Iodine fortification may influence the age-related change in thyroid volume: a longitudinal population-based study (DanThyr)

Anne Krejbjerg, Lena Bjergved1,2, Inge Bülow Pedersen, Allan Carlé, Torben Jørgensen1,3,4, Hans Perrild2, Lars Ovesen5, Lone Banke Rasmussen6, Nils Knudsen2 and Peter Laurberg

Department of Endocrinology, Aalborg University Hospital, Sdr. Skovvej 15, DK-9000 Aalborg, Denmark, 1Research Centre for Prevention and Health, The Capital Region of Denmark, Glostrup, Denmark, 2Department of Endocrinology and Gastroenterology, Bispebjerg Hospital, Copenhagen, Denmark, 3Faculty of Medicine, Aalborg University, Aalborg, Denmark, 4Faculty of Health Sciences, Copenhagen, Denmark, 5Department of Gastroenterology, Slagelse Hospital, Slagelse, Denmark and 6Department of Nutrition, National Food Institute, Technical University of Denmark, Søborg, Denmark

Correspondence should be addressed to A Krejbjerg
Email annekrejbjerg@rn.dk

Abstract

Objective: To assess the individuals’ thyroid volume changes after the mandatory nationwide iodine fortification (IF) program in two Danish areas with different iodine intake at baseline (Copenhagen, mild iodine deficiency (ID) and Aalborg, moderate ID).

Design: A longitudinal population-based study (DanThyr).

Methods: We examined 2465 adults before (1997) and after (2008) the Danish IF of salt (2000). Ultrasonography was carried out by the same sonographers using the same equipment, after controlling performances. Participants treated for thyroid disease were excluded from analyses.

Results: Overall, median thyroid volume had increased in Copenhagen (11.8–12.2 ml, \( P < 0.001 \)) and decreased in Aalborg, although not significantly (13.3–13.1 ml, \( P = 0.07 \)) during the 11 years of follow-up. In both regions, there was an age-related trend in individual changes in thyroid volume from baseline to follow-up; thyroid volume increased in women <40 years of age and decreased in women >40 years of age. In a multivariate regression model, higher age at entry was a predictor \( (P < 0.05) \) for thyroid volume decrease >20% during the follow-up period (women aged 40–45 years: odds ratio (OR) 4.3 (95% CI, 2.2–8.2); women aged 60–65 years: 5.8 (2.9–11.6)) and individuals of higher age were also less likely to have an increase in thyroid volume (women aged 40–45 years: OR 0.2 (0.1–0.3); women aged 60–65: OR 0.3 (0.2–0.4)).

Conclusions: Age-dependent differences in thyroid volume and enlargement had leveled out after the Danish iodization program. Thus, the previously observed increase in thyroid volume with age may have been caused by ID.

Introduction

Low dietary iodine may give rise to goiter and other iodine deficiency (ID)-related thyroid diseases (1, 2, 3). Until recently, the iodine intake of many European populations, including the Danish, was below the level recommended by the WHO (4). However, during the last 20 years, progress has been made in the elimination of ID and in 2004 almost 70% of households worldwide had access to iodized salt (5).

The iodine status in Denmark was evaluated in 1995 by a group of experts in nutrition and thyroid diseases (6). The group concluded that the iodine intake in Denmark should be increased by introducing iodized salt.
Furthermore, it was recommended that the iodization program should be accompanied by a monitoring program.

The monitoring program ‘The Danish investigation on iodine intake and thyroid diseases’ (DanThyr) was initiated in 1997 and in 2000 mandatory iodine fortification (IF) of salt was introduced with iodine added to a level of 13 μg/g. The program includes a number of cross-sectional and register studies (7). The first cross-sectional study (C1a) was performed in 1997–1998 before IF was implemented. The second cross-sectional study (C2) took place 4–5 years after mandatory iodization (2004–2005). The monitoring program was designed for a later follow-up of the C1a cohort after iodization, which was a unique possibility to observe the association between iodization and individual changes in thyroid volume.

A number of longitudinal studies on thyroid volumes in a population are available (8, 9, 10) and some studies have investigated thyroid volume in relation to differences in iodine intake (11, 12, 13) However, we know of no other longitudinal population-based studies with systematic follow-up information on thyroid volume before and after IF.

This study is an 11-year follow-up of the first cross-sectional DanThyr study (C1a). The main goal was to assess the individual thyroid volume changes after a mandatory nationwide IF program in two areas with different iodine intake at baseline, and to clarify the main predictors of these changes. Furthermore, we wanted to elucidate the relation between thyroid volume and aging. For this, we used both the longitudinal data and comparisons between age-associated differences in thyroid volume in the two population cohorts studied in the DanThyr program.

**Subjects and methods**

**Study population and design**

The first cross-sectional study (C1a) commenced in 1997 as a population surveillance conducted in two regions in Denmark with mild (Copenhagen, eastern Denmark) and moderate ID (Aalborg, western Denmark), representing the regional differences in iodine intake caused by different iodine content of the groundwater (14). The participants were selected in specific age and sex groups. Because of the high frequency of thyroid abnormalities among women, and the importance of adequate iodine intake during pregnancy, an overrepresentation of women was chosen for the study. The age groups represented women before the childbearing age (18–22 years), in the childbearing age (25–30 years) and after the childbearing age, and both pre- (40–45 years) and postmenopausal age (60–65 years). A group of men (60–65 years) was selected for comparisons between sexes. All subjects were identified in the civil registration system where all inhabitants of Denmark are registered by a unique ten-digit number. A computer program gave random numbers to the 40 233 subjects living in the selected areas, and 9274 subjects were drawn from the system to be invited for participation. A total of 4649 subjects (50.1%) participated: 2429 in Copenhagen and 2220 in Aalborg. The study is described in detail elsewhere (15), and all the procedures were similar in the current 11-year follow-up study.

A second cross-sectional study (C2) was carried out 4–5 years after iodization of salt (2004–2005). In this study, 3570 (46.6%) subjects participated, and the participants were randomly selected in the same two regions and in the same age groups as in Cohort 1a. The median urinary iodine excretion was 108 μg/l in Copenhagen and 93 μg/l in Aalborg, making Copenhagen iodine sufficient and Aalborg mildly iodine deficient (4). Details of the study have been published previously (13).

C1b was carried out from February 2008 to February 2010. Of the 4649 participants in the baseline C1a-study, 475 subjects were impossible to reach due to emigration (out of the country) or death, leaving 4174 to be invited for participation in our follow-up study (Fig. 1). In case of no response to the invitation, a letter with a further invitation was sent and if still no response, no further attempts were made to persuade the subjects to participate. The examinations took place at the ‘Centre for Prevention of Goitre and Thyroid Diseases’ at either Aalborg Hospital in Aalborg, western Denmark or Bispebjerg Hospital in the region of Copenhagen, eastern Denmark.

The two teams each comprised a physician and a sonographer who carried out the examinations. The participants answered health, food frequency and food supplements questionnaires, underwent physical examination, and gave blood and urine samples. Furthermore, a thyroid ultrasonography as well as an interview was carried out.

**Ultrasonography**

Both the sonographers and the ultrasonography apparatus (Sonoline Versa Pro 7.5 MHz 70 mm linear transducer, Siemens, Munich, Germany) were the same as used in the baseline study. The two sonographers’ comparability were
A Krejbjerg and others
Iodine fortification and thyroid volume

The Danish Investigation of Iodine Intake and Thyroid diseases (DanThyr) 1997–1998
Cohort 1a
n = 4649
Aalborg n = 2220
Copenhagen n = 2429

Deceased n = 403
Emigrated n = 72

Invited for 11 years follow-up
2008–2010
n = 4174
Aalborg n = 2039
Copenhagen n = 2135

No participation n = 1709

Full participation
Cohort 1b
n = 2465 (59.1%)
Aalborg n = 1229
Copenhagen n = 1236

Treated for thyroid disease n = 228
Missing values of thyroid volume or treatment for thyroid disease n = 29

Included in the analysis for thyroid volume
n = 2208
Aalborg n = 1087
Copenhagen n = 1121

The Danish Investigation of Iodine Intake and Thyroid diseases (DanThyr) 1997–1998
Cohort 1a
n = 4649
Aalborg n = 2220
Copenhagen n = 2429

Deceased n = 403
Emigrated n = 72

Invited for 11 years follow-up
2008–2010
n = 4174
Aalborg n = 2039
Copenhagen n = 2135

No participation n = 1709

Full participation
Cohort 1b
n = 2465 (59.1%)
Aalborg n = 1229
Copenhagen n = 1236

Treated for thyroid disease n = 228
Missing values of thyroid volume or treatment for thyroid disease n = 29

Included in the analysis for thyroid volume
n = 2208
Aalborg n = 1087
Copenhagen n = 1121

Figure 1
Flow diagram illustrates participants in the DanThyr Cohort 1.

Blood and urine samples were collected during the time interval from 0800 to 1730 h. Serum and urine samples were kept frozen (−20 °C) until study end and analyzed in random order with regard to age, sex, region, and time of examination (both baseline and follow-up).

The spot urine samples were analyzed for iodine concentrations (µg/l) by the Ce⁴⁺/As³⁺ method after digestion by alkaline ashing as described previously (17, 18). When a urine sample containing 93.9 µg/l was measured in triplicate in 18 assays, the intra-assay coefficient of variation (CV) for single determinations was 2.1% and the inter-assay CV was 2.7% (19). The iodine laboratory was certified by the U.S. Centers for Disease Control and Prevention’s EQUIP Program. Thyroid-stimulating hormone (TSH) was analyzed at baseline with LUMIfest assays (BRAHMS, Berlin, Germany). Thyroid peroxidase antibody (TPO-Ab) was measured by RIA (Dynotest anti-TPO, BRAHMS Diagnostica) as has been described in detail previously (20).

Definition of variables

The change in thyroid volume from baseline to follow-up was analyzed in three categories for each individual participant: i) no change in thyroid volume, ii) thyroid volume decrease, and iii) thyroid volume increase. The change in thyroid volume was defined as a more than 20% increase or decrease from the baseline to follow-up by ultrasonography. The interobserver variability in thyroid volume measurements by ultrasonography is around 10% (16).

The thyroid enlargement was defined as a thyroid volume exceeding 18 ml for women and 25 ml for men, which corresponds to the mean + 3 s.d. in iodine-sufficient populations (21). Hypoechogenicity was defined as markedly or slightly decreased echogenicity. Only thyroid nodules with a diameter of 10 mm or larger were included in the variable multinodularity at baseline.

The change in smoking status was categorized into four groups: i) current smokers at baseline and follow-up, ii) former smokers at baseline and follow-up, iii) participants who stopped smoking during follow-up, and iv) participants who had never smoked. The few subjects who started smoking during follow-up (n = 23) and occasional smokers (n = 256) were excluded from the analysis (22).

BMI was analyzed in three categories: i) BMI < 18.5 kg/m², ii) 18.5 kg/m² ≤ BMI < 25 kg/m², and iii) BMI ≥ 25 kg/m². TPO-Ab ≥ 30 kU/l was considered...
antibody positive (20). TSH was analyzed in three categories: i) TSH <0.4 mU/l, ii) 0.4 mU/l ≤ TSH ≤ 3.6 mU/l, and iii) TSH >3.6 mU/l (23).

Statistical analysis

All data processing was done with the STATA version 11.0 (Stata Corp., College Station, TX, USA). The non-parametric tests were used to compare thyroid volume at baseline with follow-up (Wilcoxon Signed-rank test) and in between the regions (Mann–Whitney U test), as the distribution of thyroid volumes was skewed toward higher volumes. Comparisons between frequencies were done using McNemars test for related samples and χ²-test for comparisons between regions. Two-sided P <0.05 was considered significant.

The participants previously treated for thyroid disease (current or previous treatment with medicine (n = 154), surgery (n = 51), or radioactive iodine therapy (n = 23)) at baseline or at follow-up and the participants with no information on treatment for thyroid disease or thyroid volume (n = 29) were excluded leaving 2208 subjects for the analysis.

To investigate the age-related effect on thyroid volume in the DanThyr cohorts C1a, C2, and C1b, with different iodine intake, we used a simple linear regression model, restricted to women aged 30–45 years and compared the slopes. In addition, two age intervals (age 30–32 and 40–42 years) present in each of the DanThyr cohorts were chosen for comparison between geometric means of the thyroid volume in each cohort with an independent two-sample t-test before and after stratifying by region. Predictors of thyroid volume change during the 11-year follow-up were identified in a multivariate logistic regression model and only women were included in this analysis. Interactions between relevant variables were investigated and significant interaction between age group and region was observed.

A separate multivariate logistic regression model was used to determine if sex was a predictor of thyroid volume change. This analysis was limited to subjects from 60 to 65 years of age at baseline. The model included: change in smoking status; familial disposition; and at entry: region, BMI, TSH, TPO-Ab; hypoechogenicity; thyroid enlargement; and multinodularity.

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) and the study was conducted in accordance with the Declaration of Helsinki. All participants gave written informed consent.

Results

Study population

The current study comprised 2465 participants (59.1% of the invited) and the mean follow-up time was 11.2 years (range: 10.1–12.8 years). Participants differed somewhat from the non-participants on some central variables as presented in detail in Table 1.

As might be expected, participants of C1a who deceased during the follow-up period also differed from participants in many of the central variables (Table 1). Smoking status, BMI, and presence of TPO-Ab at baseline remained significantly different between deceased and participants in a multivariate model adjusted for age.

The subjects excluded from the analysis because of treatment for thyroid disease (n = 228) differed significantly from participants in all the central ultrasonographic variables, family history, presence of TPO-Ab, and urinary iodine excretion (data not shown).

Iodine excretion

Urine samples from 2453 subjects (99.5%) were analyzed for iodine concentration. At the follow-up, median iodine excretion had increased from 53 to 83 μg/l in Aalborg and from 68 to 84 μg/l in Copenhagen (after exclusion of subjects taking individual iodine supplementation, this was from 45 to 76 μg/l and from 61 to 73 μg/l respectively). According to the criteria outlined by WHO (4), the Aalborg area had changed status from moderate to mild ID and the Copenhagen area still had mild ID despite an increase in median iodine excretion at follow-up.

Thyroid volume

Overall, median thyroid volume increased in Copenhagen during the 11 years of follow-up, whereas thyroid volume in Aalborg decreased, although not significantly (Table 2). Median thyroid volume in women <40 years of age at entry increased in both areas during follow-up (Copenhagen: from 9.8 to 11.6 ml, P <0.001; Aalborg: 10.7 to 12.0 ml, P <0.001). A different pattern was seen in women >40 years of age at entry, as thyroid volumes in Copenhagen were unaltered (12.7–12.8 ml, P = 0.28),
whereas a decrease was observed in Aalborg (14.3–12.9 ml, \(P < 0.001\)). After iodization, the overall median thyroid volume in Copenhagen remained significantly lower than in Aalborg.

In Fig. 2, the age and sex dependence of thyroid volume in the DanThyr cohorts are depicted. Higher values, in moderately iodine-deficient Aalborg, were apparent in all age groups in the baseline Cohort 1a study carried out before iodization. In the DanThyr Cohort 2 study (4–5 years after iodization), there was no regional difference in median thyroid volume in the age groups younger than 45 years, and again in this current follow-up study, Cohort 1b, a region-related difference was not observed in the youngest age groups. In the groups of men, a statistically significant difference was still observed, similar to the Cohort 1a investigation. On the other hand, the regional difference between elderly females was no longer statistically significant (\(P = 0.06\) (Fig. 2).

In a linear regression model restricted to women aged 30–45 years, thyroid volume difference per year of age was calculated for each cohort. A significantly lower thyroid volume increase per year of age was detected in C1b compared with C1a (C1b: –0.02 (CI –0.15 to 0.10), C1a: 0.22 (CI 0.12–0.32), \(P = 0.02\)). C2 was in between, and there was no significant difference in thyroid volume change per year of age when comparing C2 with C1a and C1b (data not shown).

In analyses limited to women aged 30–32 and 40–42 years, we compared geometric mean thyroid volume in participants of C1a, C2, and C1b. In C1a, thyroid volume was significantly higher among women aged 40–42 years compared with women aged 30–32 years (12.3/14.5 ml, \(P < 0.001\)), whereas no significant difference was observed in C2 (11.4/11.8 ml, \(P = 0.3\)) or in C1b (12.7/12.4 ml, \(P = 0.5\)). The same pattern was observed when stratifying by region; there was a higher thyroid volume among 40–42-year-old women compared with 30–32-year-old women in C1a, but no significant difference between the age groups in C2 and C1b (data not shown).

### Table 1: Baseline characteristics at the first examination (Cohort 1a) of participants in the follow-up study (Cohort 1b), non-participants, and people who deceased during the follow-up period. Data are presented \(n\) (%) and median (25th–75th percentiles). Comparisons between participants and non-participants were made using \(\chi^2\)-test for categorical variables and Mann–Whitney’s \(U\) test for medians of continuous variables.

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>Participants ((n=2465))</th>
<th>Non-participants ((n=1709))</th>
<th>(P^*)</th>
<th>Deceased during follow-up ((n=403))</th>
<th>(P^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women, 18–22</td>
<td>489 (19.8)</td>
<td>434 (25.4)</td>
<td>&lt;0.001*</td>
<td>2 (0.5)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Women, 25–30</td>
<td>514 (20.9)</td>
<td>391 (22.9)</td>
<td></td>
<td>7 (1.7)</td>
<td></td>
</tr>
<tr>
<td>Women, 40–45</td>
<td>657 (26.7)</td>
<td>237 (13.9)</td>
<td></td>
<td>24 (6.0)</td>
<td></td>
</tr>
<tr>
<td>Women, 60–65</td>
<td>381 (15.5)</td>
<td>366 (21.4)</td>
<td></td>
<td>142 (35.2)</td>
<td></td>
</tr>
<tr>
<td>Men, 60–65</td>
<td>424 (17.2)</td>
<td>281 (16.4)</td>
<td></td>
<td>228 (56.6)</td>
<td></td>
</tr>
<tr>
<td>Daily smokers</td>
<td>793 (32.2)</td>
<td>668 (39.1)</td>
<td>&lt;0.001*</td>
<td>238 (59.4)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>BMI (kg/m²) &lt;18.5</td>
<td>85 (3.5)</td>
<td>65 (3.8)</td>
<td>0.16</td>
<td>25 (6.2)</td>
<td></td>
</tr>
<tr>
<td>18.5–24.9</td>
<td>1439 (58.6)</td>
<td>947 (55.6)</td>
<td></td>
<td>133 (33.1)</td>
<td></td>
</tr>
<tr>
<td>≥25</td>
<td>933 (38.0)</td>
<td>692 (40.6)</td>
<td></td>
<td>244 (60.7)</td>
<td></td>
</tr>
<tr>
<td>Treated for thyroid disease</td>
<td>104 (4.2)</td>
<td>84 (4.9)</td>
<td>0.29</td>
<td>37 (9.2)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Family history of thyroid disease</td>
<td>507 (20.6)</td>
<td>309 (18.1)</td>
<td>0.05</td>
<td>70 (17.4)</td>
<td>0.14</td>
</tr>
<tr>
<td>TPO-Ab ≥30 kU/l</td>
<td>391 (16.1)</td>
<td>224 (13.4)</td>
<td>0.02</td>
<td>41 (10.4)</td>
<td>0.003*</td>
</tr>
<tr>
<td>TSH (mU/l) &lt;0.4</td>
<td>126 (5.2)</td>
<td>87 (5.2)</td>
<td>0.41</td>
<td>32 (8.1)</td>
<td></td>
</tr>
<tr>
<td>0.4–3.6</td>
<td>2183 (89.5)</td>
<td>1517 (90.4)</td>
<td></td>
<td>348 (87.7)</td>
<td></td>
</tr>
<tr>
<td>&gt;3.6</td>
<td>130 (5.3)</td>
<td>74 (4.4)</td>
<td></td>
<td>17 (4.3)</td>
<td></td>
</tr>
<tr>
<td>Thyroid volume (ml)</td>
<td>12.6 (9.6–17.4)</td>
<td>12.2 (9.2–16.6)</td>
<td>0.01</td>
<td>15.7 (11.3–22.1)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Thyroid enlargement (&gt;18/25 ml)</td>
<td>468 (15.0)</td>
<td>282 (16.6)</td>
<td>0.04</td>
<td>112 (27.8)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Multinodularity</td>
<td>279 (11.3)</td>
<td>199 (11.7)</td>
<td>0.73</td>
<td>88 (21.8)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Urinary iodine excretion (µg/l)</td>
<td>Including subjects taking iodine supplements</td>
<td>61 (34–101)</td>
<td>0.26</td>
<td>54 (32–93)</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Excluding subjects taking iodine supplements</td>
<td>52 (29–80)</td>
<td>0.03</td>
<td>44 (26–73)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

\(^*P<0.05\) in multivariate model including all variables with \(P<0.05\) in univariate model.

\(^*P\) values for comparisons between participants and non-participants.

\(^*P\) values for comparisons between participants and deceased during the follow-up period.
Thyroid enlargement
In both regions there was an age-related trend in thyroid enlargement; however, this was not significant for all groups. In men younger than 40 years, the frequency of thyroid enlargement at follow-up was higher than in men older than 40 years. Overall, women had a higher frequency of thyroid enlargement at follow-up, whereas men younger than 40 years and women older than 40 years had a higher frequency of thyroid enlargement at follow-up.

Predictors of thyroid volume change
Higher age, at entry, was a predictor for thyroid volume change. Higher age at entry was associated with an increase in thyroid volume. In a multivariate logistic regression model restricted to men and women aged 60–65 years, no significant association was found between sex and change in thyroid volume (data not shown).

In women, having change from moderate to mild ID (Aalborg) was a predictor of individual thyroid volume increase. In men with thyroid multinodularity at baseline, there was a significantly higher incidence of thyroid enlargement during the follow-up period and individuals with thyroid multinodularity at baseline were more likely to have an increase in thyroid volume. This association was found in a multivariate logistic regression model and confirmed in a univariate logistic regression model (Table 4).

In men, there was an age-related trend in thyroid volume; however, this was not significant for all groups. There were significant differences in the prevalence of thyroid volume change between the two regions. Thyroid enlargement was more frequent in Aalborg than in Copenhagen among the youngest and oldest women. However, no difference in incidence rates between the two regions was found in men (incidence ratio 1.3 (95% CI 0.9–2.0)).

In women younger than 40 years, the frequency of thyroid enlargement at follow-up was higher than in women older than 40 years. Overall, women had a higher frequency of thyroid enlargement at follow-up, whereas men younger than 40 years and women older than 40 years had a higher frequency of thyroid enlargement at follow-up.
introduction of mandatory IF of salt in Denmark (13 μg/g) and 11.2 years after its first examination. Based on participants’ median urinary iodine excretion, Aalborg had changed its status from moderate to mild ID, whereas Copenhagen has remained mildly iodine deficient despite an increase in the median iodine excretion at follow-up. In the 11-year follow-up period, the median thyroid volume had increased in Copenhagen and decreased in Aalborg. This reduced the region-related difference in thyroid volume compared with baseline data, although the difference remained significant. Both regions witnessed an age-related trend with a thyroid volume increase among women younger than 40 years and a decrease among women above 40 years of age. The change in thyroid volume per year of age in women aged 30–45 years was found to be smaller in the DanThyr Cohort 1b than in Cohort 1a. The frequency of thyroid enlargement was overall higher at follow-up than at baseline, and somewhat unexpectedly this was most pronounced among women in the youngest age group.

Higher age and thyroid enlargement at baseline were predictors for an individual thyroid volume decrease among women during the follow-up period. In addition, women with multinodularity at baseline and those who had a family history of thyroid disease were less likely to experience a decrease in their thyroid volume during follow-up.

Iodine and thyroid volume

The relation between iodine intake and thyroid volume has been described in a number of previous studies carried out in areas with ID. Most of these studies were cross-sectional and reported an inverse relationship between iodine intake and thyroid volume, as well as a lower frequency of thyroid enlargement after IF (12, 13, 15). However, these studies were not designed to evaluate the effect of iodine at the level of the individual. Only very few longitudinal studies investigated the thyroid volume in relation to IF (10, 24), but they did not obtain baseline data before IF. A longitudinal study, with 5 years of follow-up, was conducted in three regions in China with different regional iodine intakes; mildly deficient iodine, more than adequate iodine and excessive iodine (24). The study was initiated 3 years after the introduction of IF of salt and reported an iodine-related decrease in goiter prevalence between the three regions. This is consistent with the results of this study, where both baseline and follow-up data showed an overall higher frequency of thyroid enlargement in Aalborg (moderate ID at baseline) than

Figure 2
Median thyroid volume (ml) by region in the DanThyr Cohorts: C1a (n = 4649), C2 (n = 3570), and C1b (n = 2465). Note that participants in the C1b cohort were on average 11.2 years older. The subjects treated for thyroid disease were excluded (C1a: n = 228, C2: n = 192, and C1b: n = 228). Data were missing for 12 subjects in C1a, 11 in C2, and 29 in C1b. To assist visualization, graphical lines have been added between various age groups of women for each region.
in Copenhagen (mild ID at baseline). However, at follow-
up where difference in iodine excretion between the two
regions was less pronounced, regional differences in
thyroid volume were only evident in the youngest and
the oldest age groups of women and in the group of men.
Yu et al. (24) detected a higher incidence of diffuse goiter
in the regions with mildly deficient and excessive iodine
intake, than in the region with adequate iodine intake.
This relation was not detected in our study, where the
regional difference in iodine excretion had practically
disappeared after IF.

Overall, we found a different pattern of thyroid
volume change between the regions, with a median
thyroid volume decrease in Aalborg (although not
statistically significant) but increase in Copenhagen.
Before iodization of salt, the population in Aalborg
suffered from moderate ID, whereas Copenhagen was
only mildly deficient. Thus, the explanation for the
difference in change of thyroid volume could be that IF
had the prime effect on individuals exposed to the highest
level of ID. This may explain the decrease in median
thyroid volume in Aalborg despite participants getting 11
years older, in contrast to Copenhagen where the level of
iodine intake was always higher.

Thyroid baseline enlargement predicted a volume
decrease during follow-up, whereas baseline multinodu-
ularity predicted a volume increase. These conflicting
results may indicate that the effect of IF on thyroid
volume is limited in individuals with multiple nodules, or
that a longer observation time was necessary to determine
effect, keeping in mind that regression toward the mean
(25) might explain some of the volume decrease seen in
individuals with thyroid enlargement at baseline.

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Formerly mild ID (Copenhagen)</th>
<th>Formerly moderate ID (Aalborg)</th>
<th>Copenhagen vs Aalborg, after iodization, P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
<td>Before iodization n=1121 (%)</td>
<td>After iodization n=1121 (%)</td>
<td></td>
</tr>
<tr>
<td>Women (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18–22</td>
<td>2.1</td>
<td>7.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>25–30</td>
<td>4.5</td>
<td>9.5</td>
<td>0.01</td>
</tr>
<tr>
<td>40–45</td>
<td>26.5</td>
<td>21.8</td>
<td>0.06</td>
</tr>
<tr>
<td>60–65</td>
<td>19.2</td>
<td>15.3</td>
<td>0.48</td>
</tr>
<tr>
<td>Men, 60–65 years</td>
<td>15.9</td>
<td>9.2</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Before iodization n=1087 (%)</td>
<td>After iodization n=1087 (%)</td>
<td></td>
</tr>
<tr>
<td>Women (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18–22</td>
<td>8.5</td>
<td>17.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>25–30</td>
<td>9.4</td>
<td>13.9</td>
<td>0.02</td>
</tr>
<tr>
<td>40–45</td>
<td>28.2</td>
<td>26.3</td>
<td>0.44</td>
</tr>
<tr>
<td>60–65</td>
<td>29.7</td>
<td>25.4</td>
<td>0.19</td>
</tr>
<tr>
<td>Men, 60–65 years</td>
<td>29.0</td>
<td>20.1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Values are age at baseline. After iodization participants were on average 11.2 years older.

### Age and thyroid volume

In this study, participants were on average 11.2 years older
at follow-up than at baseline, and both the higher iodine
intake and the higher age may have affected thyroid
volume. Several studies conducted in iodine-deficient
countries, including the present baseline study (15),
found an increased thyroid weight and volume with age
(26, 27). Thus, the increase in thyroid volume observed in
the youngest participants of our study could be explained
by the fact that they had become 11 years older. However,
the oldest age groups had a decrease in thyroid volume
during follow-up despite getting older. Correspondingly,
thyroid enlargement was more frequent after iodization
among the youngest age groups and less frequent among
the oldest age groups.

In contrast to ID areas, some studies carried out in
iodine-sufficient countries have reported that thyroid
volume decreased with age (28, 29). To investigate the
association between age and thyroid volume in relation
to iodine intake further, we compared age-dependent
differences in thyroid volume in the DanThyr cohorts C1a,
C2, and C1b. The increase in thyroid volume with
aging was lower in C1b (median iodine excretion:
83 µg/l) than in C1a (median iodine excretion: 61 µg/l).
This outcome was corroborated by our results from the
analysis on 30–32- and 40–42-year-old women in C1a, C2,
and C1b. Here, we found a higher thyroid volume
in women aged 40–42 years compared with women aged
30–32 years in C1a but not in C2 or C1b. Our results
indicate that the age-related changes in thyroid volume
in adults may be dependent on iodine intake. Thus, in
iodine-deficient countries thyroid volume may increase

www.eje-online.org
with aging whereas volume may be stable or even decrease with aging in iodine-sufficient countries. As depicted in Fig. 2, such a trend was apparent for both regions when comparing C1a, C2, and C1b, and such a difference in age dependence was also in accordance with our results from the multivariate analysis.

**Strengths and limitations**

The 59.1% rate of participation introduces a possible selection bias, and based on baseline characteristics, the participants and non-participants differed on some central variables. However, in a multivariate model adjusting for age, only smoking status and the presence of TPO-Ab remained significantly different between the two groups.

The strength of the study was the prospectively planned longitudinal design with investigation of participants both before and after mandatory IF of salt. Moreover, all procedures in the baseline and at the follow-up study were similar and ultrasonography was carried out by the same two sonographers on the same apparatus at both baseline and follow-up. A complicating factor in the interpretation of data is that iodine intake had changed (but it was still somewhat low) and in addition all participants in the follow-up study had become 11 years older. The ideal way to study the age-related effect would

---

**Table 4** Predictors of thyroid volume change (±20%) by ultrasonography at 11-year follow-up (n=1526). The DanThyr Cohort 1 was examined at baseline (1997–1998) and re-examined at follow-up 11 years later (2008–2010). Subjects treated for thyroid disease (n=228), subjects with a missing value of treated for thyroid disease (n=22), subjects with no thyroid volume measurement (n=7), and men (n=403) were excluded from the analysis. Multivariate logistic regression model with change in thyroid volume from baseline to follow-up as the dependent variable (>20% decrease, >20% increase and no change in thyroid volume as the reference category). The model included change in smoking status and at baseline: age, region (proxy for iodine intake), TPO-Ab status, ultrasound hypoechogenicity, thyroid enlargement, multinodularity, and familial disposition as independent variables. In this model, no attempts were made to distinguish between confounding and effect modifying variables (30).

<table>
<thead>
<tr>
<th>predictor</th>
<th>thyroid volume decrease n</th>
<th>odds ratio (95% CI)</th>
<th>p</th>
<th>thyroid volume increase n</th>
<th>odds ratio (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agea (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women, 18–22</td>
<td>379</td>
<td>1.00 (ref.)</td>
<td>1.00 (ref.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women, 25–30</td>
<td>394</td>
<td>1.84 (0.91, 3.70)</td>
<td>0.09</td>
<td>0.42 (0.31, 0.58)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Women, 40–45</td>
<td>508</td>
<td>4.28 (2.24, 8.17)</td>
<td>&lt;0.001</td>
<td>0.20 (0.13, 0.29)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Women, 60–65</td>
<td>245</td>
<td>5.78 (2.89, 11.56)</td>
<td>&lt;0.001</td>
<td>0.28 (0.17, 0.44)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Region</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aalborg</td>
<td>721</td>
<td>1.71 (1.23, 2.37)</td>
<td>0.001</td>
<td>0.78 (0.60, 1.01)</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Copenhagen</td>
<td>805</td>
<td>1.00 (ref.)</td>
<td>1.00 (ref.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI at baseline (kg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;18.5</td>
<td>69</td>
<td>1.42 (0.63, 2.30)</td>
<td>0.39</td>
<td>1.14 (0.63, 2.05)</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>18.5–24.9</td>
<td>985</td>
<td>1.00 (ref.)</td>
<td>1.00 (ref.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥25</td>
<td>468</td>
<td>1.26 (0.90, 1.78)</td>
<td>0.18</td>
<td>0.81 (0.59, 1.10)</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>TSH at baseline (mU/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.4</td>
<td>57</td>
<td>0.52 (0.21, 1.27)</td>
<td>0.15</td>
<td>0.70 (0.33, 1.49)</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>0.4–3.6</td>
<td>1391</td>
<td>1.00 (ref.)</td>
<td>1.00 (ref.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;3.6</td>
<td>58</td>
<td>0.79 (0.32, 1.94)</td>
<td>0.61</td>
<td>1.33 (0.70, 2.54)</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>Hypoechogenicity (ultrasonography at baseline)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>99</td>
<td>0.75 (0.40, 1.40)</td>
<td>0.37</td>
<td>0.61 (0.32, 1.17)</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1426</td>
<td>1.00 (ref.)</td>
<td>1.00 (ref.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroid enlargement at baseline (volume &gt;18/25 ml by ultrasonography)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>246</td>
<td>2.01 (1.34, 3.02)</td>
<td>0.001</td>
<td>0.62 (0.38, 1.01)</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1280</td>
<td>1.00 (ref.)</td>
<td>1.00 (ref.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multinodularity at baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>150</td>
<td>0.48 (0.28, 0.85)</td>
<td>0.01</td>
<td>1.72 (1.02, 2.91)</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>No (including solitary nodules)</td>
<td>1376</td>
<td>1.00 (ref.)</td>
<td>1.00 (ref.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Familial history of thyroid disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>321</td>
<td>0.60 (0.39, 0.92)</td>
<td>0.02</td>
<td>0.99 (0.72, 1.35)</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1205</td>
<td>1.00 (ref.)</td>
<td>1.00 (ref.)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a*Age at baseline.
be to assess thyroid volume according to age in an area with iodine sufficiency throughout the lifetime of the inhabitants.

To compare thyroid volume change per year of age between the different DanThyr cohorts, we used linear regression even though the age of our participants was not equally distributed throughout the timeframe used. This might bias our results. However, similar trends were apparent when looking at the two different regions in each cohort.

We have follow-up data on a relatively large cohort, but we cannot generalize our results to the entire population because the study was primarily based on women in specific age strata.

After the initiation of the mandatory iodization of table salt and bread salt in the year 2000 there has been a clear increase in urinary iodine excretion in the population. However, to prevent side effects to a sudden abrupt increase in iodine intake, the iodization was very cautious (7) and the population is still mildly iodine deficient according to WHO criteria (4). Possibly, even larger changes in thyroid volume and goiter frequency would have been observed if the iodization program had been more intensive.

Conclusions
Age-dependent differences in thyroid volume and enlargement had leveled out after the Danish iodization program. Thus, the previously observed increase in thyroid volume with age may have been caused by ID.

At the level of the individual, participants with thyroid enlargement tended to normalize thyroid volume after the iodization program. This was most pronounced in the cohort with the lowest iodine intake at baseline. On the other hand, thyroid multinodularity at baseline predicted that thyroid enlargement would not disappear after the increase in iodine intake. Probably, it takes a generation before the full benefits of the iodization program are in effect.

Acknowledgements
The authors thank Ingelise Leegaard and René Fiege for carefully performing the ultrasonographies and the laboratory work.

References


18 Laurberg P. Thyroxine and 3,5,3'-triiodothyronine content of thyroglobulin in thyroid needle aspirates in hyperthyroidism and hypothyroidism. Journal of Clinical Endocrinology and Metabolism 1987 64 969–974. (doi:10.1210/jcem-64-5-969)


