2465; follow-up to C1a). The cross-sectional studies were conducted within the aforementioned cohort areas (figure 1). The prevalence of subclinical and overt thyroid dysfunction among representative samples of the general population remains to be investigated past the year 2010. Median UIC values for subjects residing in the areas marked in figure 1 also remain to be investigated past this point.

III. Various treatments for thyroid disease (radio-iodine therapy, thyroid surgery, anti-thyroid medication and levothyroxine therapy) have been continuously monitored on a national level since before salt iodization was introduced and is still ongoing[21,40–42].

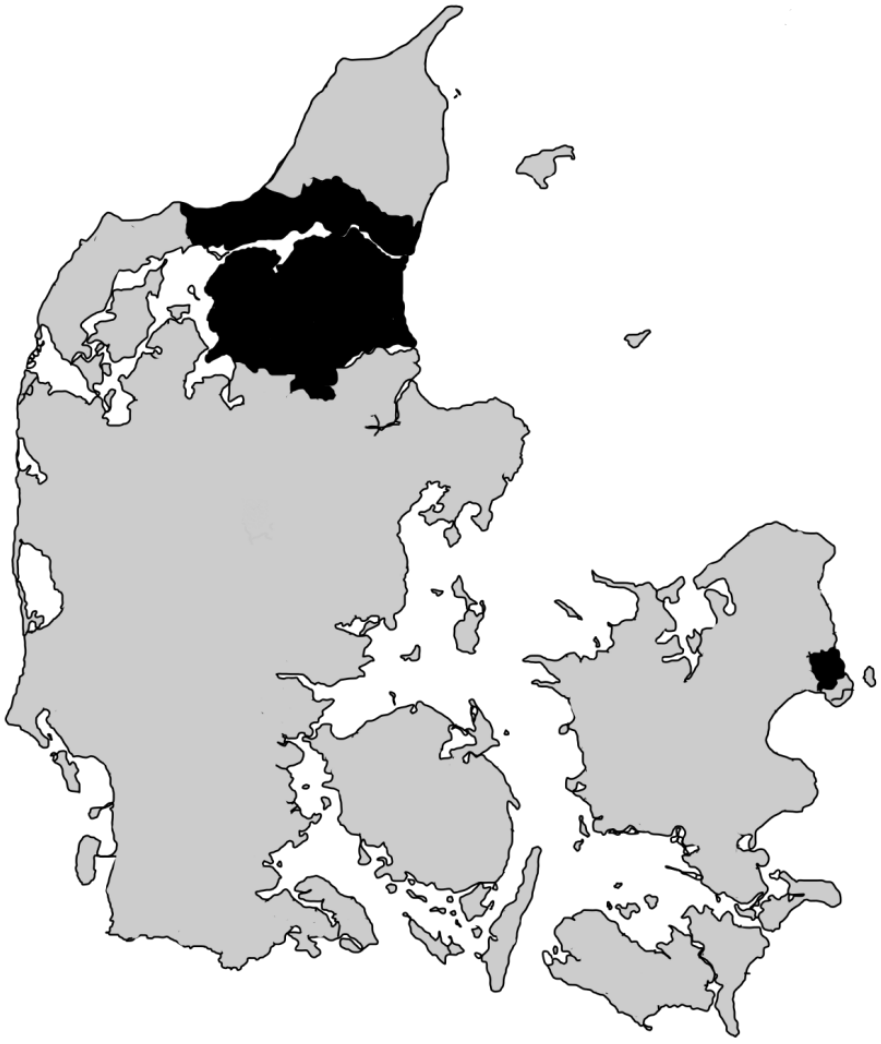

Figure 1: The two cohort areas utilized in the DanThyr studies. The Western cohort located in Northern Jutland encompassed a total of 309,434 subjects by January 1st, 1997, while the Eastern cohort located in the Danish capital Copenhagen contained 224,535 subjects. The population of the Western cohort had a median urinary iodine concentration (UIC) of 45 µg/l (moderate iodine deficiency) in 1997-98 among subjects not using iodine containing supplements, while the population of the Eastern cohort had a median UIC of 61 µg/l (mild iodine deficiency) during the same period.

1.2 Aims of this PhD thesis

This PhD thesis aims to expand our existing knowledge about the impact of the Danish iodine fortification program on the changes in incidence rate of overt thyroid dysfunction by adding more than a decade to the previously published results from the DanThyr cohort studies[15,19]. Furthermore, the thesis aims to reveal how the occurrence of specific nosological subtypes of thyrotoxicosis and hypothyroidism has changed after the introduction of mandatory salt iodization in Denmark. The importance of follow-up investigation of cases with overt thyroid dysfunction will be evident when incidence rates from the monitoring program are compared to those obtained by our follow-up study. Widely different incidence rates may be discovered when normalization of thyroid function in relation to initiation of treatment are included in the verification process.

The diagnostic and therapeutic blood sampling activity before and after mandatory salt iodization will also be subject to investigation. As will the TFT levels of overtly hypothyroid patients at the time of diagnosis.

Chapter 2: Methods

2.1 Study I & II

2.1.1 Purpose

The purpose of the first and second study, included in the present dissertation, was to monitor the development in the incidence rate of diagnosed overt biochemical thyroid dysfunction in two areas of Denmark with different preexisting levels of iodine deficiency before and long after the initiation of salt iodization. The first study deals with overt biochemical thyrotoxicosis, while the second study investigates overt biochemical hypothyroidism.

2.1.2 Study Setting

Two open cohorts were used for monitoring the diagnostic incidence rate of overt thyrotoxicosis and hypothyroidism before and after implementation of salt iodization (figure 1). The first (Western) cohort was located in Northern Jutland and included Aalborg city with some surrounding municipalities (n=309,434 by January 1st, 1997). The second (Eastern) cohort was located in the Danish capital Copenhagen (n=224,535 by January 1st, 1997). Based on data from the DanThyr cross-sectional studies, the median urinary iodine concentration (UIC) within the Western cohort was determined to be 45 µg/l (moderate ID) among subjects not using iodine-containing supplements before introduction of any salt iodization (1997-98), while the median UIC within the Eastern cohort during the same period was 61 µg/l (mild ID)[37]. A second cross-sectional study was conducted in 2004-05 and determined that salt iodization successfully increased the median UIC significantly within both cohort areas (86 vs. 99 µg/l for the Western and Eastern cohort respectively)[38]. A follow-up to the first cross-sectional study was conducted in 2008-10 and found the median UICs for the Western and Eastern cohorts slightly decreased (73 and 76 µg/l)[39].

The first study (monitoring the incidence rate of overt thyrotoxicosis) covers a study period of 21 years (1997-2017), while the second study (monitoring the incidence

rate of overt hypothyroidism) covers a 20 year study period (1997-2016). The period before implementation of voluntary IF (1997-June 1998) was used as the baseline period in both studies. Due to a national structural reform, the boundaries of the Danish municipalities were restructured in January 2007, resulting in a small decrease in the size of the Western cohort (n=261,569 by January 1st, 2007), while the Eastern Cohort was left unaffected. Detailed information on the composition of the cohorts were provided yearly by Statistics Denmark[43].

2.1.3 Data Collection

All TFTs performed within the cohort areas were collected and evaluated in a specially designed register database. Four laboratories handled the analysis of all TFTs sampled within the cohort areas; these were the laboratories at Aalborg University Hospital, Frederiksberg Hospital, Bispebjerg Hospital and the General Practitioners Laboratory in Copenhagen.

In Denmark, all general practitioners (GPs), hospital departments and private practice specialists have unique referral identification numbers for laboratory services. All Danish citizens possess unique identification numbers in the Centralized Person Register (CPR), which is used for all interactions with the healthcare sector. The unique referral identification numbers and the CPR numbers allowed for the inclusion of only TFTs sampled from patients who resided inside the cohort areas. Less than one percent of subjects living within the cohort areas were consulting GPs outside the cohort areas[35].

All potential new cases of overt biochemical thyroid dysfunction were identified by subjecting all TFTs collected from patients residing within the cohort areas to the diagnostic algorithms specified below:

Overt biochemical thyrotoxicosis: serum TSH < 0.2 mU/l combined with elevated serum total T_3 and/or elevated serum total T_4.

Overt biochemical hypothyroidism: serum TSH > 5.0 mU/l combined with decreased serum total T_4.

Details on the reference ranges of the total T_3 and total T_4 assays used by each laboratory have been described previously[35]. To evaluate the diagnostic performance of the four laboratories, a serum reference panel was collected from 100 healthy subjects (50 men and 50 women) with ages between 20 and 45 years. This reference panel was stored in micotubes at ÷80°C to be used for assay comparison between the

four laboratories. These comparisons were made each time a new assay was introduced by one of the four laboratories.

When patients were identified as potential new cases of overt biochemical thyroid dysfunction by the above-mentioned diagnostic algorithms, the present GP of each patient was contacted for confirmation of the patient's status as a new case of overt thyroid dysfunction. If the patient had previously been diagnosed with overt thyroid dysfunction, the patient would be excluded from further analysis. In cases where the hospital records of the patients clearly indicated previous overt thyroid dysfunction contact to the GP was deemed unnecessary. Manual confirmation of each potentially new patient's area of residence was conducted.

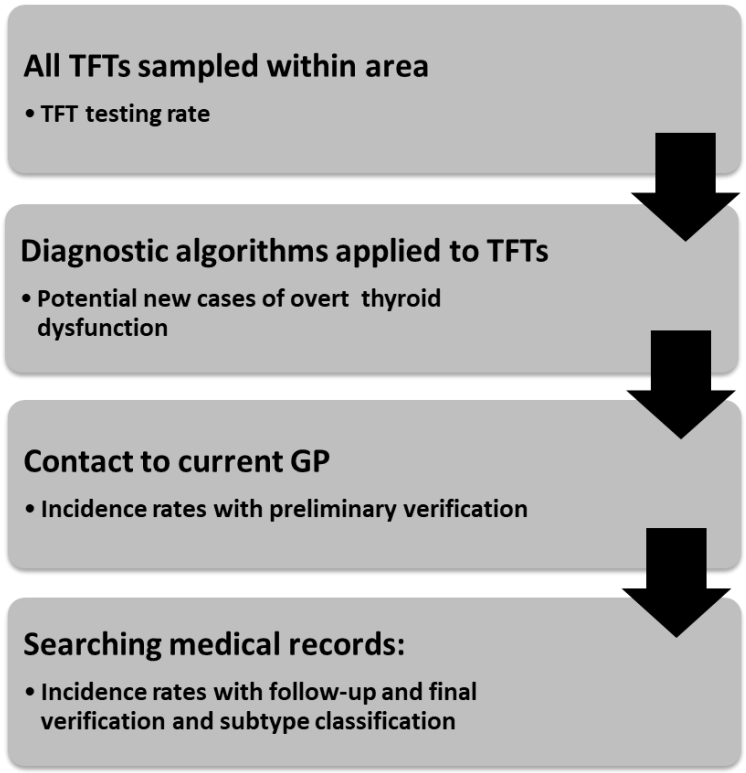

Figure 2: The different levels of data extraction from the DanThyr cohort studies. The database collects all thyroid function tests (TFTs) from within the cohort area (general practitioners (GPs), hospital departments and specialists with private practice). Diagnostic algorithms are then applied to select potential new cases of overt thyroid dysfunction. The GPs of each potential new case is then contacted for preliminary verification. The monitoring program uses data from this preliminary verification to assess incidence rates (Study I & II). Finally the medical records of cases with preliminary verification may be searched within

limited time periods to gain final verification including follow-up and subtype classification (Study III).

2.1.4 Statistics

Changes in the sex and age composition of the cohort were adjusted using the method of direct standardization[44] and thus the standardized incidence rates (SIRs) were calculated according to:

1) $$\text{SIR} = \frac{\sum (\text{age and gender specific rates} \times \text{standard weights})}{\sum (\text{standard weights})}$$

The Danish population at January 1st, 2005 was used as the standard population. The SIR was noted in cases per 100,000 per year. Significance to baseline SIR was calculated using the 95% confidence intervals (95% CI) of the standardized incidence rate ratio (SIRR)[44] in accordance with:

2) $$\text{Upper and lower 95\% CI of SIRR} = \frac{\text{SIR}}{\text{Baseline SIR}}^{1 \pm (1.96 / \frac{\text{SIR} - \text{Baseline SIR}}{\sqrt{\text{SE (SIR)}^2 + \text{SE (Baseline SIR)}^2}})}$$

Results were considered significant if the 95% CI for the SIRR did not include 1. For the statistical analysis IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp was used.

2.2 Study III

2.2.1 Purpose

The purpose of the third study in the present dissertation was to investigate changes in the incidence rates of specific nosological subtypes of overt thyrotoxicosis and hypothyroidism in an area with previous moderate ID after introduction of cautious mandatory salt iodization. Furthermore, a verification procedure involving follow-up

on TFT normalization and initiation of treatment was conducted for all patients discovered by our monitoring program within the selected time periods (figure 2).

2.2.2 Study Setting

An open cohort located in Northern Jutland including Aalborg City with surrounding municipalities, (n=309,434 by January 1st, 1997) was utilized for this study (the Western cohort from Study I&II; figure 1). Potential new cases of overt thyrotoxicosis and hypothyroidism discovered within the cohort area by our surveillance program (see section 2.1.3) during two specific periods (1997-00 vs. 2014-16) were selected for follow-up investigation and subtype classification. The 2014-16 period was selected because peak incidence rate of overt hypothyroidism was discovered by our monitoring program (Study I&II) during those years, meanwhile the greatest reduction in the incidence rate of thyrotoxicosis was observed. The study cohort comprised 272,954 subjects at the initiation of the post IF study period (January 1st, 2014), while the total amount of person-years covered by the post IF study period (2014-16) was 825,842. Median UIC levels within the cohort area were determined in 1997-98 (45 µg/l; moderate ID)[37] and again in 2008-10 (73 µg/l; mild ID)[39]. Detailed information on the composition of the cohort were provided yearly by Statistics Denmark[43].

2.2.3 Data Collection

Follow-up investigation and subtype classification was performed for all new cases of overt thyroid dysfunction discovered within the cohort area by our diagnostic algorithms and subsequently verified through contact to their current GP between the years 1997-00 and 2014-16 (see section 2.1.3 for further details on the identification of potential new cases).

The distribution of the nosological subtypes of overt thyroid dysfunction were described in detail for the cohort population during the years 1997-2000 before mandatory salt iodization[10,36]. In total 1069 potential new cases of overt thyroid dysfunction (thyrotoxicosis: 666, hypothyroidism: 403) were identified by our register database and subsequently confirmed as such by their current GPs during the years 2014-16. Correspondingly, during the years 1997-00 (before mandatory IF of salt) a total of 2011 potential new cases of overt thyroid dysfunction were

identified (thyrotoxicosis: 1601, hypothyroidism: 410). Thorough examination was performed of each patient's hospital records, medicinal database (used by private practitioners and hospital departments), subsequent TFTs, TSH receptor antibodies (TRAb) measurements and thyroid scintigraphies.

A follow-up procedure was implemented for all the 1069 cases of overt thyroid dysfunction identified by our surveillance program during the years 2014-16 (figure 3). The patient would be conclusively verified (otherwise excluded) if any of the following were discovered to be true during the follow-up investigation:

I) Sustained overt biochemical thyroid dysfunction, i.e. a confirmatory TFT at least 3 weeks later.

II) Normalization of thyroid function due to treatment for hypothyroidism (levo-thyroxine therapy) or thyrotoxicosis (anti-thyroid medication, radioiodine therapy or thyroid surgery).

III) Normalization of thyroid function without treatment but with a medical history suggesting a condition of transient thyroid dysfunction, such as: postpartum thyroid dysfunction (PPTD), subacute thyroiditis (SAT), silent thyroiditis, radioiodine-induced thyroid dysfunction, radiation-induced thyroid dysfunction, medication-induced thyroid dysfunction (amiodarone, lithium, interferons, interleukins and monoclonal antibodies) or surgical manipulation of the thyroid gland.

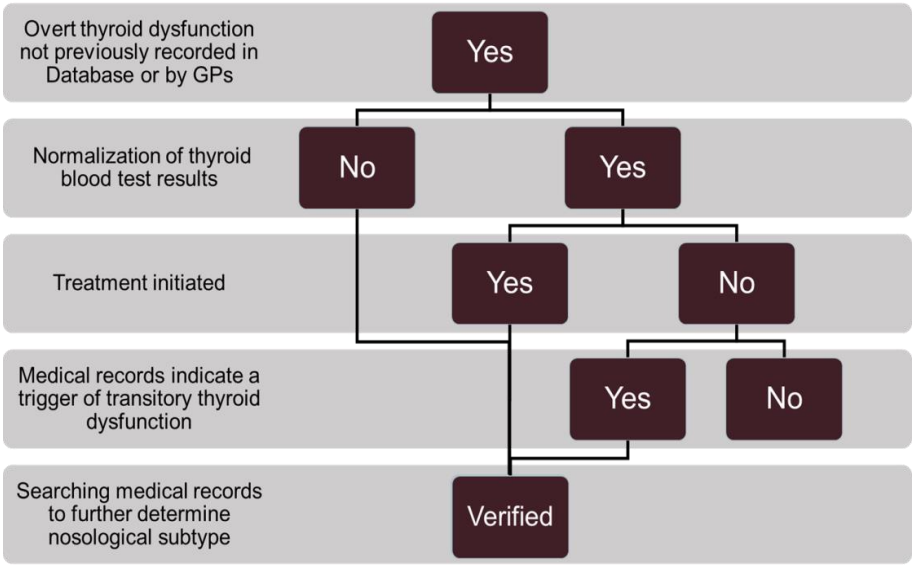

Figure 3: Flowchart showing the process of verification for cases of overt thyroid dysfunction. Patients identified by the diagnostic algorithms within the cohort area, who had

not been registered with overt thyroid dysfunction before by either the patients' current general practitioner (GP) or by the register database, constituted the pool of cases for further evaluation. Cases were first evaluated with respect to normalization of thyroid function tests, and then according to whether this normalization was the result of treatment or a case of spontaneous normalization. Cases of spontaneous normalization were verified as true hypothyroid or thyrotoxic patients if their medical history suggested a known condition of transient thyroid dysfunction (e.g. subacute thyroiditis, post partum thyroid dysfunction or one of several iatrogenic causes) otherwise they were excluded. All verified cases were further scrutinized to determine their nosological subtype. Copied from Study III.

A total of 201 cases failed to meet any of the three above mentioned criteria and were thus excluded. Furthermore, 209 cases were excluded due to other specific exclusion criteria listed below:

- Patients had previously suffered from overt thyroid dysfunction (n=9).
- Patients were receiving levothyroxine or anti-thyroid medication at the time of diagnosis (n=115).
- Presence of gestational transient thyrotoxicosis (n=22).
- An elevated level of thyroid hormone binding globulin (TBG) present due to either pregnancy or estrogen therapy (n=11).
- No confirmative blood test result in a patient who survived beyond 2 months (n=6).
- Amiodarone treatment where the thyrotoxic patients did not have an elevated total T_3 (n=19); elevated total T_4 should be expected in a patient with subclinical thyrotoxicosis receiving amiodarone.
- Treatment was initiated after the patient had shifted from overt to subclinical thyroid dysfunction (n=14).
- Other reasons (n=13), these included cases with pituitary disease, children having different reference intervals of total T_4 and erroneously being included as cases of overt thyrotoxicosis by our algorithm, cases where overt thyroid dysfunction occurred several years after spontaneous normalization, one case where the thyroid gland was surgically removed before any confirmatory TFT could be performed.

Thus, the follow-up procedure allowed for the verification of 659 patients with overt thyroid dysfunction out of 1069 (thyrotoxicosis: 408, hypothyroidism: 251).

A number of patients were contacted by our research team following their diagnostic TFT and invited for a comprehensive investigation at our research center. This included: several systematic questionnaires about their medical history, ultrasonographic examination of the thyroid gland, thyroid scintigraphy (among

thyrotoxic cases), blood tests (TBG, thyroglobulin, thyrotropine receptor antibodies (TRAb), thyroid peroxidase antibodies (TPO-Ab), and thyroglobulin antibodies (Tg-Ab)). Out of 1069 potential new cases discovered in 2014-16, 511 patients were examined at our research center (48%). During the years 1997-00, 38% of all new cases with overt thyroid dysfunction were examined. Full consent was obtained from each patient after a thorough explanation of the nature and purpose of all the procedures used.

Based on their medical history, TRAb measurements and thyroid scintigraphies each verified case of thyroid dysfunction were classified into one of the following categories:

a) Graves' disease (GD) with thyrotoxicosis: positive TRAb measurement (TRAb+, TRAb > 1.0 IU/l) and/or a nonsuppressed homogeneous TcO_4^- uptake within the entire thyroid gland on scintigraphy (n=181).

b) Multinodular toxic goitre (MNTG): a heterogeneous uptake on thyroid scintigraphy with at least two nodules of enhanced TcO_4^- accumulation combined with absent or diminished uptake in the rest of the gland. If TRAb was negative or not measured, the diagnosis was MNTG (n=74).

c) 'Mixed type' thyrotoxicosis (Marine-Lenhart syndrome): patients with a positive TRAb measurement but a MNTG like pattern on thyroid scintigraphy (n=71).

d) Solitary toxic adenoma (STA): a single nodule with enhanced TcO_4^- uptake combined with absent or low TcO_4^- accumulation in rest of the thyroid gland (n=13).

e) SAT (subacute thyroiditis/de Quervain thyroiditis): transient thyrotoxicosis and/or hypothyroidism, with no medical history which could otherwise explain the transient period of thyroid dysfunction (amiodarone, lithium, interferon, interleukin, monoclonal antibodies, radioiodine treatment, radiation or surgery) and with at least two of three SAT criteria fulfilled: anterior neck pain; absent or low TcO_4^- uptake with no visible thyroid nodules on scintigraphy; or elevated erythrocyte sedimentation rate / C-reactive protein (thyrotoxicosis: n=24, hypothyroidism: n=1).

f) Silent thyroiditis: transient thyrotoxicosis with absent or low TcO_4^- uptake and no presence of anterior neck pain or elevated erythrocyte sedimentation rate / C-reactive protein. Similar to SAT, no other explanation for the transient period of thyroid dysfunction should be detectable in the patient's medical history (n=6).

g) Postpartum thyroid dysfunction (PPTD): overt thyroid dysfunction presenting within one year after delivery. If TRAb was negative or not measured in the case of thyrotoxicosis, PPTD was the diagnosis. If TRAb was positive, the patient was classified as GD (thyrotoxicosis: n=15, hypothyroidism: n=23).

h) Amiodarone-associated thyroid dysfunction: overt thyrotoxicosis or hypothyroidism diagnosed during or within 12 months after amiodarone treatment (thyrotoxicosis: n=6, hypothyroidism: n=11).

i) Radioiodine-associated thyroid dysfunction: transient overt thyrotoxicosis developed within a month after radioiodine treatment of non-toxic goitre was performed, or overt hypothyroidism developed within one year (thyrotoxicosis: n=4, hypothyroidism: n=5).

j) Lithium-associated thyroid dysfunction: overt thyroid dysfunction in patients previously (<12 months) or currently treated with lithium (thyrotoxicosis: n=5, hypothyroidism: n=4).

k) 'Manipulation thyroiditis' with thyrotoxicosis: transient thyrotoxicosis developed shortly after thyroid manipulation during surgery on thyroid or parathyroid gland (n=3).

l) Thyroid dysfunction associated with previous (<12months) or current treatment with interferon (IFN), interleukin (IL) or monoclonal antibodies (thyrotoxicosis: n=4, hypothyroidism: n=5).

m) Radiation-associated thyroid dysfunction: overt thyrotoxicosis within 3 months after any radiation therapy against the neck region or overt hypothyroidism within one year (thyrotoxicosis: n=2, hypothyroidism: n=7).

n) Surgically induced hypothyroidism: overt hypothyroidism within one year after hemithyrodectomi or total thyrodectomi (n=12). Patients who underwent sufficient L-T_4 substitution immediately after surgery would logically not emerge as hypothyroid.

o) Congenital hypothyroidism: identified through the Danish neonatal screening program (n=3).

***p) Spontaneous hypothyroidism: overt hypothyroidism in patients without any of the above described conditions (n=180). We have previously shown that this combined group of patients with hypothyroidism due to Hashimoto's or Ort's disease almost exclusively (>99%) harbored TPO-Ab and/or Tg-Ab*[45].**

Textbox 1: Definitions of the different subtypes of overt thyroid dysfunction included in Study III and the number of cases of each subtype during the years 2014-16. The content of the textbox was copied directly from Study III of the present dissertation.

2.2.4 Statistics

Using the criteria described above, subtype classification was possible for 380 out of 408 verified cases of overt thyrotoxicosis. A small number of patients however, (n=28) had no TRAb measurement or thyroid scintigraphy performed. Based on our examination of their medical histories, the entities e-m could be ruled out. Similar to the method used in our previous study, we performed nearest neighbor hot deck-

imputation[46] to classify this small group of patients (6.9%) into the subgroups a-d (GD/MNTG/mixed-type/STA: n=10/9/8/1). Nearest neighbor hot deck-imputation did not have any effect on the results of the analysis. Similar to the first and second study the standardized incidence rates (SIRs) was calculated according to the principle of direct standardization[44]. However, the Danish population as of January 1st, 1999 was used as the standard population to allow for comparison with data from the previous study during the years before initiation of effective IF (1997-2000).

2.3 Study IV

2.3.1 Purpose

The purpose of the fourth study was to evaluate the biochemical severity (TSH and total T_4 levels) of overt chronic autoimmune hypothyroidism at the time of diagnosis before and after introduction of mandatory IF in an area with pre-fortification moderate ID.

2.3.2 Study Setting

The open cohort in Northern Jutland utilized in the third study was also utilized in the fourth study with similar study periods: 1997-00 and 2014-16 (n=309,434 January 1st, 1997; n=272,954 January 1st, 2014). The median UIC levels for the cohort population mentioned in section 2.1.2 and 2.2.2 remain indicative for the ID level during these two study periods (1997-98: 45 µg/l; moderate ID[37]; 2008-10: 73 µg/l; mild ID[39]) . Detailed information on the composition of the cohort were provided yearly by Statistics Denmark[43].

2.3.3 Data Collection

Cases with sustained overt chronic autoimmune hypothyroidism identified within the cohort area using the methods described in section 2.2.3 during the two time periods were chosen for comparison of TSH and total T_4 levels at the time of diagnosis. A total of 274 and 180 patients fitting these criteria were identified during

the years 1997-00 and 2014-16 respectively. The TSH and total T_4 assays utilized during the two periods are described below:

- Baseline (1997-00): Lumitest by Brahms Diagnostica (TSH reference interval: 0.3-4.5 mU/l), Ria Kit by Ortho-Clinical Diagnostics (total T_4 reference interval: 60-140 nmol/l) and ADVIA Centaur by Bayer (TSH reference interval: 0.550-4.780 mU/l, total T_4 reference interval: 60-140 nmol/l)
- Follow-up (2014-16): Cobas 8000 modular by Roche Diagnostics (TSH reference interval: 0.27-4.2 mU/l, total T_4 reference interval: 60-140 nmol/l).

Thorough examination of the differences between various TSH and total T_4 assays can be found elsewhere[47,48].

2.3.4 Statistics

Comparison between baseline and follow-up median values for serum TSH and total T_4 among cases of overt chronic autoimmune hypothyroidism at the time of diagnosis was performed using Mann-Whitney U tests. Significance level was set at 0.05.

Chapter 3: Results

The following section reports the results of our monitoring program which includes preliminary verification of potential new cases of overt thyrotoxicosis and hypothyroidism through contact to the patient's current GP (see figure 2). Thus, these incidence rates represent patients for whom TFT measurements indicating overt thyroid dysfunction were evident while no record of previous overt thyroid dysfunction was present with the patient's current GP. No follow-up procedure investigating normalization of thyroid function and initiation of treatment was performed.

3.1 Monitoring program for thyrotoxicosis

The SIR of overt thyrotoxicosis within the two cohorts at baseline (1997-mid 98) before introduction of voluntary salt iodization was 128.5 and 80.1/100,000 per year (moderate vs. mild ID, SIRR: 1.60 (95% CI: 1.37-1.87)). Significant increases sharply followed by substantial decreases were observed in both cohorts (figure 4). Peak incidence was reached in mid 2000-01 within the moderately iodine deficient Western cohort (SIRR to baseline: 1.39 (1.24-1.55)) and in 2004-05 within the mildly iodine deficient Eastern cohort (SIRR to baseline: 1.52 (1.31-1.76)). A marked decline in SIR ensued within both cohorts, however due to the shorter follow-up period the SIR for the Eastern cohort did not reach below pre-fortification level at the conclusion of the study period in 2008 (SIRR to baseline: 1.12 (0.90-1.38)). A substantial decline significantly below baseline level was however observed in the Western cohort which seemed to stabilize from 2010 and onward at around 30-40% below baseline level with final values from 2016-2017 33% below (SIRR: 0.67 (0.58-0.76)). The previously observed significant difference in SIR of thyrotoxicosis between the two cohorts at the initiation of the study was no longer significant by the years 2006-2007 (SIRR: 1.08 (0.96-1.23)).

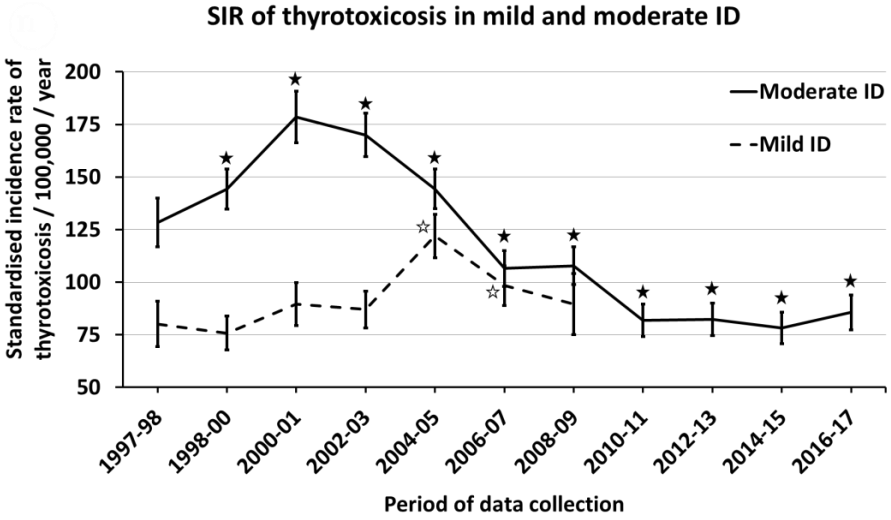

Figure 4: Age and gender standardized incidence rates of thyrotoxicosis per 100,000 per year in the two cohorts from 1997 to 2017. Voluntary salt iodization: initiated in July 1998 with 8 µg/g iodine in table salt and salt used by the food industry. Mandatory salt iodization: initiated in July 2000 with 13 µg/g iodine in all table salt and salt used for the production of bread. The error bars indicate the 95% confidence intervals (CI) for the incidence rates. Stars indicate statistically significant differences to baseline (1997-mid 1998; solid stars: moderate ID cohort, empty stars: mild ID cohort). Solid line: Western cohort in and around Aalborg City with moderate iodine deficiency (ID) (45 µg/L) prior to salt iodization. Dotted line: Eastern cohort in Copenhagen with mild iodine deficiency (61 µg/L) prior to salt iodization. The study period for the cohort with mild iodine deficiency was not possible beyond 2008. Copied from Study I

The incidence rate of thyrotoxicosis was three to four times higher among women compared to men throughout the entire study period and within both cohort areas (figure 5). With data pooled from both cohorts the female preponderance in thyrotoxicosis incidence (female/male ratio) was 3.80 during the baseline period and remained around this level for the remainder of the study period.

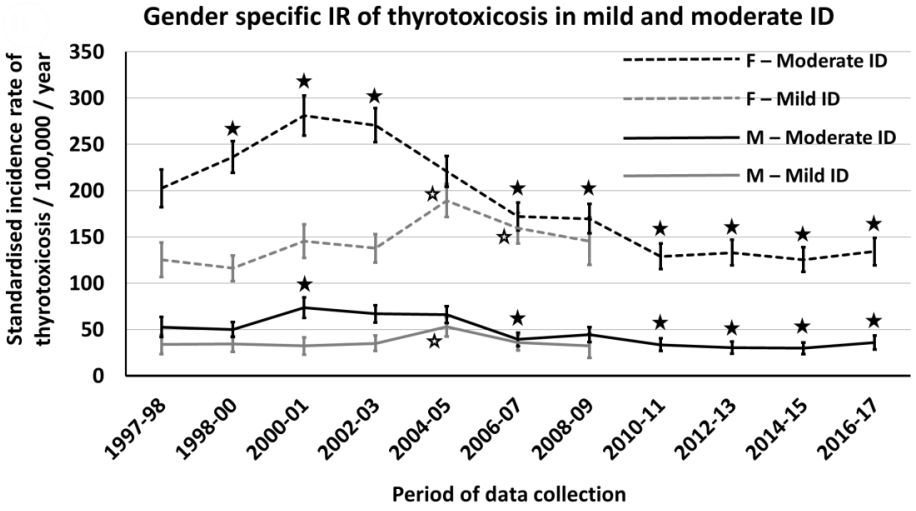

Figure 5: Gender-specific incidence rates of thyrotoxicosis per 100,000 per year in the moderately and mildly iodine deficient cohorts from 1997 to 2017. Error bars represent 95% confidence intervals. Data were age standardized. Stars indicate statistically significant differences to baseline (solid stars: moderate ID cohort, empty stars: mild ID cohort). M = Males. F = Females. Grey lines = mild ID cohort. Black lines = moderate ID cohort.
In both cohorts the incidence rate of thyrotoxicosis was strongly correlated with age during the baseline period. In the Western cohort with moderate ID, the age-specific incidence rates of thyrotoxicosis among three age groups: young (20-39 years), middle aged (40-59 years) and elderly (60 + years) were 49.3, 141.4 and 359.0/100,000 per year, respectively. Age-specific incidence rates for the same three age groups within the Eastern cohort with mild ID were 40.8, 100.1 and 192.4/100,000 per year, respectively. Copied from Study I

Significant increases in incidence rates were observed in all three age groups following IF within both cohorts (figure 6). Peak incidence rate of thyrotoxicosis within the Western cohort was observed in 2002-03 for the young and middle aged (SIRR to baseline, young: 2.22 (1.69-2.92); middle aged: 1.49 (1.24-1.78)) while peak incidence occurred immediately following mandatory IF (mid 2000-01) among the elderly (SIRR: 1.32 (1.14-1.52)). In the Eastern cohort peak incidence rate was observed in 2004-05 among all three age groups (SIRR to baseline, young: 2.50 (1.85-3.38); middle aged: 1.43 (1.08-1.90); elderly: 1.34 (1.08-1.65)).

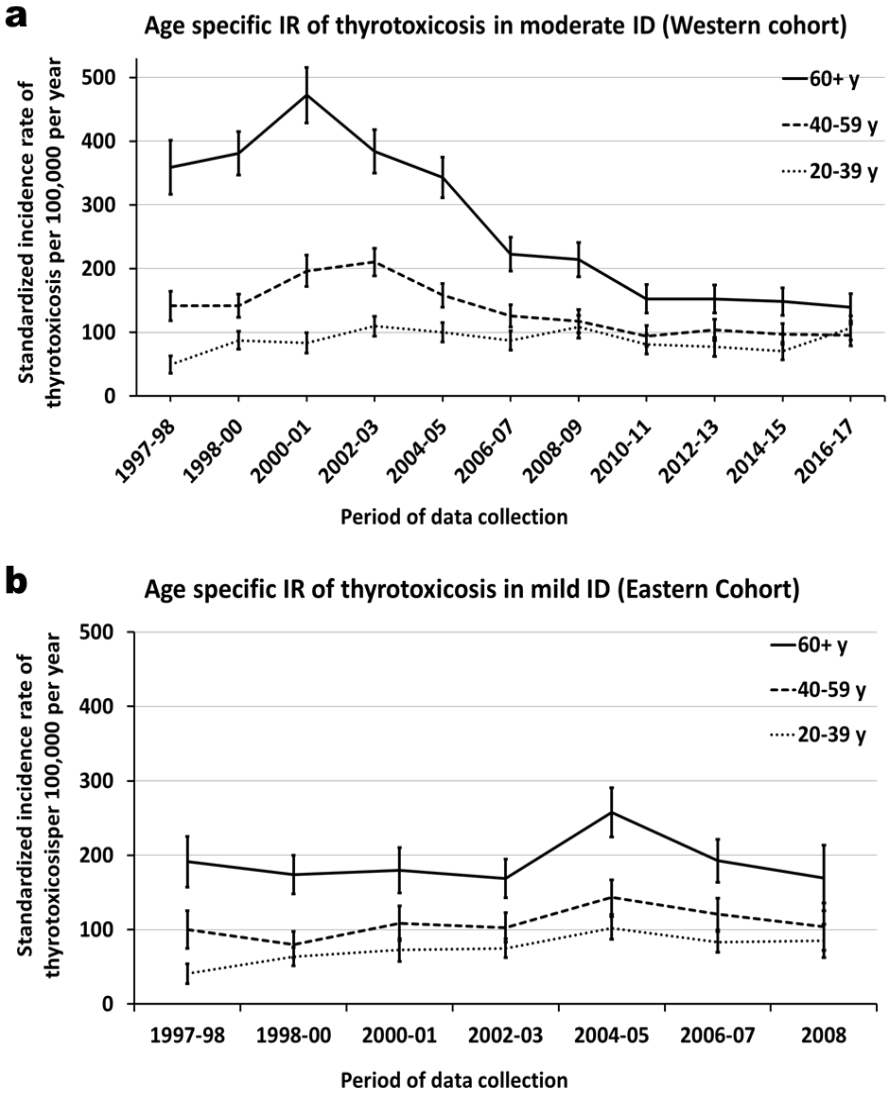

Figure 6 a-b: Age-specific incidence rates of thyrotoxicosis per 100 000 per year. Subjects have been split into three age groups: 20-39, 40-59 and 60 + years. Error bars represent 95% confidence intervals. Upper panel (a) shows data from the cohort with moderate iodine deficiency from 1997 to 2017. Lower panel (b) shows data from the cohort with mild iodine deficiency from 1997 to 2008. Copied from Study I

Within both cohorts the SIR of thyrotoxicosis among the younger age groups did not reach baseline level by the end of the study periods (SIRR to baseline, moderate ID: 2.16 (1.59-2.95) in 2016-17; mild ID; 2.08 (1.35-3.20) in 2008). In the Eastern cohort with mild ID, the middle aged and elderly were at baseline level by 2008 (SIRR to baseline, middle aged: 1.04 (0.69-1.54); elderly: 0.88 (0.65-1.20)) (figure 6b). In the Western cohort with moderate ID, the SIR among the middle aged and elderly were substantially below baseline level by the end of the study period: 2016-17 (SIRR to baseline, middle aged: 0.68 (0.53-0.87); elderly: 0.39 (0.32-0.48)). Differences in incidence rates were no longer detectable between the three age groups in the Western cohort by the end of the study period (figure 6a). Thus, a noticeable shift in age-specific incidence rates was seen in the cohort with previous moderate ID. The incidence rate of thyrotoxicosis was strongly correlated with age prior to IF, this was however no longer the case by the end of the study period where the incidence rate of overt thyrotoxicosis remained constant from the age of 30 and upward (figure 7).

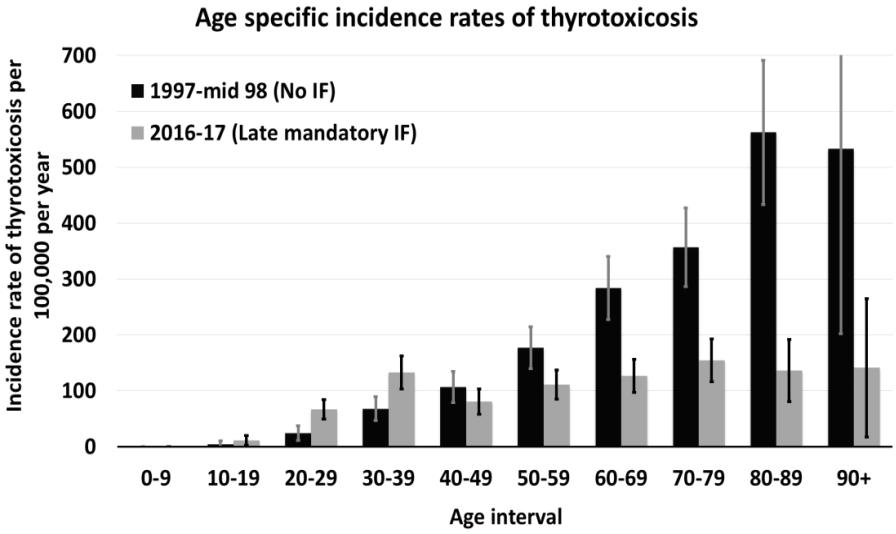

Figure 7: Age-specific incidence rates of thyrotoxicosis per 100 000 per year from: 1997-mid 1998 (prior to voluntary salt iodization) and 2016-17 (long after mandatory salt iodization) in the Western cohort with moderate iodine deficiency prior to iodine fortification (IF). Error bars represent 95% confidence intervals. Copied from Study I

3.2 Monitoring program for hypothyroidism

In the Western cohort with moderate ID, the baseline SIR of hypothyroidism was 32.9 compared to 47.3/100,000 per year in the Eastern cohort with mild ID (SIRR: 0.69 (95% CI: 0.54-0.90)). Following mandatory IF, the SIR of hypothyroidism increased significantly in both cohorts (figure 8). Peak incidence rate was observed in the Western cohort during the last three years of the study period (2014-16) with a SIRR to baseline: 1.50 (1.25-1.81). The incidence rate of hypothyroidism within the Eastern cohort was significantly increased from the years 2004-05 (SIRR to baseline: 1.30 (1.06-1.60)) and onward to 2006-07. Peak incidence rate within the Eastern cohort was actually observed in 2008, the difference to baseline level was however not statistically significant.

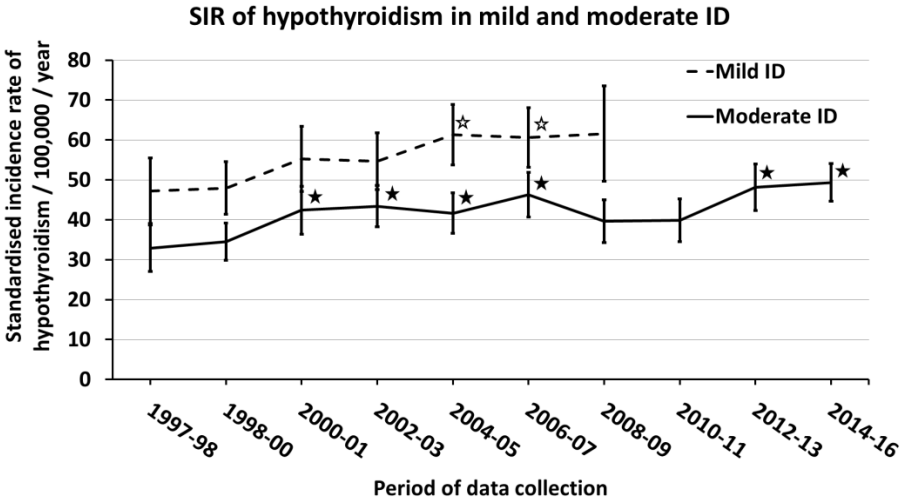

Figure 8: The standardized incidence rates of hypothyroidism per 100,000 per year in the cohorts with mild (dotted) and moderate (solid) iodine deficiency. The rates are sex and age standardized to the Danish population of the year 2005. The error bars indicate the 95 % confidence intervals (CI) for the incidence rates. Voluntary IF: initiated in July 1998 with 8 µg/g iodine in table salt and salt used by the food industry. Mandatory IF: initiated in July 2000 with 13 µg/g iodine in all table salt and salt used for the production of bread. Stars indicate statistical significance to baseline (solid stars: moderate ID cohort, empty stars: mild ID cohort). The study period for the mildly iodine deficient cohort was concluded by the end of September 2008. Copied from Study II

The SIR of hypothyroidism among women prior to voluntary IF was 53.8 and 72.2 /100,000 per year (moderate vs mild ID) (figure 9). The SIR among women in the Eastern cohort with mild ID increased significantly after introduction of mandatory IF with peak incidence in 2004-05 (SIRR: 1.29 (1.02-1.62)). Surprisingly, the increase in SIR among women in the Western cohort was not statistically significant until the final years (2014-16) of the study period (SIRR: 1.31 (1.06-1.63)). The SIR of hypothyroidism among men prior to voluntary IF was 11.4 vs. 21.8 /100,000 per year (moderate vs. mild ID). A significant increase in incidence rate of hypothyroidism after introduction of salt iodization was observed among men only within the Western cohort with previous moderate ID. This increase was statistically significant from the years 2001-02 and onward. Peak incidence was observed in 2012-13 (SIRR: 2.54 (1.66-3.90)). No significant increase in incidence rate of hypothyroidism among men was seen in the Eastern cohort with mild ID.

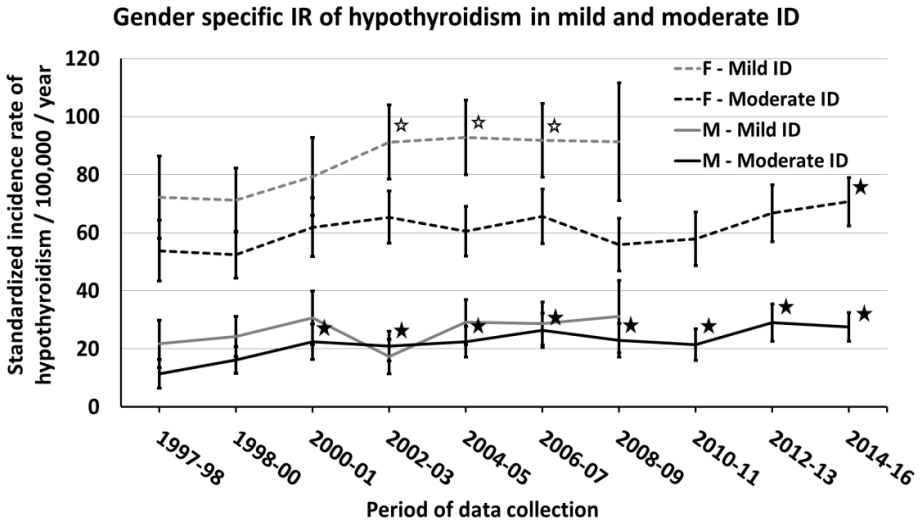

Figure 9: Gender specific incidence rates of hypothyroidism per 100,000 per year among men (M) and women (F) in the Western cohort with moderate iodine deficiency (ID) and the Eastern cohort with mild ID. Stars indicate statistical significance to baseline (solid stars: moderate ID cohort, empty stars: mild ID cohort). Incidence rates were standardized for age. Error bars represent 95% confidence intervals. Copied from Study II

The baseline SIRs among three age groups (young: 20-39y, middle aged: 40-59y and elderly: 60+y) within the Western cohort with moderate ID were: 14.5, 30.8 and

92.2 /100,000 per year respectively (figure 10). A gradual increase in the incidence rate of hypothyroidism among the young was observed until 2012-13 where peak incidence was reached (SIRR to baseline: 2.80 (1.67-4.70)). A significant increase in SIR of hypothyroidism was also observed among the middle aged in the Western cohort, reaching peak incidence in 2014-16 (SIRR to baseline: 2.16 (1.55-3.01)). No significant changes in incidence rate of hypothyroidism were observed among the elderly throughout the study period.

The baseline SIR of hypothyroidism among the young (20-39 y), middle-aged (40-59 y) and elderly (60+ y) in the Eastern cohort with previous mild ID were: 16.4, 42.2 and 148.3/100,000 per year respectively. The SIR increased significantly among both the young and the middle-aged following mandatory IF. Peak incidence rate in the young age group was observed in 2008 (SIRR to baseline: 2.62 (1.36-5.04)). Peak incidence rate among the middle aged was reached in 2006-07 (SIRR to baseline: 2.19 (1.49-3.23)). Similarly to the pattern observed in the Western cohort, no significant changes in incidence rate of hypothyroidism were seen among the elderly within the Eastern cohort.

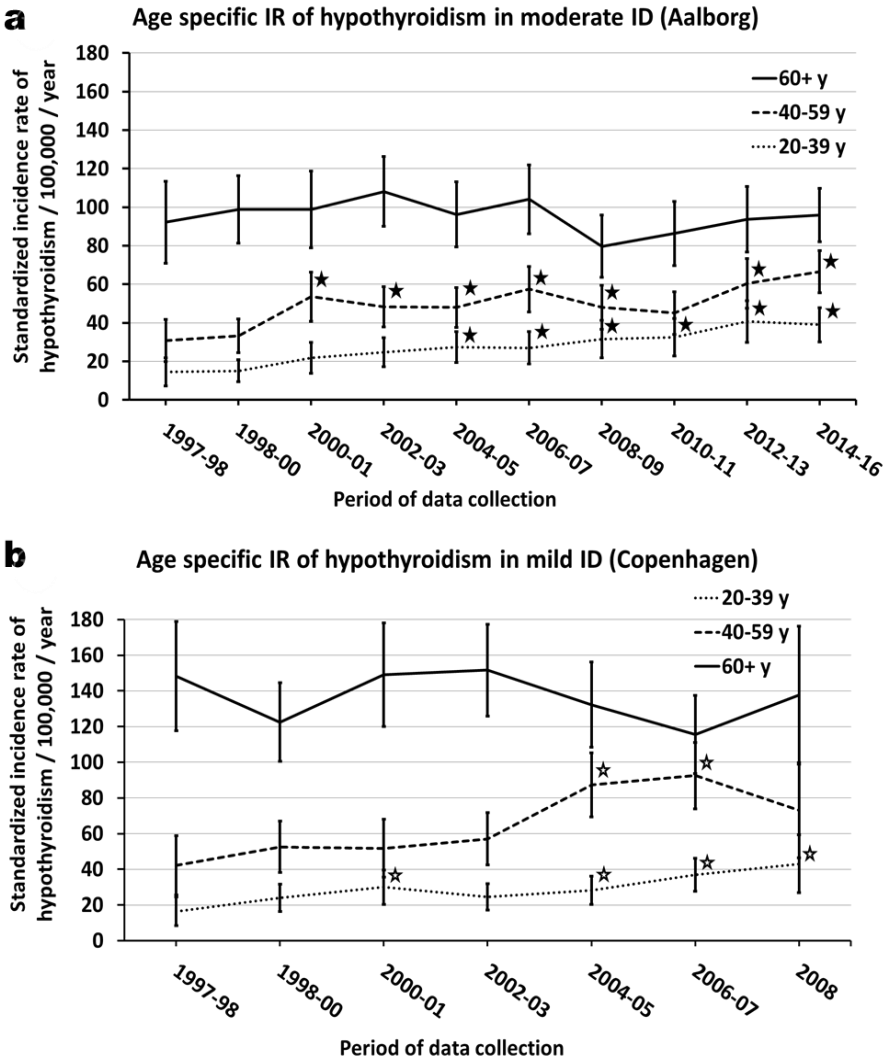

Figure 10 a-b: Age specific incidence rates of hypothyroidism per 100,000 per year within the following age groups: 20-39, 40-59 and 60+ years for the area of moderate iodine deficiency (ID) (a) and mild ID (b). Stars indicate statistical significance to baseline (solid stars: moderate ID cohort, empty stars: mild ID cohort). Incidence rates were standardized for age and gender. Error bars represent 95% confidence intervals. Copied from Study II

3.3 Follow-up and subtype classification

The incidence rates found in this section recounts the results obtained through our manual scrutiny of hospital records for all potential new cases of overt thyroid dysfunction discovered within the Western cohort area (previous moderate ID) during the years 1997-00 and 2014-16. Thus, a follow-up procedure for TFT normalization in relation to initiation of treatment was part of the final verification process and almost entirely explains any differences to the results obtained from our monitoring program (figure 2).

3.3.1 Manually verified thyrotoxicosis

Within the Western cohort with previous moderate ID the overall SIR of manually verified thyrotoxicosis decreased markedly from 97.5/100,000 per year in 1997-00 (before mandatory IF was in effect) to 48.8 in 2014-16, (SIRR: 0.50 (95% CI: 0.45-0.56)) (figure 11). Decreases in SIR were observed for MNTG from 44.5 to 8.2 (SIRR: 0.18 (0.15-0.23)), for STA from 6.1 to 1.6 (SIRR: 0.26 (0.16-0.43)) and surprisingly for GD from 33.2 to 22.2 (SIRR: 0.67 (0.56-0.79)). No significant changes in SIR were observed for iatrogenic thyrotoxicosis (SIRR: 1.09 (0.64-1.86)), post partum thyrotoxicosis (SIRR: 1.03 (0.53-1.99)) or mixed-type thyrotoxicosis (SIRR: 1.14 (0.83-1.58)). Surprisingly, the SIR of SAT increased significantly during the study period from 1.8 to 3.7 (SIRR: 2.07 (1.15-3.72)). The overall SIR of manually verified overt thyrotoxicosis among men declined from 33.0 to 21.3 between 1997-00 and 2014-16 (SIRR: 0.65 (0.51-0.82)) while the SIR for women declined from 160.4 to 75.6 during the same years (SIRR: 0.47 (0.42-0.53)).

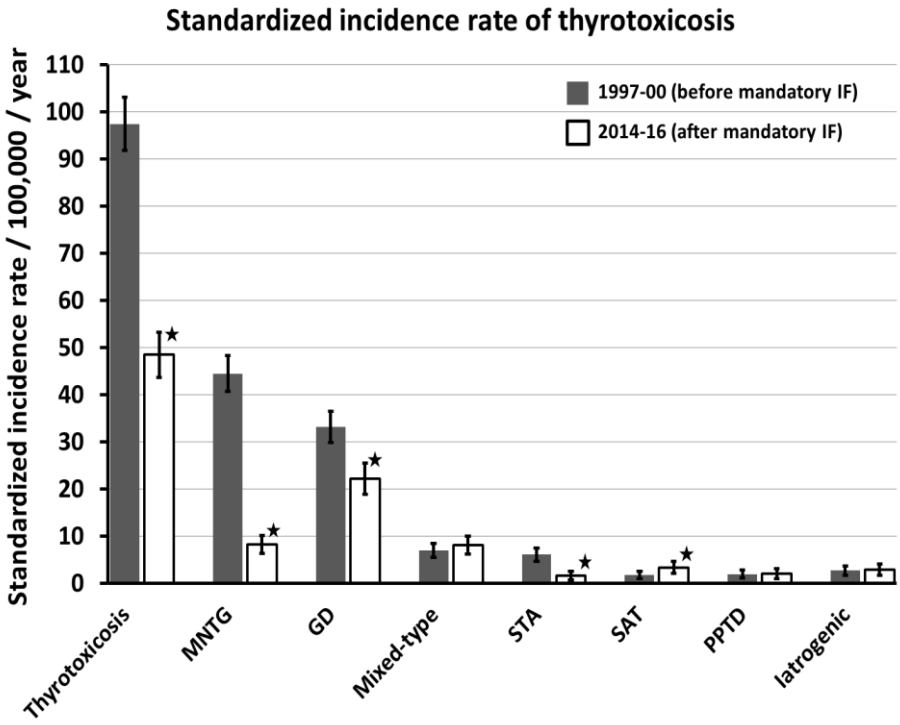

Figure 11: Standardized incidence rates of overt thyrotoxicosis and nosological subtypes per 100,000 per year between 1997-00 (pre-iodine fortification period) and 2014-16 (after iodization of salt). Grey columns represent data from 1997-00 and white columns data from 2014-16. Stars indicate statistical significance to baseline value (1997-00). Error bars represent 95% confidence intervals. Copied from Study III

Decreases in SIRs of manually verified overt thyrotoxicosis were evident in all age groups investigated (young, middle-aged and elderly: 20-39, 40-59 and 60+ years), these were however only statistically significant among the middle-aged (40-59 y) and elderly (60+ y). From 1997-00 to 2014-16 the following changes in incidence rates were observed among the three age groups: young: (SIRR: 0.80 (0.63-1.02)), middle-aged (SIRR: 0.67 (0.56-0.80)) and elderly (SIRR: 0.30 (0.25-0.35)) (figure 13).

3.3.2 Manually verified hypothyroidism

The overall SIR of verified overt hypothyroidism did not change between the years 1997-00 and 2014-16 (29.4 vs. 30.3/100,000 per year; SIRR: 1.03 (0.87-1.22)) when follow-up procedures were included in the verification process (figure 12). The SIR of chronic autoimmune hypothyroidism declined non-significantly from 23.3 to 21.6 (SIRR: 0.93 (0.77-1.12)). The SIR of iatrogenic hypothyroidism increased non-significantly from 3.1 to 4.8 (SIRR: 1.58 (1.00-2.50)) and post-partum hypothyroidism from 1.8 to 3.3 (SIRR: 1.83 (0.97-3.44)). A non-significant decline was observed in the SIR of hypothyroidism due to other causes (SAT and congenital hypothyroidism) from 1.3 to 0.6 (SIRR: 0.44 (0.17-1.16)). The SIR of overt hypothyroidism among men increased non-significantly from 12.6 to 16.9 (SIRR: 1.34 (0.96-1.86)) while it decreased non-significantly among women from 45.8 to 43.4 (SIRR: 0.95 (0.78-1.15)).

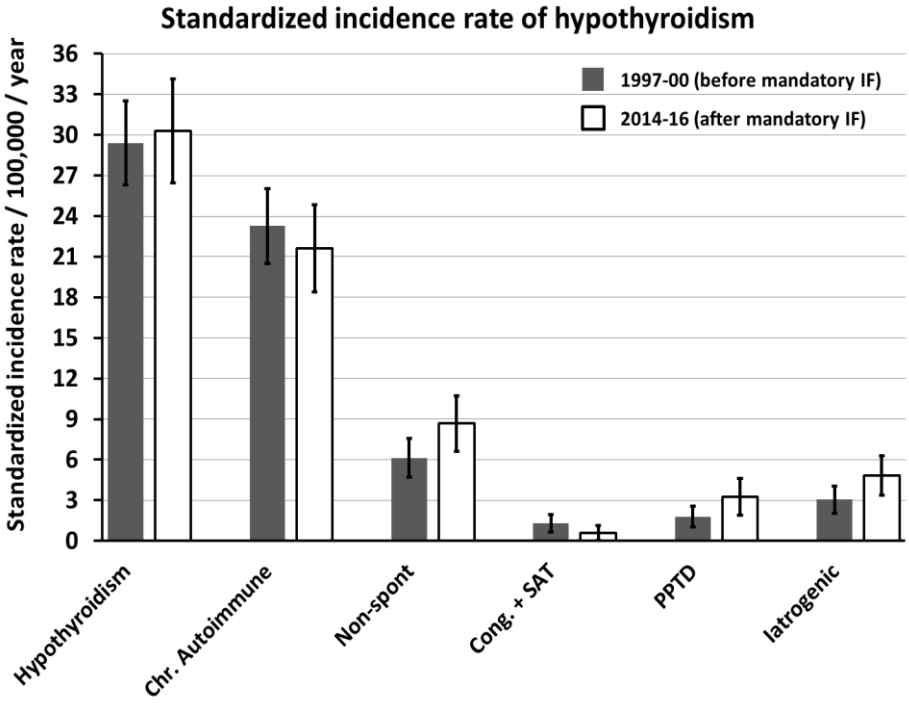

Figure 12: Standardized incidence rates of overt hypothyroidism and nosological subtypes per 100,000 per year between 1997-00 (pre-iodine fortification period) and 2014-16 (after iodization of salt). Grey columns represent data from 1997-00 and white columns data from 2014-16. Error bars represent 95% confidence intervals. Copied from Study III

The impact of mandatory IF on the SIR of overt verified hypothyroidism among the three aforementioned age groups was surprisingly different (figure 13). Among the young (20-39 y) a significant increase in SIR was present from 14.7 to 26.5 (SIRR: 1.81 (1.20-2.72)), while a non-significant increase was evident among the middle-aged from 33.0 to 43.8 (SIRR: 1.33 (1.00-1.77)). A significant decrease in SIR was however present among the elderly (60+ y) from 76.5 to 48.1 (SIRR: 0.63 (0.49-0.80)). These opposing changes in SIR of overt verified hypothyroidism observed between the three age groups after introduction of mandatory IF prevented an increase in the overall SIR of hypothyroidism for the whole population.

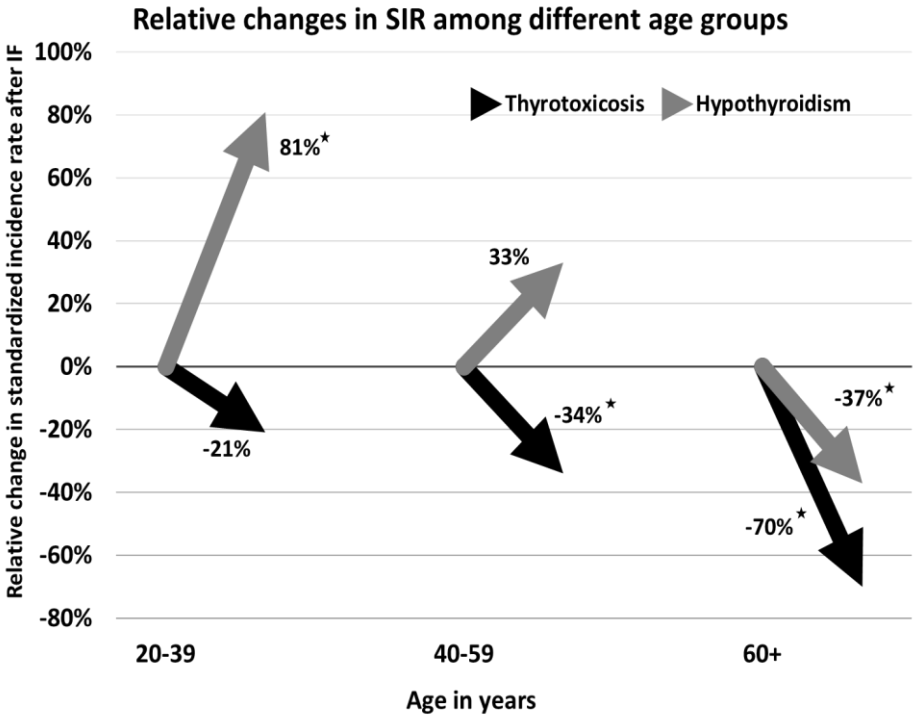

Figure 13: Relative change in standardized incidence rate of overt thyrotoxicosis and hypothyroidism between 1997-00 and 2014-16 among three age groups (20-39, 40-59 and 60+ years). Black arrows represent overt thyrotoxicosis and grey arrows represent overt hypothyroidism. Stars indicate significant change from baseline value (1997-00). Copied from Study III

3.4 Thyroid function test results in hypothyroidism

Median serum TSH among manually verified patients with overt chronic autoimmune hypothyroidism at the time of diagnosis was 40.4 mU/l in 1997-00 (before IF) and 20.5 mU/l after IF in 2014-16 (P<0.001) (table 1). Median TSH among men were 49.1 mU/l before IF vs. 16.2 mU/l after IF (P<0.001) while corresponding values among women were 37.8 mU/l before IF vs. 24.7 mU/l after IF (P<0.01). The TSH medians among different age groups (20-39, 40-59, 60-79, 80+ years) were lower after introduction of mandatory IF (2014-16) compared to before (1997-00), though only significant for the age groups 40-59, 60-79 and 80+ years (P-values<0.05) (figure 14). This was likely due to insufficient statistical power within the youngest age group (20-39 years).

TFT values among hypothyroid patients before and after IF			
Chronic autoim. HT		1997-00	2014-16
N		274	180
Median age (years)		64	55
TSH (mU/l)	Minimum	5.3	5.1
	1st quartile	20.0	8.1
	Median	40.4	20.5
	3rd quartile	88.0	52.4
	Maximum	341	419
TT4 (nmol/l)	Minimum	<8	<8
	1st quartile	18.8	42
	Median	41	52
	3rd quartile	52	56
	Maximum	59	59

Table 1: Serum thyroid stimulating hormone (TSH mU/l) and total thyroxine (TT4 nmol/l) values among patients with overt chronic autoimmune hypothyroidism (Hashimoto's

thyroiditis: HT) from 1997-00 (before IF) and 2014-16 (after IF). Mann-Whitney U test for median difference between 1997-00 and 2014-16: P < 0.001 (TSH and TT4 both). Copied from Study IV

Among patients with overt chronic autoimmune hypothyroidism the median serum total T4 at the time of diagnosis was 41 nmol/l in 1997-00 (before IF), and 52 nmol/l in 2014-16 (after IF) (P<0.001) (table 1). Median total T_4 among men was 34 nmol/l before mandatory IF and 52 after (P= 0.021). Corresponding values among women were 42 nmol/l before IF and 52 after IF (P< 0.001). Among all the age groups (20-39, 40-59, 60-79, 80+ years) the total T_4 medians were higher in 2014-16 than in 1997-00, though only statistically significant for the 40-59, 60-79 and 80+ year age groups (figure 15). Exclusion of women with high estrogen levels (birth control or hormone replacement) from the analysis did not change any of the results.

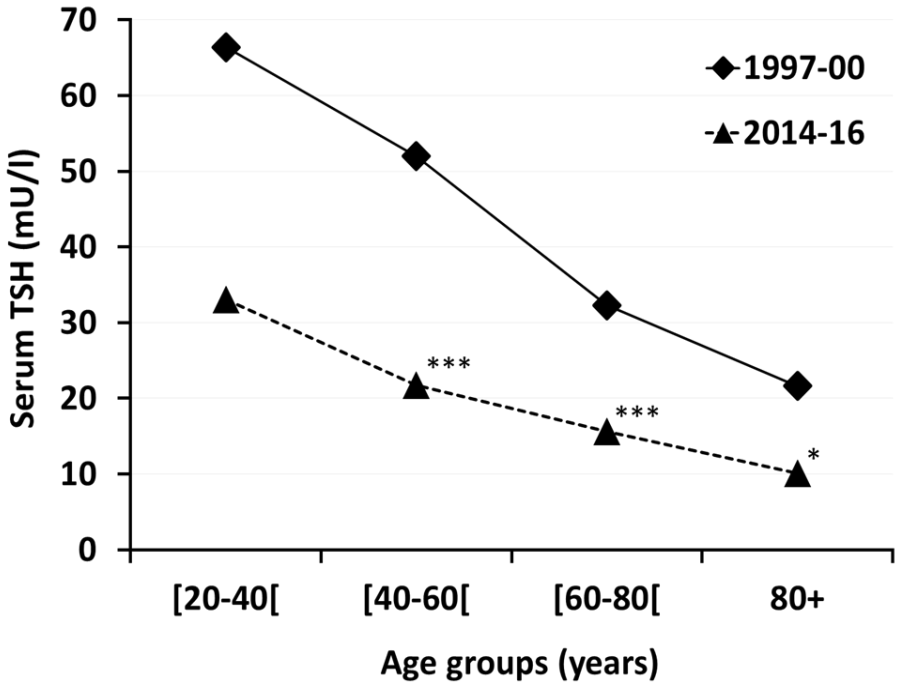

Figure 14: Median serum thyroid stimulating hormone (TSH mU/l) among patients with overt chronic autoimmune hypothyroidism at the time of diagnosis within specific age groups

before (1997-00) and after (2014-16) introduction of mandatory salt iodization in Denmark. Varying degrees of statistical significance to the baseline values (1997-00) are indicated as follows: * P-value < 0.05. ** P-value < 0.01. *** P-value < 0.001. Copied from Study IV

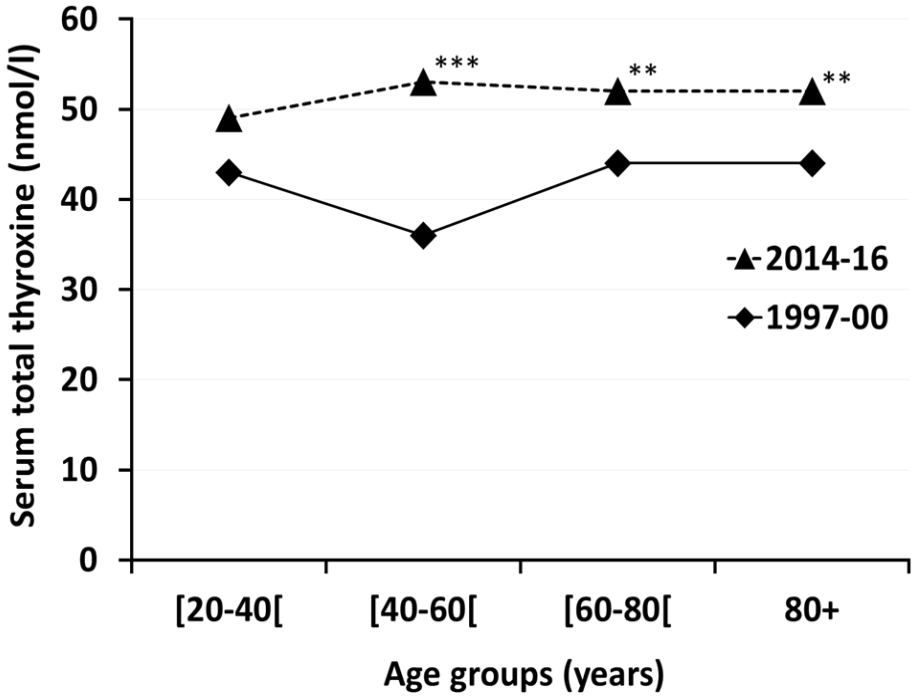

Figure 15: Median serum total T_4 among patients with overt chronic autoimmune hypothyroidism at the time of diagnosis within specific age groups before (1997-00) and after (2014-16) introduction of mandatory salt iodization in Denmark. Varying degrees of statistical significance to the baseline values (1997-00) are indicated as follows: * P-value < 0.05. ** P-value < 0.01. *** P-value < 0.001. Copied from Study IV

3.5 TSH testing rate within the Western cohort

The TSH testing rates (diagnostic and therapeutic testing combined) within the Western cohort area increased substantially during the study period for both genders and all age groups above 20 years (table 2). For men and women respectively, the total TSH testing rate (diagnostic and therapeutic testing combined) increased from 1,560 and 4,018 per 10,000 per year at baseline (1997-00) to 4,839 (+210%) and 7,837 (+95%) during follow-up (2014-16).

TSH testing rate before and after mandatory IF						
	Men		Women		Total gender	
	1997-00	2014-16	1997-00	2014-16	1997-00	2014-16
20-39 y	575	1,701 +196%	1,849	4,229 +129%	1,187	2,904 +145%
40-59 y	1,397	4,170 +198%	3,750	7,040 +88%	2,561	5,591 +118%
60-79 y	3,278	8,708 +166%	6,753	11,173 +65%	5,145	9,976 +94%
80+ y	4,882	16,431 +237%	8,288	17,840 +115%	7,088	17,292 +144%
Total age*	1,560	4,839 +210%	4,018	7,837 +95%	2,804	6,335 +126%

Table 2: Thyroid stimulating hormone (TSH) test rate (per 10,000 per year) among men and women at different ages before mandatory salt iodization (1997-00) and after (2014-16). Relative increase (%) in TSH test rate from 1997-00 to 2014-16 are shown. An asterix indicates the exclusion of individuals below the age of 20 years, thus the TSH testing rate in the bottom row marked "Total age" only includes adult individuals (20+ years). Copied from Study IV

Chapter 4: General discussion

4.1 Main findings

In the area with previous moderate ID, a substantial reduction in the incidence rate of overt thyrotoxicosis was observed after the initiation of mandatory IF. Our partially automated monitoring program estimated this reduction to be around 33% between 1997-98 and 2016-17. Correspondingly, our manual follow-up procedure for cases identified by our monitoring program during the years 1997-00 and 2014-16 revealed a 50% reduction in the incidence rate of overt thyrotoxicosis, when cases with spontaneous normalization of thyroid function and no medical history suggesting a condition with transitory thyroid dysfunction were excluded from the analysis. This decline in incidence rate of overt thyrotoxicosis was the result of marked decreases in the MNTG, STA and GD incidences. Unsurprisingly, both our monitoring program and our manual follow-up study revealed major effects among the elderly and minor effects among the young.

Marked increases in the incidence rate of overt hypothyroidism were discovered by our monitoring program in both cohort areas. These effects were most pronounced among the young and entirely absent among the elderly. Interestingly, our manual follow-up procedure for cases discovered by our monitoring program revealed that the overall incidence rate of overt hypothyroidism had remained unchanged between 1997-00 and 2014-16, when cases with spontaneous normalization of thyroid function and no medical history suggesting a condition with transitory thyroid dysfunction were excluded from the analysis. Significant differences were however present between the different age groups investigated. A marked increase in incidence rate of overt hypothyroidism was found among the young while a marked decrease was discovered among the elderly, resulting in an unaltered overall incidence rate for the entire population.

A 126% increase in the TSH testing rate was discovered in the Western cohort area between 1997-00 and 2014-16. This is the most likely explanation for the markedly lower median TSH value and markedly higher total T_4 value observed among patients with chronic autoimmune hypothyroidism at the time of diagnosis after introduction of mandatory salt iodization.

4.2 Comparison with other studies

4.2.1 Thyrotoxicosis

The reductions in thyrotoxicosis incidence observed in the studies of the present dissertation are in accordance with observations from several other countries. In 1980, salt iodization was increased from 7.5 to 15 µg/g in Switzerland[49]. This was accompanied by a 56% decrease in the overall incidence rate of thyrotoxicosis[49]. MNTG and GD incidence rates declined 73% and 33% respectively, while 82% and 33% decreases was observed in Study III of the present dissertation. These result are however not entirely comparable as the population of Switzerland changed from mild ID to adequate iodine intake during the course of the study while our cohort area increased from moderate to mild ID as a result of mandatory salt iodization. Furthermore, the results from the Swiss study are based exclusively on referral of patients to a single hospital, whereas our data were obtained from GPs, hospital departments and specialists with private practices, thus leaving no possibility of cases with overt thyroid dysfunction evading our algorithm.

Salt iodization in Austria was increased from 7.5 to 15 µg/g in 1990 and all cases of thyrotoxicosis referred to 14 departments of nuclear medicine were recorded from 1987-1995[17]. The observed pattern of initial increase and subsequent decline in thyrotoxicosis incidence in the Austrian study does somewhat resemble the pattern observed through our monitoring program. However, no clear comparison can be made due to the many methodological discrepancies and the very short follow-up period after increased salt iodization in the Austrian study.

A major rise in thyrotoxicosis incidence was observed in Tasmania after iodization of bread salt and introduction of iodophores in the dairy industry in the year 1966[14,50]. The incidence rate increased markedly from 24 / 100,000 / year before IF to reach peak incidence rate in 1967 at 125 / 100,000 / year. Similar to most other studies the results from Tasmania were based on hospital referrals and thus direct comparison to our monitoring program remains difficult. A more than fivefold increase in incidence rate of thyrotoxicosis does appear extraordinary compared to the 39% and 52% increases discovered in our cohort areas. The explanation for a difference of this magnitude remains elusive.

In 1999 salt iodization in Slovenia was increased from 10 to 25 µg/g. Referrals of GD, MNTG and STA patients to the University Medical Centre Ljubljana were

monitored from 1999 to 2009[51]. By 2009 the MNTG and STA incidence had decreased by 27% while no change in GD incidence occurred.

The prevalence of MNTG, STA and GD was investigated in a rural community in Northern Italy before and after introduction of voluntary salt iodization at a level of 30 µg/g in 2005[22]. There was no significant difference in prevalence of MNTG, STA or GD between 1995 and 2010.

The first and second cross-sectional studies within DanThyr (see section 1.1.3) found decreased prevalence of subclinical thyrotoxicosis between 1997-98 and 2004-05 but no significant decrease in the prevalence of overt thyrotoxicosis. This seems in accordance with the findings of our cohort studies where a significantly decreased incidence rate of overt thyrotoxicosis did not occur before the years 2006-07. A lower frequency of solitary and multiple thyroid nodules were discovered between the first cross sectional study in 1997-98 and the follow-up study performed in 2008-10, when comparing participants of similar age at the time of ultrasonographic examination[52]. A lower frequency of thyroid nodules among participants of the same age ought to be expected given the substantial reductions in MNTG and STA incidence rates observed in our study.

The marked decline in MNTG and STA incidence observed in Study III of this dissertation, should account for the substantial reduction in the use of radioiodine treatment observed on a national scale in Denmark between 1997 and 2015 (47.7 vs. 33.1 / 100,000 / year)[21]. Furthermore, the use of anti-thyroid medication declined 23% on a national scale between 1997 and 2014[21]. The incidence rates of MNTG and STA should not be expected to have decreased equally in all areas of Denmark as significant differences in the occurrence of these conditions existed between areas with mild and moderate ID prior to the introduction of mandatory salt iodization[10]. Thus, the results from the national registries (mild and moderate ID prior to IF) are not incompatible with the results of our studies in which only the cohort with previous moderate ID could be investigated during the years 2014-16.

4.2.2 Hypothyroidism

The results from our monitoring program of hypothyroidism incidence within the moderately iodine deficient cohort area (Study II) could superficially seem contradictory to those of our follow-up study (Study III), as the former found a 50% increase in the incidence rate of overt hypothyroidism during the years 2014-16 while the latter found this increase to be completely absent during the same years.

These results are however not at all contradictory when scrutinizing the details. There was indeed a 50% increase in the number of new patients with overt hypothyroidism (TSH>5.0 mU/l and total T_4<60 nmol/l) in the cohort area. However, when cases with spontaneous normalization of thyroid function (i.e. without treatment) and no medical history suggesting a typical condition causing transitory thyroid dysfunction were excluded, this 50% increase vanished entirely. Thus, the criteria used for categorizing hypothyroidism in a study population appear to be of paramount importance for the comparability of the results to those of other studies, as the conclusion drawn from a study can be entirely different depending on the criteria used to define hypothyroidism (transitory hypothyroidism with spontaneous normalization vs. sustained hypothyroidism requiring treatment). Evaluating the impact of various IF programs on the incidence rate of hypothyroidism is further complicated by several other possible differences in study design. Such differences may involve: inclusion of both subclinical and overt hypothyroidism, including only referred cases of hypothyroidism, including specific thyroid auto-antibodies levels and ultrasonographic patterns in the definition of hypothyroidism, differences in the level of ID prior to IF and the magnitude of IF.

In Italy, the prevalence of Hashimoto's thyroiditis (HT) increased significantly after introduction of voluntary salt iodization in 2005[22]. The prevalence of HT rose from 2.8% in 1995 to 5.0% in 2010, though this increase was entirely driven by a greater number of cases among children (<15 years). An increased prevalence of thyroid autoantibodies was also reported between 1995 and 2010 (12.6% vs. 19.5%). Straight comparison to our results is complicated by the criteria used to define HT in the Italian study. A mixture of TFTs, ultrasonographic pattern (hypoechoic) and concentrations of thyroid auto-antibodies were utilized for the identification of HT cases. These compound criteria allowed for the inclusion of subclinically hypothyroid or even euthyroid cases in certain circumstances.

From 1999 to 2009 the incidence rate of Hashimoto's thyroiditis increased 127% in Slovenia presumably as a result of salt iodization having been initiated in 1999[51]. These results are however not comparable to ours as both subclinical and overt hypothyroidism was included in the analysis and the incidence rates were based solely on referral to a single hospital department.

The prevalence of overt and subclinical hypothyroidism was investigated in three areas of Hungary and Slovakia with different median UICs (72, 100 and 513µg/g creatinine)[53]. The prevalence of overt hypothyroidism in these areas was 0.8%, 1.5%, and 7.6% respectively, while the prevalence of subclinical hypothyroidism was 4.2%, 10.4%, and 23.9% respectively. These results are in fact somewhat comparable to our results as we found significant increases in incidence rate of

hypothyroidism among the younger age groups but not among the elderly. Even if the overall incidence rate of hypothyroidism for the entire population remains constant, a shift in the age distribution towards the young would cause the prevalence of overt hypothyroidism to increase over time, as the elderly patients presumably cannot be expected to survive with the condition for as long as young patients.

In Denmark the incident use of thyroid hormone therapy nearly doubled from 1997-2014[21]. Given that the incidence of overt treatment-requiring hypothyroidism did not increase from 1997-00 to 2014-16 (Study III), the explanation for this vast increase in thyroid hormone therapy use is likely to be found in increased treatment of subclinical hypothyroidism (see section 5 for further elaboration on this point).

4.3 Possible mechanisms involved

4.3.1 Thyrotoxicosis

The current conception of how autonomous thyroid nodules develop as a result of iodine deficiency is summarized below[54]:

Iodine deficiency and reduced substrate availability for thyroid hormone production could cause increased TSH secretion from the pituitary gland. TSH then induces hyperplasia and increased functional activity among the thyrocytes. This may in turn lead to increased mutagenesis through the formation large quantities of H_2O_2 and other reactive oxygen species (ROS). Gain of function mutations could then occur in genes regulating thyrocyte growth and thus lead to the emergence of autonomous thyrocyte clones[54].

Correction of iodine deficiency should hence reduce the development of autonomously functioning nodules among a previously iodine deficient population. This seemingly accounts for the substantial reduction in MNTG and STA incidence rates observed in our study. The reduction in the frequency of non-toxic multiple and solitary thyroid nodules observed in the C1b follow-up study (DanThyr) among subjects of similar age at the time of examination are likely to be caused by the same mechanism.

The reduced incidence rate of GD observed in Study III may be explained by two factors:

1) The incidence rate of overt thyrotoxicosis did in fact increase significantly when voluntary salt iodization was introduced and peaked around the period mid 2000-01. This increase was most pronounced among the younger age group and likely the product of increased GD occurrence as MNTG is extremely rare in this age group. Correspondingly, only a relatively small increase in thyrotoxicosis incidence was discovered among the elderly for whom MNTG was the leading cause of overt thyrotoxicosis. Thus, this initial increase in thyrotoxicosis incidence was likely to be caused by GD and not MNTG. If the otherwise insufficient voluntary salt iodization managed to cause a transitory increase in GD incidence this would result in an overestimation of GD incidence prior to mandatory IF. This seems unlikely to be relevant for MNTG due to the aforementioned skewed age distribution of the increased incidence rate of thyrotoxicosis, and not likely to be important for hypothyroidism at all since the voluntary salt iodization did not result in any increased incidence rate.
2) Smoking is positively associated with the severity and onset of GD[55]. Hence the decreased frequency of smoking among the Danish population might be of some importance in explaining the decreased incidence rate of GD observed in Study III of this dissertation.

4.3.2 Hypothyroidism

Alterations in the level of thyroid autoimmunity and accompanying thyroid failiure could be a tempting explanation for the impact of IF on the incidence rate of hypothyroidism. In the DanThyr cross-sectional studies, the prevalence of thyroid peroxidase antibody (TPO-Ab) positivity increased significantly from 14.3% in 1997-98 to 23.8% in 2004-05[23]. Furthermore, a clear association between elevated TSH and TPO-Ab level has been established[23,24]. The largest increase in prevalence of TPO-Ab positivity was found among the young while only small increases were found among the elderly[23]. This is in accordance with the findings of our current studies in which increased incidence rate of hypothyroidism was discovered among the young but not among the elderly.

Several other studies have also suggested autoimmunity as the likely explanation for the rise in occurrence of hypothyroidism following salt iodization[9,56–58]. Some evidence suggests that auto-regulatory processes within the thyroid gland involving

arachidonic acid derivates might be of importance in inducing hypothyroidism following IF as a way to protect against iodine overload[59].

During the course of the last two decades a marked reduction in the frequency of smoking has occurred in Denmark[60]. This is likely of importance to our results as a negative association between smoking and TPO-Ab positivity was observed in the DanThyr cross-sectional studies[61]. Thus, the decreased frequency of smoking should be accompanied by an increased prevalence of TPO-Ab positivity.

The lower median serum TSH and higher median serum total T_4 observed among patients with chronic autoimmune hypothyroidism at the time of diagnosis during the years 2014-16 is quite likely to be the direct result of the vastly increased rate of TSH testing discovered in the cohort area between 1997-00 and 2014-16. As chronic autoimmune thyroiditis causes a gradual decline in the capacity for thyroid hormone production, TSH should be expected to slowly increase while total T_4 subsequently decreases among affected individuals. An increased TSH testing rate should allow for earlier detection of these TSH and total T_4 derangements, thus resulting in lower median TSH and higher median total T_4 values at the time of diagnosis.

TSH testing rates differ considerably between various countries. Studies from Great Britain[62], Scotland[62] and New Zealand[63] revealed TSH testing rates of around one in six adults per year. The TSH testing rate among adults in Australia in 2016-17 was 2,874 per 10,000 per year[64]. This highly resembles the TSH testing rate in our Western cohort before mandatory salt iodization was in effect (1997-00). The TSH testing rate among adults increased to a remarkable 6,335 per 10,000 per year in the Western cohort during the years 2014-16 (Study IV).

Discovering hypothyroidism at an earlier stage could have important beneficial effects as overt hypothyroidism is associated with increased mortality rates[65] and specifically the duration of overt hypothyroidism is correlated with all-cause mortality[66]. Early diagnosis and subsequent treatment of these patients should reduce their mortality rate as well as their risk of psychiatric disease and their likelihood of receiving disability pension[67,68]. Importantly, subclinical hypothyroidism does not seem to be associated with increased mortality rates[66].

4.4 Implications for IF programs

Even a very cautious salt iodization program (<50 µg increase in average daily intake) managed to successfully reduce the incidence rate of nodular toxic goitre (MNTG: ÷82% and STA: ÷74%) among a population with previous moderate ID,

thus causing an overall decline in incidence rate of overt thyrotoxicosis of around 50% in only 14-16 years. A less pronounced effect on thyrotoxicosis incidence ought to be expected in areas with less severe ID as the disease burden of nodular toxic goitre should be significantly lighter in such areas. A transient increase in the incidence rate of overt thyrotoxicosis during the initial period following salt iodization seem unavoidable and appears to be particularly impactful among the younger age groups.

A significant increase in the incidence rate of overt hypothyroidism among individuals <40 years should be expected when introducing mandatory salt iodization of this magnitude, though this may to some extent be compensated by fewer cases among the elderly in areas of moderate ID. This increased incidence rate of overt hypothyroidism among the young might be rendered less impactful by an increased TSH testing rate as diagnosis can be made earlier and at less severe stages of TFT derangement.

Increasing the Danish salt iodization further should be engaged with caution. Increasing the median UIC of the population to the 150 µg/l recommended by WHO might be undesirable among the Danes, as a further reduction in the burden of nodular toxic goitre seems unobtainable and an increased incidence rate of overt hypothyroidism seems unavoidable among the young. A median UIC just above 100 µg/l could represent the level of iodine intake with the lowest frequency of overt thyroid dysfunction among the Danish population.

Chapter 5: Methodological considerations

It appears quite likely that treatment of subclinical thyroid dysfunction and subclinical hypothyroidism in particular has become more frequent during the course of our study period[69]. This would be a limitation of considerable importance as initiation of treatment among subclinically hypothyroid patients would prevent them from becoming overtly hypothyroid. This would naturally lead to an underestimation of the increase in incidence rate of hypothyroidism caused by introduction of salt iodization as properly treated hypothyroid patients would be prevented from proceeding to overt hypothyroidism. Chronic autoimmune hypothyroidism should be of particular interest with regards to the effects of initiating treatment of subclinical thyroid dysfunction as the condition encompasses a slow gradual decline in thyroid hormone production and thus ample time for intervention and initiation of treatment prior to transitioning to overt hypothyroidism. The increased TSH testing rate discovered in Study IV would lead to the discovery of more subclinically hypothyroid patients in our cohort area. If this effect was to be combined with a lower s-TSH threshold for initiation of treatment among hypothyroid individuals we should expect that a larger quantity of patients, who would otherwise become overtly hypothyroid during our study period, could be prevented from transitioning to overt hypothyroidism. Some evidence suggest that the s-TSH threshold for initiating L-T_4 treatment among hypothyroid patients has decreased in the Danish capital Copenhagen between 2001 and 2015[69]. Among hypothyroid patients the median s-TSH at which L-thyroxine treatment was initiated decreased from 10 mU/l in 2001 to 6.8 mU/l in 2015. Meanwhile, the annual s-TSH testing rate increased 164% between 2001 and 2015[69].

The increased s-TSH testing rate discovered in our Western cohort between 1997-00 and 2014-16 should lead to an inflated incidence rate of overt thyroid dysfunction in the later parts of our study period. An increased s-TSH testing rate ought to diminish the pool of undiagnosed patients with overt thyroid dysfunction. Indeed, the prevalence of undiagnosed overt thyrotoxicosis decreased 61% between 1997-98 and 2004-05 while a 91.2% decrease in the prevalence of undiagnosed overt hypothyroidism was discovered during the same period[70]. Though we are unable to separate diagnostic from therapeutic TFT sampling, we would consider it quite unlikely that the more than doubling in s-TSH testing rate was the sole product of increased s-TSH sampling among patients with known overt thyroid dysfunction. In an urban population in Exeter in the year 2012, only 17% of s-TSH tests were

sampled from subjects who were currently or previously under treatment for thyroid dysfunction[71]. Similarly, in the San Antonio Military Health System 30% of TSH measurements were sampled from patients with a known history of hypothyroidism[72].

The incidence rates reported in the studies of this dissertation should correctly be termed "diagnostic incidence rates" as true incidence rates require continues testing of one group of individuals over the course of several years to determine the exact number of subjects who develop the condition in question. Monitoring all the subjects within our cohort areas with yearly TFTs would require insurmountable resources and monitoring a much smaller area in the same fashion would render a number of events too small for any meaningful analysis given the relatively low frequency of overt thyroid dysfunction. Thus, we had to accept utilizing the cases of overt thyroid dysfunction discovered through the diagnostic activity of private practices and hospital departments in our cohort areas.

The iodine intake of our cohort populations may not have been entirely stable after the introduction of mandatory salt iodization. Though the median UIC within the Western cohort had increased from 45 µg/l in 1997-98 to 86 µg/l in 2004-05, a slight decline in median UIC was observed during the follow-up study in 2008-10 (73 µg/l)[73]. This decline in median UIC could be the result of reduced iodine content in diary products[73]. Newer studies regarding the iodine intake in the Western cohort are required to investigate this.

The impossibility of continuing our surveillance of the Eastern cohort with previous mild ID is unfortunate and allows for a greater degree of ascertainment bias in our evaluation of the impact of mandatory salt iodization on overt thyroid dysfunction. A technical difficulty combined with staff limitations made continues verification of incident cases impossible beyond the year 2008 in the Eastern cohort. Thus, the effect of mandatory salt iodization on the incidence rates of over thyroid dysfunction under conditions of mild ID remains to be elucidated.

As all TFTs sampled among GPs, hospital departments and specialists with private practice within our cohort areas were included, our method substantially reduces the impact of the referral biases[74] typically associated with studying incidence rates of thyroid dysfunction in relation to IF programs[14,17,49–51].

Chapter 6: Conclusions

Mandatory salt iodization in Denmark caused a less than 50 µg increase in average daily iodine intake yet striking reductions in the incidence rate of nodular toxic goitre ensued (MNTG: ÷82% and STA: ÷74%) leading to a 50% decline in the overall incidence rate of overt thyrotoxicosis. Even if the incidence rate of overt hypothyroidism did not increase among the population as a whole, the incidence rate among the young (<40 years) did increase markedly (+81%). This age group should be granted special attention when initiating IF programs in the future. The s-TSH testing rate among the Danish population has more than doubled in less than 20 years which is the likely explanation for the observed reduction in median s-TSH among overtly hypothyroid patients at the time of diagnosis. Monitoring thyroid dysfunction in an area using simple TFT algorithms and subsequent verification with GPs can be almost fully automated and should provide a reasonable estimate for the impact of IF among an iodine deficient population. Manual scrutiny of the medical records of all possible new cases of thyroid dysfunction within an area is a labor and time consuming process, though important knowledge about the severity, duration, need for treatment and nosological subtype of thyroid dysfunction is only obtainable this way.

Increasing the level of salt iodization in Denmark would be unlikely to succeed in reducing the burden of nodular toxic goitre further in any significant degree. Whereas an increased incidence rate of overt hypothyroidism among young subjects (<40 years) seems likely. Thus, aiming for a median UIC of 150 µg/l as recommended by WHO could be undesirable among the Danish population. A median UIC just above 100 µg/l would still be within the normal range and may ensure less of a thyroid dysfunction related disease burden among the young.

Chapter 7: Perspectives and future research

The impact of salt iodization on the incidence rate of subclinical thyroid dysfunction remains to be elucidated and seems to be a topic of increasing concern. Our current Register Database unfortunately cannot provide accurate information on the number of new cases of subclinical of thyroid dysfunction before and after the initiation of salt iodization in Denmark. The DanThyr cross-sectional studies report the prevalence of subclinical thyroid dysfunction before and after IF yet we have no indication of the prevalence of these conditions past the year 2010. A new cross-sectional study could provide crucial information on this increasingly important topic.

The effects of salt iodization on the incidence rates of overt thyroid dysfunction among subjects with previous mild ID remain to be investigated past the year 2008. This should be of considerable interest given how widespread mild ID was among the Danish population prior to initiation of IF and because the effects of IF on thyroid dysfunction in mild ID areas likely differ considerably from those seen in areas with moderate ID.

Around one third to one half of all subjects presenting with overt thyroid dysfunction within the cohort area with previous moderate ID were interviewed and examined at our research centre in Aalborg. Thyroid auto-antibodies (TRAb, TPO-Ab and Tg-Ab) and other thyroid related blood tests (TBG and thyroglobulin) were measured among all subjects and the data from these examinations remain to be analyzed. Furthermore, data on: smoking status, diet, medicine, pregnancies and ultrasonographic examination of the thyroid are available for all of the subjects interviewed in our Research Centre. These data may provide answers to many questions of interest with regard to the impact of mandatory IF on thyroid function.

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