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An 11-year DanThyr follow-up investigation

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BY
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DISSERTATION SUBMITTED 2015

AALBORG UNIVERSITY
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CV

During medical school I had two employments in 2007 and in 2008-2009 at the Department of Endocrinology, Aalborg University Hospital. Here Professor Peter Laurberg introduced me to endocrine research and after I obtained my medical degree at Aarhus University in 2011, I was enrolled as a PhD student at Aalborg University on September 15, 2011. The main focus of my research has been on iodine status and thyroid ultrasonography. In this PhD thesis the implications of iodine fortification for the individual inhabitant of a population is addressed. Additionally, research on benign thyroid nodules examined with ultrasonography and ultrasound elastography has been of great interest and has resulted in one published and one submitted paper within this field. Throughout the years as a PhD student I have participated and presented my research at numerous national and international conferences.
October 1, 2015 I will begin my medical internship at the Department of Acute Medicine, Aalborg University Hospital.

ENGLISH SUMMARY

Background: Iodine is an essential mineral for the synthesis of thyroid hormones. Low iodine intake levels are associated with goitre and other iodine deficiency (ID)-related disorders that have affected billions of people worldwide. Until recently the iodine intake of many European populations, including the Danish, was below the recommended level and iodine fortification (IF) programs are internationally recommended to ensure sufficient iodine intake of the affected populations.

Aim: The overall aim of this PhD thesis was to evaluate individual changes in thyroid related variables (thyroid volume, thyroid nodularity and serum thyroglobulin (Tg)) associated with a mandatory nationwide IF program.

Methods: We performed a prospectively planned longitudinal population-based study (DanThyr) in two regions of Denmark with different iodine intake at baseline: Aalborg (moderate ID) and Copenhagen (mild ID). We examined 2,465 adult participants before (1997) and after (2008) the Danish mandatory IF of salt (2000).

Results: Regional residence as a proxy of baseline iodine intake was of major importance for the individual changes observed in thyroid volume, thyroid nodularity and serum Tg. At follow-up the regional difference in urinary iodine concentration had disappeared. Correspondingly, the regional differences observed at baseline in both thyroid volume and serum Tg had levelled out at follow-up. Contradictory results were found regarding thyroid nodules where especially lower normalization rate of multiple nodules in Aalborg was evident. This may indicate that thyroid nodularity is more resistant to an increase in iodine intake compared with thyroid volume and serum Tg. At the follow-up investigation both regions were classified as mildly iodine deficient and median Tg among non-users of iodine containing supplements were higher than among iodine supplement users.

Conclusions: The results of these investigations suggest that even small differences in baseline iodine intake play an important role for the effect of an IF program**.** Furthermore, our findings raise the question if a moderate increase in the level of iodine added to salt could be beneficial for the Danish population.

DANSK RESUME

Baggrund: Jod indgår i dannelsen af stofskiftehormoner og et for lavt jodindtag er forbundet med struma og andre jodmangel sygdomme som har påvirket milliarder af mennesker verden over. Jodmangel har også været et problem i mange europæiske lande, inklusiv Danmark, og jodberigelsesprogrammer anbefales internationalt for at sikre sufficient jodindtag.

Formål: Formålet med dette Ph.d. projekt var at undersøge individuelle ændringer i thyroidea relaterede variabler (thyroidea volumen, thyroidea knuder og serum thyroglobulin (Tg)) forbundet med et obligatorisk nationalt jodberigelsesprogram.

Metode: Vi udførte et prospektivt planlagt longitudinelt populations-baseret studie (DanThyr) i to områder af Danmark med forskellig jodindtag ved baseline: Aalborg (moderat jodmangel) og København (mild jodmangel). Vi undersøgte 2.465 personer både før (1997) og efter (2008) den obligatoriske jodberigelse af salt i år 2000.

Resultater: Niveauet af jodmangel før jodberigelsen viste sig at være af stor betydning for de individuelle ændringer vi observerede i både thyroidea volumen, thyroidea knuder samt serum Tg. Ved follow-up undersøgelsen var jodindtaget steget og den regionale forskel i jodindtag var forsvundet. Tilsvarende var de observerede regionale forskelle i thyroidea volumen og serum Tg også udjævnet ved follow-up. Undersøgelsen af thyroidea knuder viste modstridende resultater hvor specielt en lavere normaliserings rate af multiple knuder i Aalborg var tydelig. Dette kunne indikere at thyroidea knuder er mere resistente overfor et øget jodindtag end thyroidea volumen og serum Tg. Ved follow-up undersøgelsen blev begge områder klassificeret til at have mild jodmangel og median serum Tg hos deltagere der ikke tog jodtilskud lå højere end hos de deltagere der tog et jodtilskud.

Konklusion: Vore resultater indikerer at selv små forskelle i jodindtag ved baseline har en stor og vigtig betydning for effekten af et jodberigelsesprogram. Derudover rejser vore fund spørgsmålet om hvorvidt den danske befolkning kunne have gavn af en moderat stigning i niveauet af jod der bliver tilsat salt.

ACKNOWLEDGEMENTS

The work included in this PhD thesis was performed during my research fellowship in the Department of Endocrinology, Aalborg University Hospital and I would like to thank both Aalborg University and the North Denmark Region for the facilities and funding to do my research. The project was a part of the Danish Investigation on Iodine Intake and Thyroid Disease (DanThyr) initiated and organized by the steering group: Peter Laurberg, Torben Jørgensen, Lars Ovesen and Hans Perrild, and later also Lone Banke Rasmussen.

I owe a special to Ingelise Leegaard, René Fiege, Lena Bjergved Sigurd and Mathilde Svendstup for their part of the careful and dedicated collection of data at the follow-up investigation and to Inge Bülow Pedersen and Nils Knudsen for collecting and giving me access to data from the baseline investigation. Also, I would like to thank Maggie Bloch for valuable help with logics prior and during the data collection of the follow-up study.

My main supervisor Peter Laurberg introduced me to research and has inspired me both professionally and personally. Your door has always been open for discussions and advice regardless of how busy your schedule was, and I could not have asked for a better main supervisor. I would also like to thank my co-supervisor Inge Bülow Pedersen for your valuable advice and great travel company to congresses.

During my years in this research unit I have been fortunate to have fantastic colleagues. Thanks to my roommates Ingelise Leegaard and Allan Carlé for great discussions and encouragement and to Stine Linding Andersen for having the patience to teach me how to use STATA, for inspiring discussions and for being my roommate at various congresses. Also, thank you to my lunch-date Louise Kærholm Schæbel for excellent company and conversations.

Finally, I have been blessed with a wonderful family. Thank you to my parents for always helping out whenever needed and to my husband Ehsan Motavaf and our daughters Andrea and Ellen for love and patience.

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LIST OF ABBREVIATIONS

DanThyr	The Danish Investigation on Iodine Intake and Thyroid Disease
ID	Iodine deficiency
IF	Iodine fortification
US	Ultrasonography
OR	Odds ratio
UIC	Urinary iodine concentration
Tg	Thyroglobulin
Tg-Ab	Thyroglobulin autoantibodies
TPO-Ab	Thyroid peroxidase autoantibodies
TSH	Thyroid stimulating hormone
BMI	Body mass index
WHO	World Health Organization
UNICEF	United Nations Children's Fund
IGN	Iodine Global Network

TABLE OF PUBLICATIONS

1.	**Iodine fortification may influence the age-related change in thyroid volume: a longitudinal population-based study (DanThyr)** Anne Krejbjerg, Lena Bjergved, Inge Bülow Pedersen, Allan Carlé, Torben Jørgensen, Hans Perrild, Lars Ovesen, Lone Banke Rasmussen, Nils Knudsen & Peter Laurberg. *European Journal of Endocrinology*, 170 (4), 507-17, 2014.
2.	**Thyroid Nodules in an 11-Year DanThyr Follow-Up Study.** Anne Krejbjerg, Lena Bjergved, Inge Bülow Pedersen, Nils Knudsen, Torben Jørgensen, Hans Perrild, Lars Ovesen, Lone Banke Rasmussen, & Peter Laurberg. *The Journal of Clinical Endocrinology & Metabolism,* 99 (12), 4749-57, 2014
3.	**Serum Thyroglobulin before and after iodization of salt – An 11-year DanThyr follow-up study.** Anne Krejbjerg, Lena Bjergved, Inge Bülow Pedersen, Allan Carlé, Hans Perrild, Lars Ovesen, Lone Banke Rasmussen, Nils Knudsen & Peter Laurberg.

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CHAPTER 1. INTRODUCTION

Iodine (I) is an essential mineral for the synthesis of thyroid hormones. Low iodine intake levels are associated with an increased risk of ID-related disorders (1-3) and have affected billions of people worldwide (4). Iodine is found in small amounts in many foods, but major sources are the marine food group, milk and dairy products and in some areas also drinking water (5).

1.1. MEASURES OF IODINE STATUS IN A POPULATION

Different methods can be used to measure the iodine status of a population.

1.1.1. URINARY IODINE

The majority of ingested iodine is absorbed as iodide (I^-) in the gastrointestinal tract and finally at least 90 % is excreted in the urine (6). Therefore, UIC can be used as an indicator of recent (hours to days) iodine intake. However, iodine excretion varies considerably from day to day in an individual and UIC in a spot urine sample depends on fluid intake (7,8). Thus, UIC from solitary spot urine samples can only estimate the level of iodine intake in a population and not in an individual – unless repeated sampling is performed (9). Median UIC in spot urine samples is recommended by WHO, UNICEF and IGN as a measure of current iodine status in a population (10). The examined population is considered iodine deficient if the median UIC in the population is below 100 µg/l (Table 1-1).

Table 1-1. Assessment of iodine nutrition in a population based on median urinary iodine concentration (UIC) of school-age children or non-pregnant and non-lactating adults (10).

Iodine nutrition	**Median UIC (µg/l)**
Severe iodine deficiency	< 20
Moderate iodine deficiency	20-49
Mild iodine deficiency	50-99
Optimal iodine nutrition	100-199
More than adequate iodine intake	200-299
Excessive iodine intake	> 300

1.1.2. THYROID VOLUME

Thyroid gland size and goitre rate is used as a measure of long-term (months to years) iodine status in a population (10). Traditionally, thyroid size was determined by inspection and palpation. However, in mild to moderate iodine deficient populations the sensitivity and specificity of palpation are poor and thyroid volume measured by US is preferable (11). US provides a more precise measure of thyroid volume, but standardized techniques are essential to avoid systematic interobserver variation.

1.1.3. THYROID NODULES

According to WHO, UNICEF and IGN, thyroid nodule prevalence is not a recommended measure of iodine intake in a population (10). However, numerous studies have reported a high prevalence of thyroid nodules in iodine deficient areas compared to iodine sufficient areas (12). An association between thyroid nodules and median UIC seems evident and it could be speculated whether thyroid nodules prevalence could contribute as a measure of current and maybe previous iodine status in a population.

1.1.4. SERUM THYROGLOBULIN

Tg is a protein synthesized only in the thyroid gland (13) and serum Tg is well correlated with the severity of ID (14). Measurement of serum Tg is recommended as in indicator of a populations long-term iodine nutrition over a period of at least months (10). When comparing serum Tg levels, attention should be paid to the assays used for measuring serum Tg and to the presence of Tg-Ab, as these factors may influence the results (15).

1.2. IODINE STATUS IN DENMARK

In 1995 knowledge on the iodine status in Denmark was gathered by a group of experts in nutrition and thyroid diseases (16). They reported a high occurrence of goitre and hyperthyroidism among elderly people caused by autonomous thyroid nodules as well as a low UIC compared with the recommended levels. The iodine status in Denmark varied within the country corresponding to the content of iodine found in the drinking water (17-19). Eastern Denmark had a generally higher content of iodine in the drinking water compared to western Denmark with the exception of Skagen where the drinking water had a high content of iodine (Fig. 1-1).

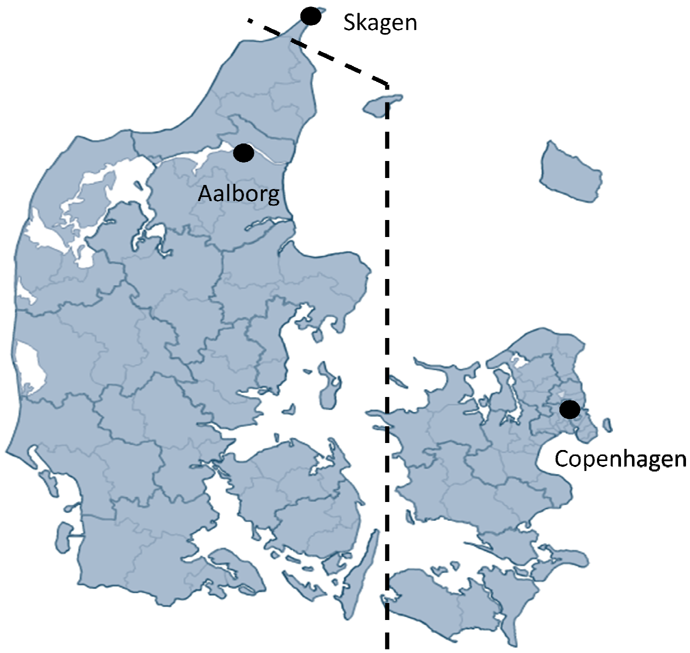

Fig. 1-1. Map of Denmark. The stippled line illustrates the regional differences in the iodine content of the Danish drinking water (17). Overall, the drinking water in eastern Denmark had higher iodine content than the drinking water in western Denmark, divided by The Great Belt. An exception was Skagen where the drinking water iodine content was the highest observed in Denmark.

To improve the Danish iodine status, a voluntary program of universal salt iodization (8 µg/g) was introduced in 1998 (20). This program turned out to be ineffective, and it was in the year 2000 replaced by a mandatory IF of household salt and salt in bread produced in Denmark (13 µg/g) (21). Iodine was added in the chemical form of potassium iodide.

A monitoring program entitled the Danish Investigation on Iodine Intake and Thyroid Diseases (DanThyr) was initiated before any iodization of salt had begun (20). This monitoring program was designed to improve knowledge on how to evaluate iodine status in a population and to evaluate the epidemiology of thyroid disorders in areas with different iodine intake as well as to study the effects of an increase in iodine intake. An important aim of monitoring was to secure that the program would not have unacceptable effects such as large increases in the occurrence of thyroid disorders. Previously, several countries experienced major increases in the incidence of hyperthyroidism after iodine fortification of salt (22).

1.3. STUDY AIM

The aim of this PhD thesis was to evaluate individual changes in thyroid related variables associated with IF. We chose thyroid volume, thyroid nodularity and serum Tg as indicators of impact and evaluated possible changes in an 11-year follow-up investigation performed before and after the mandatory IF in two regions with different iodine intake at baseline. The PhD thesis is a part of the Danish monitoring program, DanThyr.

1.3.1. SPECIFIED STUDY AIMS

Table 1-2. Specified study aims of the PhD thesis

To assess the individual thyroid volume changes after a nationwide mandatory IF program in two areas with different iodine intake at baseline, and to clarify the main predictors of these changes (Paper I).
To elucidate the relation between thyroid volume and ageing (Paper I).
To assess the changes in thyroid gland structure at 11 years of follow-up after the introduction of a mandatory IF program (Paper II).
To clarify how IF had influenced the natural history of thyroid nodules in two regions with different iodine intake at baseline (Paper II).
To investigate individual changes in serum Tg in relation to IF and examine possible predictors of these changes (paper III).

CHAPTER 2. BACKGROUND

2.1. IODINE AND THYROID VOLUME

Iodine is an essential part of thyroid hormone synthesis and both ID (12) as well as iodine excess (23) is associated with an increase in thyroid volume. An assumed goitre genesis in ID is that a fall in the blood level of thyroxine, caused by the low level of iodide in the thyroid gland, leads to an increased output of TSH from the pituitary gland. TSH increases the uptake of iodide by the thyroid gland and the increased thyroid activity is associated with hyperplasia of the follicular thyroid cells and an increase in thyroid volume (24). However, iodine also has major effects on the thyroid independent of TSH (25).

Various definitions of thyroid enlargement (goitre) have been used during the past century depending on the available methods to measure thyroid gland size. The first definitions were based on autopsy findings where studies often defined goitre as a thyroid gland exceeding 30-35 grams (12). Later, to allow investigation of people still alive, several definitions based on inspection and palpation of the thyroid gland were introduced. In the 1960s, thyroid US examinations began and different methods to calculate thyroid volume were introduced (26,27). This gave the opportunity of a much more precise thyroid volume measurement compared to inspection and palpation. Since the introduction of thyroid US examinations, the most commonly used goitre definition is a total thyroid volume exceeding the mean volume +3 SD in an iodine-replete population, corresponding to a total thyroid volume larger than 18 mL in women and 25 mL in men (28).

2.2. IODINE AND THYROID NODULES

While ID leads to an increased thyroid volume, it is also associated with an increased prevalence of thyroid nodules and nodular goitre (12). Several pathogenic models for this have been brought forward. E.g. the increase in TSH that may be seen in ID stimulates the follicular cells to proliferate. This stimulation is relatively weak and therefore only those follicular cells with a high growth potential will enter the meiotic circle and create new follicles. Follicular cells with high growth potential are not equally distributed within the thyroid gland resulting in nodule formation over time (29,30). Continued cell proliferation and nodule formation is associated with the appearance of activating TSH receptor mutations leading to thyroid nodules with autonomous hormone synthesis and secretion. Thus, nodule formation due to ID may give rise to both neck pressure symptoms as well as subclinical or overt hyperthyroidism. Another proposed model is that ID-associated auto-regulation of thyroid activity leads to an increase in thyroid H_2O_2 production and that this is a main cause of mutations and nodularity (25,31-33). Both models could play a role in severe and moderate ID but in mild ID an increase in serum TSH is limited or absent (8) and the main driving force behind thyroid cell mutation may be the ID-associated auto-regulation.

The high frequency of autonomous thyroid nodules among people living in iodine deficient areas is of concern when implementing a IF program since an increase in iodine intake may induce transient hyperthyroidism in those individuals, presumably because of the increased substrate available for thyroid hormone synthesis (22). The high prevalence of thyroid nodules in an iodine deficient population also presents a clinical challenge in the differential diagnostic between benign and malignant thyroid nodules. Because of the increased use of thyroid imaging more thyroid nodules are found. Current international guidelines recommend follow-up visits with US examination of presumably benign thyroid incidentalomas (34) and recent studies suggest that also benign nodules can look suspicious on US making the challenge for the clinicians even bigger (35-37).

2.3. IODINE AND SERUM THYROGLOBULIN

Tg is a 660 kDa protein synthesized exclusively by the thyroid follicular cells. In the thyroid follicle Tg plays a central role as a matrix in the synthesis and storage of thyroid hormone (Fig. 2-1). Previously it was assumed that Tg did not leave the thyroid gland, but in the 1960s Tg was identified in both monkey and human serum by a new sensitive radio-immunoassay method (38,39). Further studies investigated circulating Tg, and in the 1970s the association between high mean serum Tg and endemic goitre was described (40). Later, more studies confirmed and extended the results (41-44) while radio-immunoassays and screening for Tg-Ab were improved (45).

The inverse relation between iodine intake and serum Tg is well established. The mechanism behind the high level of serum Tg in an iodine deficient population is thought to be high thyroid activity as part of the iodine auto-regulation as well as a greater thyroid cell mass, since the increased amount of Tg released to the blood positively correlates with thyroid volume (46).

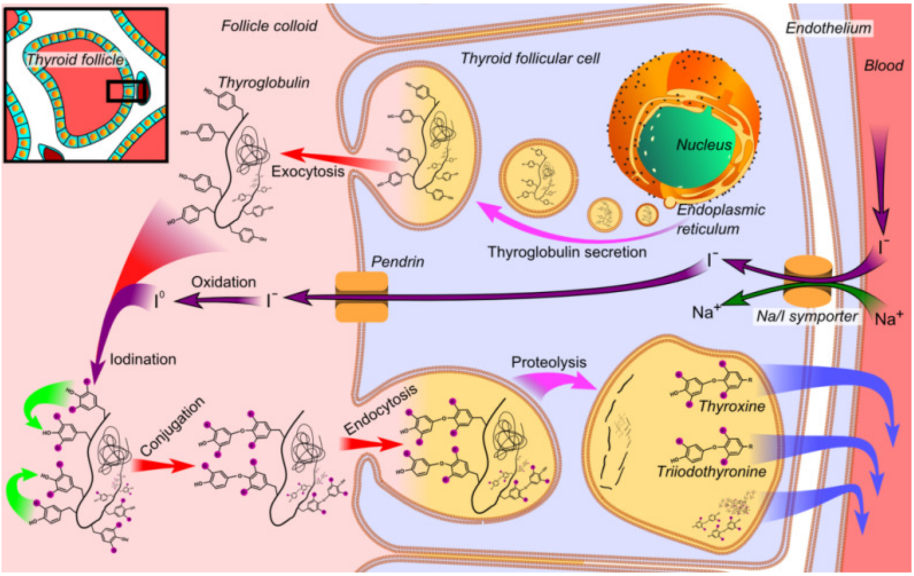

Fig. 2-1. Illustration of thyroid hormone synthesis and storage in the thyroid follicle (47). Tg is syntezised in the thyroid follicular cell and transported to the follicular colloid where it is stored. In the follicular lumen the thyrosine residues of Tg undergo iodination to produce mono- (MIT) and di-iodothyrosines (DIT) and after conjugation of these iodothyrosines Tg with Triiothyronine (T3) and Thyroxine (T4) is stored as colloid in the follicular lumen. As part of thyroid hormone secretion Tg is endocytosed into the follicular cell. Here Tg undergoes proteolyse to release T3 and T4 and both T3, T4 and Tg is secreted into the bloodstream (48).

Serum Tg can be a challenge to measure since inter-method differences are high and since results can be hampered by circulating Tg-Ab (15). In a Tg-Ab positive individual, the immunometric assay (IMA) method will typically underestimate the serum total Tg level, whereas the radio-immunoassay (RIA) typically will tend to overestimate the serum total Tg level. This is of major importance when serum Tg is used in diagnosis and follow-up of individual patients with differentiated thyroid carcinoma (15). However, the importance of Tg-Ab in population-based studies is debatable (49).

CHAPTER 3. METHODS

3.1. STUDY DESIGN

The monitoring program DanThyr was initiated in 1997 and includes a number of cross-sectional and register studies (20). As illustrated in Fig. 3-1, the first cross-sectional study (Cohort 1a (C1a)) was performed in 1997-1998 before IF was implemented. A second cross-sectional study (Cohort 2 (C2)) was performed 4-5 years after IF. The monitoring program was designed for a later follow-up of C1a after the iodization of salt (Cohort 1b (C1b)) (Fig. 3-1). This planned longitudinal design gave a unique possibility to observe the consequences of IF at an individual level. Additionally, data from C2 was included in sub-analyses of paper I and paper III.

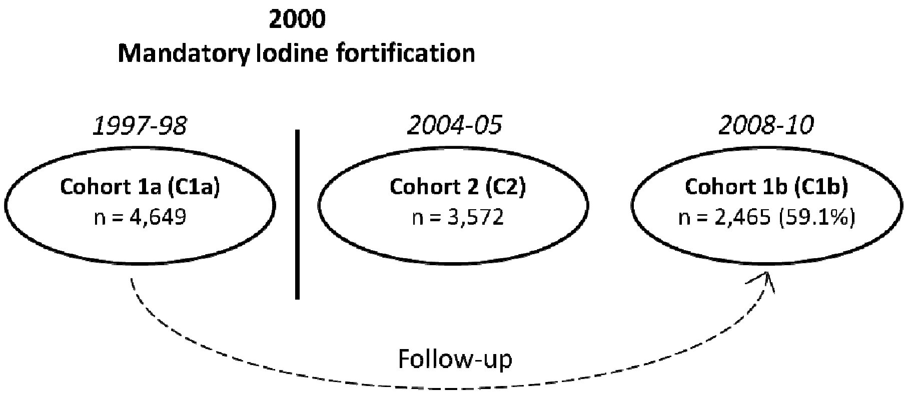

Fig.3-1. Illustration of the DanThyr cohorts investigated before and after the Danish mandatory IF of salt.

DanThyr was designed to observe the Danish population in two regions with mild (Copenhagen, eastern Denmark) and moderate ID (Aalborg, western Denmark) representing the national differences in iodine intake caused by different iodine content of the drinking water (17).

3.2. STUDY POPULATIONS

The participants of the baseline study (C1a) were selected in specific age and sex groups. An overrepresentation of woman, and especially young women, was chosen due to the high frequency of thyroid abnormalities among women and the importance of adequate iodine intake during pregnancy. A group of men was selected to allow for between sex comparisons. The age and sex groups were: women before the childbearing age (18-22 years), in the childbearing age (25-30 years), after the childbearing age, both pre- (40-45 years) and postmenopausal age (60-65 years) and men (60-65 years). All subjects living in the selected areas within the specific age and sex groups were identified in the national civil register where all Danish citizens are registered by a unique ten-digit number (n = 40,233). A computer program randomly selected 9,274 of these subjects to be invited for participation. In total, 4,649 subjects (50.1 % of those invited) participated in Cohort 1a, 2,429 in Copenhagen and 2,220 in Aalborg (50). Non-participants were asked to answer a short questionnaire concerning, among other things, previous thyroid disease. No significant difference was found between participants and non-participant on hyper- or hypothyroidism whereas bias was found regarding self-reported goitre (participants 5.9 % vs. non-participants 3.6 %, $p < 0.001$) (51).

Participants of the C2 study were randomly selected in the same regions and in the same age and sex groups as in C1a, making the two cohorts directly comparable. A total of 3,570 subjects participated in the study (46.6 % of the invited), 1785 in Copenhagen and 1785 in Aalborg (52).

The follow-up investigation C1b was carried out between February 2008 and February 2010 resulting in a mean follow-up time of 11.2 years (range 10.1-12.8 years). As illustrated in Fig. 3-2, all 4,649 participants of the baseline C1a study were identified. During the follow-up period, 403 subjects had died and 72 subjects had emigrated out of the country leaving 4,174 subjects to be invited for participation in the follow-up study. The invitations were sent by letter and in case of no response only one further invitation was sent. In total, 2,465 subjects participated in the follow-up study (59.1 % of the invited). The examinations were performed at either Aalborg University Hospital or Bispebjerg University Hospital in the region of Copenhagen. At each hospital a team including a physician and a sonographer performed the examinations. The participants answered health, food frequency and food supplement questionnaires, gave blood and urine samples and had a thyroid US examination performed. In addition, a physical examination as well as an interview was carried out.

Owing to the prospective design of DanThyr, it was possible to keep all procedures identical in all three cohorts.

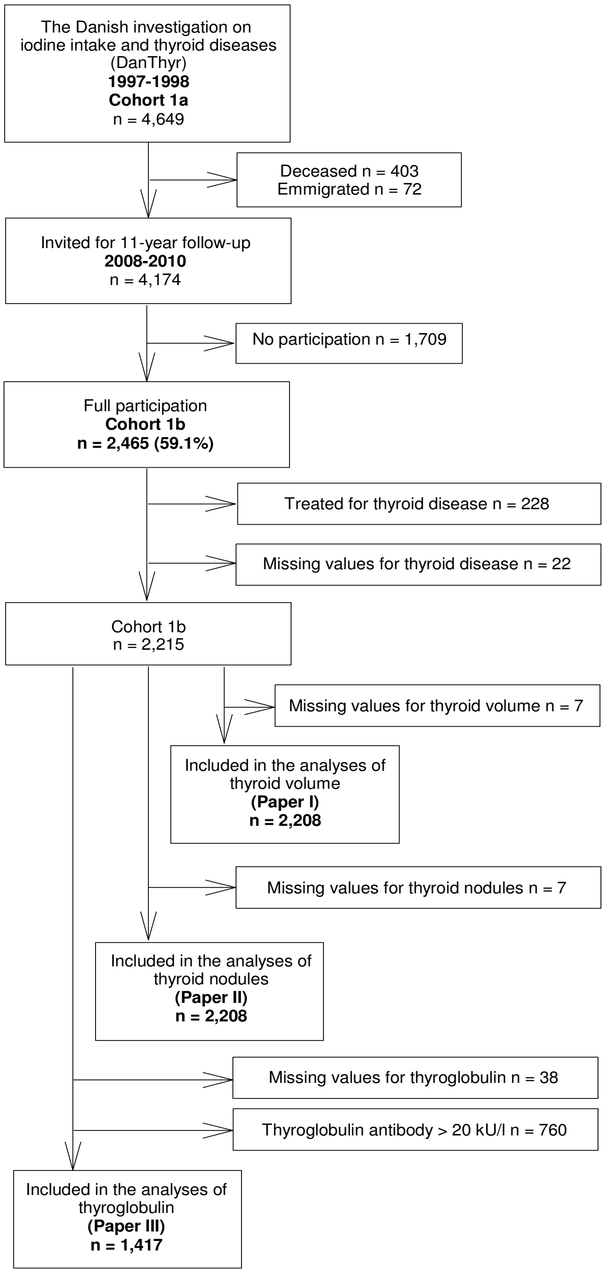

Fig.3-2.Flowchart illustrates participants included in the final study population of Paper I, Paper II and Paper III.

3.1. URINARY IODINE CONCENTRATION IN THE COHORTS

In the baseline C1a study, median UIC in Copenhagen was 68 µg/l (61 µg/l for non-users of iodine supplements) and in Aalborg median UIC was 53 µg/l (45 µg/l) (50), classifying Copenhagen as mildly iodine deficient and Aalborg as moderately iodine deficient (10).

In C2, median UIC had increased compared with C1a. In Copenhagen median UIC was 108 µg/l (99 µg/l for non-users of iodine supplements) and in Aalborg UIC was 93 µg/l (86 µg/l) (52) making Copenhagen iodine sufficient and Aalborg mildly iodine deficient (10).

In C1b, median UIC had decreased again. In Copenhagen median UIC was 84 µg/l (76 µg/l for non-users of iodine supplements) and in Aalborg it was 83 µg/l (73 µg/l) (53) classifying both Copenhagen and Aalborg as mildly iodine deficient (10).

3.2. ULTRASONOGRAPHY

All US examinations were performed by the same two sonographers using the same US apparatus (Sonoline Versa Pro 7.5-MHz 70-mm linear transducer; Siemens, Germany) in C1a, C2 and C1b.

Thyroid volume was calculated as length × depth × width × $\pi/6$ for each lobe (54). Thyroid enlargement was defined as a thyroid volume >18 mL for women and >25 mL for men, corresponding to the mean + 3 SD in an iodine sufficient population (28).

Thyroid nodules ≥ 5mm were registered and in the event of more than three nodules in one lobe, only the three largest nodules were registered. Thyroid gland structure was defined as diffuse when no nodules were registered, as solitary nodule when one nodule was registered and as multinodular when more than one nodules was registered. Thyroid nodules included solid nodules and partly cystic nodules, but not thin-walled cysts. Thyroid nodule size was defined as the largest diameter by US, and in case of multiple nodules, the diameter of the largest nodules was used.

3.3. LABORATORY PROCEDURES

Non-fasting blood and spot urine samples were collected between 8:00 AM and 5:30 PM. Samples were kept frozen (-20 °C) and analysed in random order at the study end.

3.3.1. URINARY IODINE

UIC (µg/l) was measured by the Ce^{4+}/As^{3+} method after alkaline ashing (55,56). The analytical sensitivity was 2 µg/l. The iodine laboratory is certified by the U.S. Center for Disease Control and Prevention's EQUIP Program.

3.3.2. SERUM THYROGLOBULIN

In the baseline C1a study, serum Tg was measured with an immunoluminometric assay (LUMITEST, BRAHMS Diagnostica GmbH, Berlin, Germany) using a Stratec autoanalyzer (STRATEC biomedical Systems AG, Birkenfeld, Germany). The assay's effective working range was 1-500 µg/l. In 12 consecutive assays the inter-assay coefficient of variations (CVs) for samples with average Tg concentrations of 8, 45 and 154 µg/l were 6.8, 4.5 and 3.3%.

In the C2 study and the follow-up C1b study, serum Tg was analyzed using an immunoflourescent assay (hTg KRYPTOR BRAHMS) with a functional sensitivity <0.8 ng/ml (information from manufacturer). In 115 consecutive assays the inter-assay CVs for samples with average Tg concentrations of 3.3 and 50.5 µg/l were 5.6 and 2.8 %.

We found disagreement between the two methods and a linear regression model (Tg(follow-up)=1.487+0.693×Tg(baseline)) was used to adjust baseline (C1a) Tg to the assay used in the C2 study and in the C1b follow-up study.

3.3.3. THYROGLOBULIN ANTIBODIES

In C1a Tg-Ab were analyzed using RIA (DYNOtest, BRAHMS) with a functional sensitivity at 20 kU/l. In C2 and C1b Tg-Ab were measured with an immunoflourescent assay (anti-Tgn KRYPYOR, BRAHMS). We re-measured Tg-Ab in 201 serum samples kept frozen from the baseline study with the new assay.

We found a high correlation (r_s=0.94) and a Bland-Altman plot showed a high level of agreement between the two methods. Thus, a cut-off of 20 KU/l was used to indicate Tg-Ab positivity in C1a, C2 and C1b.

3.4. STATISTICAL ANALYSES

All data processing was done with the STATA version 11.0 (Stata Corp., College Station, Texas, USA). Two-sided $p < 0.05$ was considered statistically significant. The choice of statistical tests depended on the distribution of the outcome variable and on whether data were paired or unpaired.

3.5. ETHICS

The study protocols were approved by the Danish Ethics Committee (2-16-4-0001-97 and VN 96/208mch and N-VN-19960208MCH, the Northern Danish Region Committee). The study was conducted in accordance with the Declaration of Helsinki, and all participants gave written informed consent.

CHAPTER 4. SPECIFIC STUDIES PERFORMED

4.1. THYROID VOLUME (PAPER I)

4.1.1. RESULTS

Overall, the follow-up period saw an increase in median thyroid volume in Copenhagen (11.8-12.2 ml, p=0.001) and a borderline decrease in median thyroid volume in Aalborg (13.3-13.1 ml, p=0.07). Both regions witnessed an age-related trend in individual thyroid volume changes from baseline to follow-up: Thyroid volume increased in women <40 years of age and decreased in women >40 years of age (Fig. 4-1). In addition, multivariate logistic regression models found that higher age predicted a thyroid volume decrease and that women of higher age were less likely to have an increase in thyroid volume during follow-up.

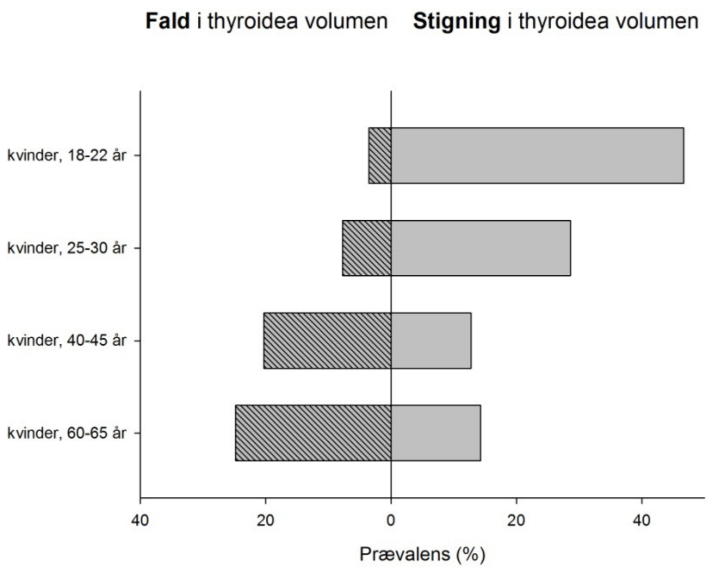

Fig. 4-1. Prevalence of thyroid volume decrease (-20%) and increase (+20%) according to female age groups at baseline.

In analyses limited to women aged 30-32 and 40-42 years, we included data from the C2 study and compared geometric mean thyroid volume in female participants of C1a, C2 and C1b. In C1a, thyroid volume was higher among women aged 40-42 years than in women aged 30-32 years (14.5/12.3ml, p<0.001), whereas no difference was observed in C2 (11.8/11.4 ml, p=0.3) or in C1b (12.4/12.7 ml, p=0.5).

In multivariate logistic regression models we found higher age and baseline thyroid enlargement to be predictors (p<0.05) of an individual thyroid volume decrease whereas baseline multinodularity and a family history of thyroid disease were predictors of a thyroid volume increase during the follow-up period.

4.1.2. DISCUSSION

During our follow-up study participants became 11 years older and had an increase in iodine intake. Thus, both age and iodine may have influenced thyroid volume in our participants. Previous studies performed in iodine deficient areas have described an increase in thyroid weight and volume with age (50,57,58). We observed an increase in thyroid volume among our youngest participants, which could be explained by them getting older. However, the oldest participants experienced a thyroid volume decrease despite getting older. Other studies performed in iodine sufficient areas suggested that thyroid volume may decrease with age (59,60). Thus, it could be speculated that iodine intake may influence the age-related variation in median thyroid volume. This notion was supported by our analyses restricted to women aged 30-32 and 40-42 years where median thyroid volume before IF was higher among the oldest women, whereas no difference in thyroid volume was observed after IF. But, if the increased level of iodine intake influenced the age-related change in thyroid volume towards a decrease, why did we then observe a thyroid volume increase among the youngest participants? We speculated if the increase in thyroid volume among the youngest women during the follow-up period could be associated with thyroid autoimmunity. To elaborate on this, we performed additional analyses of median thyroid volume among women aged 18-30 years at baseline and excluded participants positive for either Tg-Ab (Tg-Ab > 20 kU/l) or TPO-Ab (TPO-Ab >60 kU/l) at baseline, follow-up or both. These analyses showed the same results regardless of thyroid autoimmunity: an increased median thyroid volume at follow-up. Thus, thyroid autoimmunity does not seem to explain the thyroid volume increase found in the young female participants. Another explanation may be that the higher level of iodine intake had the largest impact on individuals exposed to ID in the longest period of time. Thus, the influence of IF would dominate the thyroid volume change in the oldest participants leading to a thyroid volume decrease whereas the age-related influence on the thyroid volume would dominate among the youngest participants leading to a thyroid volume increase (Table 4-1). This notion is supported by the observed regional difference in median thyroid volume: participants living in Aalborg had been exposed to the highest level of ID which resulted in an overall (although not significant) thyroid volume decrease whereas participants from Copenhagen experienced an overall thyroid volume increase.

Table 4-1. Table illustrating the theoretical influence of age and iodine on thyroid volume change in the youngest and the oldest female participants.

Women	**Age**-related thyroid volume change		**Iodine**-related thyroid volume change		**Observed** thyroid volume change
18-30 yrs	↑	+	↓	=	↑
40-65 yrs	↑	+	↓	=	↓

4.2. THYROID NODULES (PAPER II)

4.2.1. RESULTS

The follow-up period observed an overall increased prevalence of multiple thyroid nodules mainly caused by an increased prevalence in Aalborg (Fig 4-1). However, when we took age into account and compared women aged 40 years at the first investigation (1997-1998) with women aged 40 years at the second investigation (2008-2010), we found a lower prevalence of both solitary ($p=0.004$) and multiple ($p<0.001$) nodules at the second investigation.

Changes in thyroid structure during the follow-up period were common. One-third of solitary thyroid nodules identified at baseline had disappeared at follow-up and one-fifth of previous multinodular thyroid glands had a diffuse structure at follow-up. Overall, we found regional differences in both incidence and normalization rates of thyroid nodules with higher incidence and lower normalization rates in Aalborg with the lowest baseline UIC. In addition, solitary nodules had a significantly higher normalization rate than multiple nodules (normalization rate ratio 0.47 (95%CI = 0.32-0.67). The analyses were repeated for nodules with a diameter > 10 mm and showed the same results.

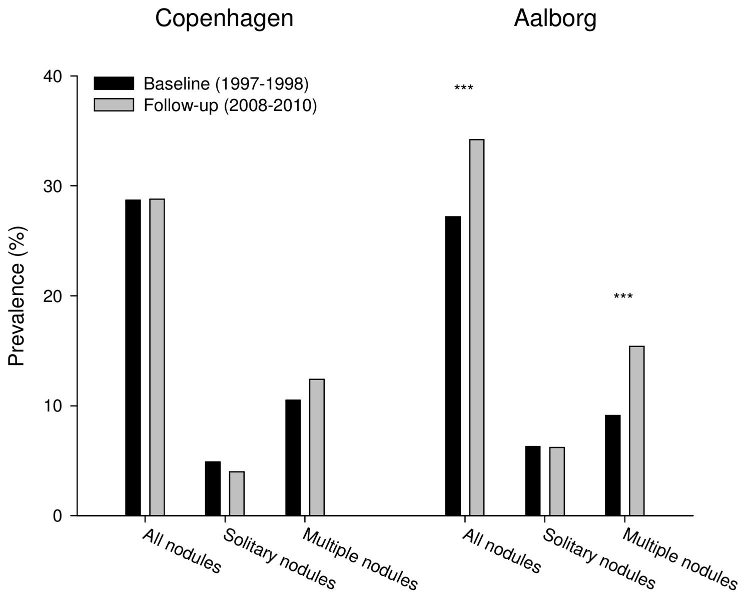

Fig 4-2. Prevalence of thyroid nodules in Copenhagen and Aalborg at baseline (1997-1998) and at follow-up (2008-2010).
****p<0.001*

4.2.2. DISCUSSION

At the follow-up investigation both regions were classified as mildly iodine deficient. Thus, owing to the mandatory IF in year 2000, Aalborg had the highest increase in median UIC compared with Copenhagen during the 11-year follow-up period. Despite this increase Aalborg had higher nodule incidence rates and lower normalization rates. This may suggest that the former longstanding level of ID still had an impact on the changes in thyroid structure. The largest regional difference was found between the normalization rates of multiple nodules, whereas no significant regional difference was found among the normalization rates of solitary nodules. Furthermore solitary nodules had higher normalization rates than multiple nodules. This demonstrates the heterogeneity among thyroid nodules with a higher reversibility of solitary nodules and it may indicate a more limited effect of IF on multiple nodules compared with solitary nodules. The reason could be structural differences or it could be that the majority of solitary nodules identified in this study were in fact the beginning of a multinodular disease and therefore more likely to normalize compared with more severe multinodular disease.

Looking at the incidence and normalization rates of thyroid nodules we did not elucidate the actual growth or shrinkage of the individual nodule investigated. To explore this we performed additional analyses where we looked at the natural history of the 46 solitary nodules present at both baseline and follow-up: 20 solitary nodules in Copenhagen and 26 solitary nodules in Aalborg. Overall, the follow-up period saw a borderline increase in median nodule diameter (15.2-16.7 mm, p=0.05). When stratifying by region, a significant increase in median nodule diameter was found in Copenhagen (14.0-18.4 mm, p=0.004) whereas no significant change was observed in Aalborg (16.2-13.6 mm, p=0.99). Thus, even though the trend with higher incidence rates and lower normalization rates in Aalborg also applied to solitary nodules, a different pattern appeared when looking at solitary nodules present at both baseline and at follow-up. Nodule growth was only evident in Copenhagen, the region with the highest pre-iodization median UIC. These analyses were unadjusted, sample size was small, and the study has no control group, however, it could be speculated that it was the larger increase in iodine intake in Aalborg compared with Copenhagen that affected the thyroid nodules and lead to nodule growth in Copenhagen and insignificant change in nodule size in Aalborg. This notion is supported by a randomized placebo-controlled trial of patients with thyroid nodules undergoing therapy with iodine (61). They found a significant reduction in nodular volume in patients treated with iodine alone compared with placebo.

The conflicting results between incidence and normalization rates on the one hand and the natural history of solitary nodules on the other hand underline the diversity of thyroid nodules. Thyroid nodule heterogeneity of morphology, function and growth has previously been described in multinodular goitre (29) and an overall difference in growth pattern between the nodules investigated in general and the solitary nodules used for the analysis of natural history could explain the inconsistent findings. Additionally, a difference in nodule susceptibility to iodine may also play a role as well as the possibility that the majority of solitary nodules used in the analyses of incidence and normalization rates actually were the beginning of multinodular thyroid disease.

4.3. SERUM THYROGLOBULIN (PAPER III)

4.3.1. RESULTS

During the 11-year follow-up period, median serum Tg had decreased in Aalborg (11.4/9.0 µg/l, $p<0.001$) whereas no change in median Tg was observed in Copenhagen (9.1/9.1 µg/l, p=0.67). In addition, the region-related differences in median Tg observed at baseline (Copenhagen/Aalborg, 9.1/11.4 µg/l, $p<0.001$) had disappeared at follow-up (Copenhagen/Aalborg, 9.1/9.0 µg/l, $p=1.00$).

In a multivariate linear regression model, living in Aalborg ($p<0.001$) and not using iodine supplements at baseline ($p=0.001$) predicted an individual decrease in serum Tg whereas high age ($p=0.003$) was associated with an increase in Tg during the follow-up period.

We found a higher median Tg among non-users of iodine containing supplements compared with iodine supplement users both before (11.2/8.1 µg/l, $p<0.001$) and after (9.4/7.9 µg/l, $p<0.001$) IF. A regional difference in median Tg was only significant among non-users of iodine supplements before IF ($p<0.001$).

4.3.2. DISCUSSION

Living in Aalborg (formerly moderate ID) was a strong predictor of a decrease in serum Tg compared with living in Copenhagen (formerly mild ID). Thus, the degree of baseline ID was very important for the individual change in serum Tg even though the difference in baseline UIC between Aalborg (53 µg/l) and Copenhagen (68 µg/l) was small. Likewise, median serum Tg only decreased in Aalborg with the largest increase in UIC, which levelled out the regional differences found before IF. These findings corroborate the results of two previous randomized trials (44,62) that showed a close end sensitive relation between individual iodine supplement intake and their serum Tg level.

Measuring serum Tg can be challenging and both the specific assay used and the Tg-Ab status of the individual is of major importance for a correct measurement (15). We excluded Tg-Ab positive participants from the analyses of this paper because Tg-Ab is known to influence the measurement of serum Tg. Though, a previous study suggested that exclusion of Tg-Ab positive participants may not influences the results of population-based studies (49). To explore this further we repeated our analyses of median serum Tg and included the Tg-Ab positive participants. As expected, this showed generally lower median Tg levels (Fig. 4-3). However, the results showed the same trends and did not change the overall conclusions. This is in accordance with Vejbjerg et al. (49) and could indicate that exclusion of Tg-Ab positive participants is not essential for the interpretation of population-based data.

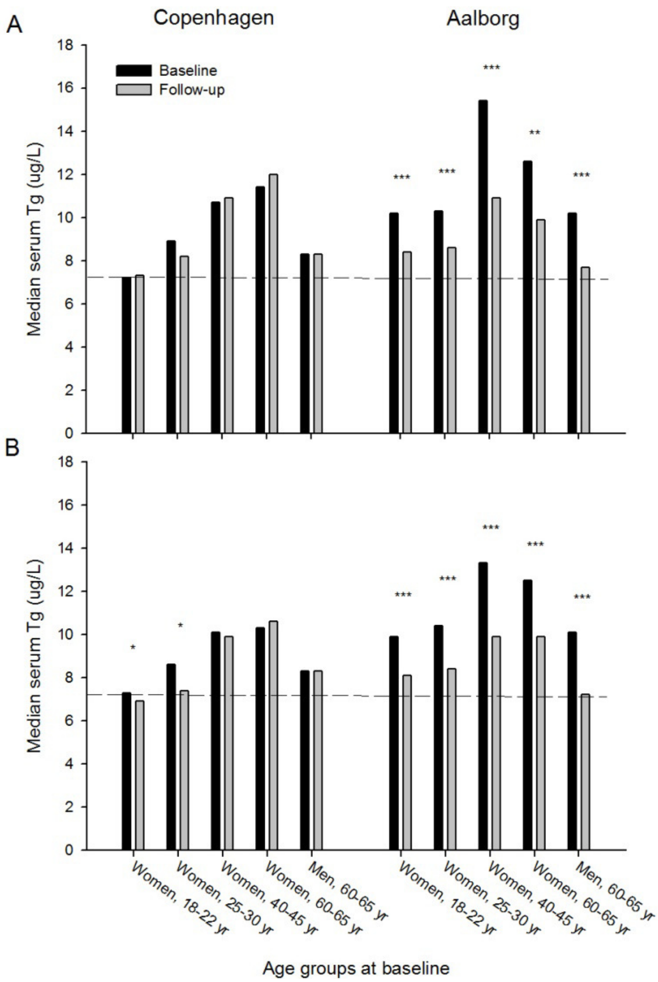

*Fig 4-3. **A** Median serum Tg (µg/l) (EXCLUDING Tg-Ab positive participants) at baseline and follow-up by age group at baseline in the two investigated regions Copenhagen and Aalborg (n=1,417). To assist visual comparison, a line (horizontal stippled) has been added corresponding to the lowest value of median Tg found at baseline in Copenhagen among 18-22 year old women (Tg=7.2 µg/l). **B** Median serum Tg (µg/l) (INCLUDING Tg-Ab positive participants) at baseline and follow-up by age groups at baseline in the two investigated regions Copenhagen and Aalborg (n=2,177). To assist visual comparison, a line (horizontal stippled) has been added corresponding to the lowest value of median Tg found at baseline in Copenhagen among antibody-negative 18-22 year old women (Tg=7.2 µg/l).*
$*p<0.05$ $**p<0.01$ $***p<0.001$

CHAPTER 5. GENERAL DISCUSSION

5.1. STUDY COMPARISONS

The three papers illuminate individual changes associated with a mandatory IF program. Overall, the small regional difference in baseline UIC played an important and dominating role for the investigated outcomes. Region predicted individual changes in all three studies although both regions were classified as mildly iodine deficient at follow-up. In paper I thyroid volume decreased in Aalborg and increased in Copenhagen and in Paper III serum Tg decreased in Aalborg whereas no change was observed in Copenhagen. These results could indicate that IF had the largest impact on those individuals who experienced the largest increase in UIC. However, when we investigated thyroid nodules (paper II) Copenhagen had the lowest incidence rates and highest normalization rates; and moreover, living in Copenhagen predicted thyroid nodule disappearance. Thus, the former longstanding level of ID appeared to have more impact on thyroid nodules than the actual increase in iodine intake during the 8 years after IF. These conflicting results were confirmed in the regression analyses performed in paper I. Here baseline thyroid enlargement predicted a thyroid volume decrease whereas baseline multinodularity predicted a volume increase, keeping in mind that regression towards the mean may explain some of the thyroid volume decrease seen in participants with baseline thyroid enlargement (63). Thus, although we found thyroid nodules to be a dynamic and not necessarily irreversible abnormality, these results may indicate that thyroid nodularity is more resistant to an increase in iodine intake compared with thyroid volume and serum Tg. A certain degree of iodine memory may be in action when it comes to thyroid nodules. Thus, thyroid structure abnormalities in a population may not only reflect the actual iodine intake but also previous iodine intake of the population (3).

5.2. THE DANISH IODINE INTAKE

After the commencement of the Danish mandatory iodization of table and bread salt there has been a clear increase in the populations UIC (Aalborg: 53 vs. 83 µg/l, Copenhagen: 68 vs. 84 µg/l) and both regions where at the follow-up investigation classified as mildly iodine deficient. However, despite this clear increase, UIC was lower than observed in the C2 study investigated 4-5 years after IF (Copenhagen:108 µg/l, Aalborg: 93 µg/l) (52). A major reason for the observed decrease may be a reduction in the iodine content of common milk and dairy products (64) and this observation stresses the importance of persistent monitoring of fortification programs in order to ensure sustainable iodine sufficiency.

Corresponding to the general increase in iodine intake with the most prominent change in Aalborg, we found a reduction in the regional differences among the investigated outcomes compared with baseline. However, at the follow-up investigation we found median Tg among non-users of iodine containing supplements to be higher than among iodine supplement users. This finding together with the decrease in UIC since the C2 investigation raise the question if a moderate increase in the level of iodine added to the salt could be beneficial for the Danish population.

The IF program initiated in year 2000 was cautious (13 µg/g) to reduce the risk of side effects (20) and WHO, UNICEF and IGN recommend that the level of iodine added to the salt should be within the range of 20-40 µg/g in order to provide 150 µg of iodine per person per day (10). Even though the Danish IF was cautious, we found significant changes in the evaluated outcomes chosen as indicators of impact, and our findings emphasize that even small changes in iodine intake is of importance. Additionally, known side-effects to an increased iodine intake (22,65,66) have been observed in the Danish population despite the relatively low level of iodine added to the salt (67-69). Thus, a cautious approach in combination with continuous monitoring would be sensible. The aim of a modest increase in the amount of iodine added to salt could be to bring the median UIC to a level around 100 µg/l as found in 2004-2005 (52).

In 2012 a Danish investigation performed by Andersen et al. (70) found insufficient iodine intake among pregnant women in Aalborg. Pregnant women are extra vulnerable to iodine deficiency because their needs for iodine are higher and since thyroid hormone plays a crucial role in early brain development of the foetus (71). This finding elaborates and confirms our results indicating a need for a moderate increase in the Danish IF. If the level of iodine added to salt was increased, a concern could be an excessive iodine intake among children due to their relatively high consumption of milk. Another study by Andersen et al. (72) included 51 children from Aalborg aged 1-14 years and found that median UIC in these children did not differ significantly from median UIC among the pregnant women. However, the number of children investigated in this study was limited and an actual investigation of schoolchildren in eastern Denmark may give the additional knowledge required to make an informed decision regarding an increase in the iodine level of the mandatory Danish IF of salt.

CHAPTER 6. METHODOLOGICAL CONSIDERATIONS

6.1. STUDY DESIGN AND POPULATION

The longitudinal design with examination of participants before and after IF gave a unique possibility to investigate individual changes associated with IF as well as identifying possible predictors of these changes. However, we have no control group (a group without IF) and therefore, no firm conclusions regarding causal relations between IF and the investigated outcomes can be drawn from this study. A few sub-analyses within Paper I and III included data from the cross-sectional study C2 and considered C1a, C2 and C1b as independent cohorts. Since C1b is a follow-up investigation of C1a, they are not independent and therefore, this is a major limitation. A cross-sectional investigation with participants from the same regions and within the same age and sex groups as in C1a and C2 would give epidemiologically more precise information on the Danish iodine status.

The long follow-up period resulted in a relatively low participation rate of 59.1%. This could introduce selection bias and participants did differ from non-participants on a few central variables. However, in Paper II we calculated inverse probability weights to account for missing at random and used these weights in our prevalence data as well as our regression models without any change in the results. Part of the study results can be difficult to interpret because participants of the follow-up study had become 11-years older. On the other hand, the long follow-up period allowed us to investigate structural changes that may developed over a long period of time such as changes in thyroid volume and nodularity.

The study population included participants in specific age groups and consisted mainly of women. Therefore, we cannot generalize our results to the entire population although we have a relatively large study population.

6.2. EXCLUDED FROM THE ANALYSES

6.2.1. SUBJECTS TREATED FOR THYROID DISEASE

We excluded participants treated for thyroid disease including those with current or previous treatment with medicine, surgery or radioactive iodine therapy from all analyses (n = 228). Exclusion took place both when therapy had been recorded at baseline and at follow-up. Information on treatment for thyroid disease was registered after an interview with a physician and no validation on self-reported data was made. As may be expected, participants excluded based on treatment for thyroid disease differed significantly from the participants included in the final study population (Table 6-1).

In this follow-up investigation we wanted to examine the general population and not thyroid patients. As depicted in Table 6-1, the participants treated for thyroid disease had higher thyroid volume, more thyroid nodules and were more frequent elderly women living in Aalborg. Thus, including them in our analyses could affect our results and cause an attenuation of the regional differences.

Table 6-1. Baseline characteristics (C1a) of participants in the follow-up investigation (C1b) included in the final study population, and participants treated for thyroid disease and excluded from the final study population. Data represent n (%) and median (25^{th}-75^{th} percentiles). Comparisons were made using χ^2-test for categorical variables and Mann-Whitney's U test for median of continuous variables.

	Participants included in the final study population (n = 2237)	**Participants treated for thyroid disease (n = 228)**	**p**
Age groups (years)			<0.001
Women, 18-22	468 (20.9)	21 (9.2)	
Women, 25-30	488 (21.8)	26 (11.4)	
Women, 40-45	585 (26.2)	72 (31.6)	
Women, 60-65	290 (13.0)	91 (39.9)	
Men, 60-65	406 (18.2)	18 (7.9)	
Region			0.002
Copenhagen	1,144 (51.1)	92 (40.4)	
Aalborg	1,093 (48.9)	136 (59.7)	
Daily smoking	715 (32.0)	78 (34.2)	0.76
Family history of thyroid disease	437 (19.5)	70 (30.7)	<0.001
TPO-Ab ≥ 30 kU/l	276 (12.6)	115 (50.9)	<0.001
Thyroid volume (ml)	12.5 (9.5-17.0)	15.0 (10.0-25.1)	<0.001
Thyroid enlargement (>18/25 ml)	479 (21.4)	91 (40.1)	<0.001
Multinodularity	223 (10.0)	56 (24.7)	<0.001

6.2.2. SUBJECTS POSITIVE FOR TG-AB

In paper III we excluded Tg-Ab positive (Tg-Ab>20 kU/l) participants because Tg-Ab is known to influence the measurement of serum Tg (15). The participants positive for Tg-Ab did differ from the final study population on two central variables: More were women (87.9 vs. 78.3 %, p<0.001) and more were living in Aalborg (53.3 vs. 47.1 %, p=0.01). However, no difference was found in regard to iodine supplements (p=0.61), thyroid volume (p=0.69) or thyroid nodularity (p=0.50).

6.3. CHANGE IN REGIONAL RESIDENCE

In total 66 participants changed regional residence during the follow-up period and were as a result examined in the opposite region at the follow-up investigation. Five of these participants were treated for thyroid disease leaving 61 participants who changed regional residence to be included in the final study population (2.8 %).

Table 6-2. Follow-up characteristics (C1b) of participants who were investigated in the same region at both baseline (C1a) and follow-up (C1b) (n=2,154) and participants who changes regional residence during the follow-up period (n=61) stratified by region at follow-up. Data represent n (%) and median (25^{th}-75^{th} percentiles). Comparisons were made using χ^2-test for categorical variables and Mann-Whitney's U test for median of continuous variables.

	Investigated in **Copenhagen** **at follow-up**			Investigated in **Aalborg** **at follow-up**		
Follow-up variables	No change in regional residence (n = 1,110)	Change in regional residence (n = 46)	p	No change in regional residence (n = 1,044)	Change in regional residence (n = 15)	p
Thyroid volume (ml)	12.2 (9.6-15.8)	12.3 (9.1-14.8)	0.66	13.1 (10.0-18.5)	13.4 (12.1-18.3)	0.53
Thyroid nodules	318 (28.8)	9 (19.6)	0.17	362 (34.8)	4 (26.7)	0.51
Serum Tg (µg/l)	8.2 (4.8-14.6)	7.4 (4.0-12.9)	0.35	8.7 (4.9-15.1)	9.3 (3.8-14.1)	0.93

In additional analyses we found no difference in our three main outcome measures between participants changing regional residence and participants investigated in the same region at both investigations (Table 6-2). Only a small percentage changed residence and even though the negative results in these analyses may be due to lack of power, these participants were unlikely to influence our results.

6.4. INTEROBSERVER VARIATION

Thyroid examination by US is influenced by both intra- and interobserver variability (73). Therefore, well-defined measuring procedures and as few observers as possible are essential for the reproducibility and reliability of thyroid US examinations.

To reduce variability in this follow-up study, US examinations were performed by the same two sonographers (one in each region) using the same equipment at both baseline and follow-up. An interobservational study was performed before the baseline investigation (54) and a confirmatory study was carried out before the follow-up investigation commenced. This showed a continued good correlation ($r=0.95$, $p<0.001$) between the two sonographers' thyroid volume measurements.

In paper I we assessed individual changes in thyroid volume and defined a change as a more than 20 % increase or decrease from baseline to follow-up by US. Due to the long period of time between examinations we defined an individual change as twice the interobserver variability found by Knudsen et al. (54).

Assessment of thyroid nodules by US is also associated with both inter- and intraobserver variability (54,74,75). We only included clearly defined thyroid nodules > 5 mm as recommended by Knudsen et al. (54) as micro-nodularity showed poor interobserver agreement.

6.5. THYROGLOBULIN ASSAYS

Two different methods were used for measuring serum Tg in the baseline and in the follow-up study. To investigate the level of agreement between the two methods we re-analysed 101 Tg-Ab negative serum samples kept frozen from the baseline study with the new assay and a Bland-Altman plot showed higher measured values with the baseline assay compared with the assay used at follow-up (Fig 6-1A). Therefore, a linear regression model was used to define the association between the two methods ($Tg(follow\text{-}up)=1.487+0.693*Tg(baseline)$) and this equation was used to adjust Tg measured at baseline to the assay used at follow-up (Fig 6-1B). Adjusted baseline Tg was used in all analyses.

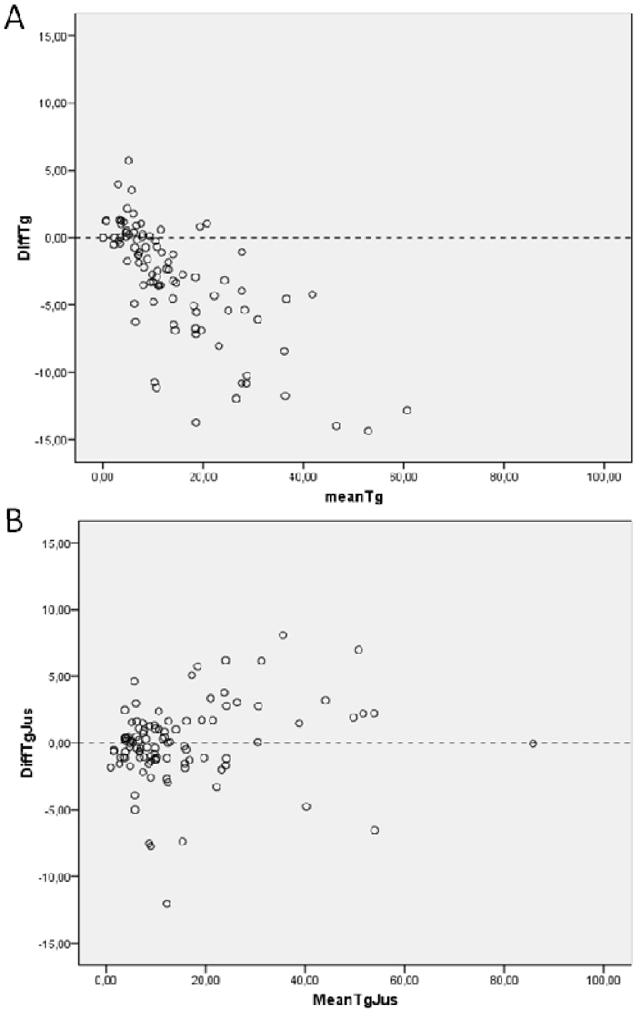

*Fig 6-1. **A** Bland-Altman plot (x-axis: average of two measures, y-axis: difference between two measures) illustrating the level of agreement between the unadjusted assay used to measure Tg in the baseline study and the new assay used in the follow-up study. 101 Tg-Ab negative serum samples from the baseline study were kept frozen and re-analysed with the new assay. **B** Bland-Altman plot illustrating the level of agreement between the adjusted baseline assay (Tg(follow-up)=1.487+0.693*Tg(baseline)) and the new assay used in the follow-up study (n=101).*

To investigate if the disagreement between the two methods could be associated with the long storage time of the frozen serum samples we performed additional Tg measurements of 100 Tg-Ab negative serum samples kept frozen from the C2 study in 2004-2005. These serum samples were kept frozen for 10 years and were re-analysed using the same method. A Bland-Altman plot (Fig 6-2) demonstrated an overall acceptable agreement between the measurements compared with the results found after re-analysing samples with a new method (Fig 6-1).

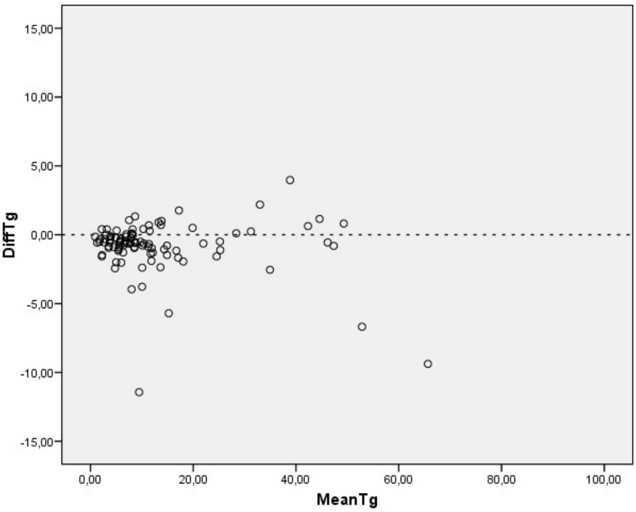

Fig. 6-2. Bland-Altman plot illustrating the agreement between the Tg values of the serum samples from C2 (2004-2005) and the re-measurement of the same serum samples after 10 years of freezer storage (2015) by the same method (n=100).

After storage, the Tg values were somewhat lower than the Tg values before storage (median 7.3 % lower, 25^{th}-75^{th} percentiles: 15.0 % lower to 0.5 % higher). This suggests that the main influence on Tg measurements in the between assay analyses was in fact the inter-assay variation and to a smaller degree the relatively long storage time. However, all baseline Tg values in Paper III were adjusted corresponding to the difference found in the inter-assay analysis and this may have over-adjusted baseline Tg values by 7%. This over-adjustment may have influenced some results of Paper III where median Tg in Copenhagen might have decreased during the follow-up period if the data had not been over-adjusted. However, even if the same assay from the same manufacturer was used when concentrations were re-measured 10 years after the first measurement, it is difficult to exclude that small changes in assay characteristics had occurred over time. Overall, this additional analysis suggests that less weight should be put on changes in Tg < 10 % if serum samples have been collected many years apart.

CHAPTER 7. CONCLUSIONS AND PERSPECTIVES

This thesis illuminates the association between IF and individual changes in thyroid volume, thyroid nodularity and serum Tg in an 11-year follow-up investigation performed in two regions with different iodine intake at baseline. Baseline information was obtained before the Danish mandatory IF of salt commenced in year 2000.

We established that regional residence as a proxy of baseline iodine intake was of major importance for the individual changes observed in all three outcome measures. This suggests that even small differences in baseline iodine intake play an important role for the effect of an IF program. At follow-up the regional difference in UIC had disappeared and both regions were classified as mildly iodine deficient. Correspondingly, regional differences in both thyroid volume (Paper I) and serum Tg (Paper II) had levelled out at follow-up. Contradictory results were found regarding thyroid nodules (paper III) where especially lower normalization rate of multiple nodules in Aalborg was evident. This may indicate that thyroid nodularity is more resistant to an increase in iodine intake compared with thyroid volume and serum Tg, and a certain degree of iodine memory may be in action.

At the follow-up investigation both regions were classified as mildly iodine deficient and median Tg among non-users of iodine containing supplements were higher than among iodine supplement users. These findings raise the question if a moderate increase in the level of iodine added to salt could be beneficial for the Danish population. A new cross-sectional investigation with participants from the same regions and within the same age and sex groups as in C1a and C2 would give valuable information on the Danish iodine status as would a schoolchildren investigation preferably performed in eastern Denmark, with the highest iodine content of drinking water.

The results of this thesis underline the importance of regional differences in iodine intake prior to an IF program where even relatively small differences in the iodine content of drinking water influenced on the individual response to IF. Furthermore, UIC were lower in our follow-up investigation compared with the cross-sectional investigation performed in 2004-2005 even though the level of iodine added to the household and industrial salt (13 µg/g) had not been changed. This stresses the importance of persistent monitoring to obtain sustainable iodine sufficiency since many factors can influence on the iodine intake in a population. Such factors may be changes in dietary habits (e.g. a lower intake of table salt) or in the iodine content of food items (e.g. a lower iodine content of dairy products).

Our study has no control group (no group without IF) and in principle no conclusions regarding causal relations between IF and individual changes in the investigated outcome measures can be drawn. However, no previous longitudinal investigation has baseline information before the implementation of an IF program. Therefore, this investigation gave unique insights into how an IF program influences the population at the level of individual inhabitants.

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SUMMARY

Iodine is an essential mineral for the synthesis of thyroid hormones. Low iodine intake levels are associated with goitre and other iodine deficiency-related disorders that have affected billions of people worldwide. Until recently the iodine intake of many European populations was below the recommended level and iodine fortification programs are internationally recommended to ensure sufficient iodine intake. Denmark was previously iodine deficient with regional differences and a mandatory iodine fortification of household salt and salt for commercial production of bread was introduced in the year 2000. A monitoring program entitled the Danish Investigation on Iodine Intake and Thyroid Diseases (DanThyr) was initiated before any iodization of salt had begun to improve knowledge on how to evaluate iodine status in a population and to study the effects of an increase in iodine intake. The aim of this PhD thesis was to evaluate individual changes in thyroid related variables associated with iodine fortification. We chose thyroid volume, thyroid nodularity and serum thyroglobulin as indicators of impact and evaluated possible changes in an 11-year follow-up investigation performed before and after the mandatory iodine fortification of salt in two regions with different iodine intake at baseline. The PhD thesis is a part of DanThyr.

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