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Thyroid nodules in an eleven-year DanThyr follow-up study

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Key terms: thyroid, thyroid nodules, ultrasonography, prospective, follow-up, iodine, food fortification.

Context: Limited longitudinal data are available on changes in the thyroid gland structure in a population and how this is influenced by iodine fortification (IF).

Objective: To clarify how IF influenced thyroid gland structure in two regions with different iodine intake at baseline (Copenhagen, mild iodine deficiency (ID); Aalborg, moderate ID).

Design and Setting: A longitudinal population-based study (DanThyr) where participants were examined before (1997) and after (2008) the Danish mandatory IF of salt (2000).

Participants: We examined 2,465 adults and ultrasonography was performed by the same sonographers using the same equipment, after controlling performances.

Main Outcome Measure: Change in thyroid gland structure.

Results: The follow-up period saw an increased prevalence of multinodularity (9.8–13.8 %, $p<0.001$), especially in the previously moderate ID region of Aalborg (9.1 -15.4 %, $p<0.001$), whereas no change in prevalence was seen for solitary nodules (5.6- 5.1 %, $p=0.34$).

In individual participants, changes in thyroid structure and disappearance of thyroid nodules during the 11 years was common with an overall normalization rate of 21.2 (95% CI 17.9–24.9) per 1000 person-years. Solitary nodules had a significantly higher normalization rate than multiple nodules (normalization rate ratio 0.47 (CI 0.32–0.67). Regional difference (Aalborg vs. Copenhagen) was seen between normalization rates of multiple nodules (normalization rate ratio 0.29 (CI 0.12–0.64)), but not for solitary nodules (normalization rate ratio 0.81 (CI 0.53–0.1.21)).

Conclusions: Changes in the thyroid gland structure with both appearance and disappearance of thyroid nodules are common after an iodization program.

Worldwide, iodine deficiency (ID) has existed for centuries (1), giving rise to thyroid nodules, goitre and other ID-related disorders (2–4). During the last decades iodine fortification (IF) programs have been initiated to prevent ID-related disorders and 70% of households worldwide had in 2004 access to iodized salt (5).

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Abbreviations:

The relation between iodine intake and the occurrence of thyroid nodules has been the focus of many previous studies (6). However, these investigations were cross-sectional and no information about how iodine affects the natural history of thyroid nodules could be obtained. Only two previous longitudinal studies have investigated the association between iodine and thyroid nodules (7, 8) and these studies did not have baseline data before IF. Thus, IF programs are initiated to prevent the development of ID-related disorders including thyroid nodules, but knowledge on how already existing thyroid nodules develop after IF is sparse.

Our study is a prospectively planned 11-year follow-up where baseline data were obtained before IF in two regions of Denmark with different iodine intake levels. The study is a part of DanThyr "The Danish investigation of iodine intake and thyroid diseases" (9) and follow-up was carried out 8.6 years after the initiation of mandatory IF of salt in Denmark (year 2000). The main goals of our study were to assess the changes in thyroid gland structure at 11-years follow-up after the introduction of a mandatory nationwide IF program, and to clarify how IF had influenced the natural history of thyroid nodules in two regions with different iodine intake at baseline (Copenhagen, mild ID and Aalborg, moderate ID).

Subjects and Methods

Design and study population

The mandatory IF of salt in Denmark was initiated in year 2000 and was accompanied by a monitoring program, DanThyr, described in detail previously (9). One of the cohorts in DanThyr was planned as a follow-up investigation with a baseline cross-sectional study in 1997–1998 before IF (10) and a follow-up study after IF (11). The follow-up examinations took place in 2008–2010, and the present study includes data from this 11-year follow-up investigation. Participants were initially chosen at random by a computer program using the civil registration system where all inhabitants of Denmark are registered by a unique 10-digit number. Subjects in specific age and sex groups (women 18–22, 25–30, 40–45 and 60–65 years and men 60–65 years) were chosen from two different regions of Denmark; Copenhagen (eastern Denmark) with mild ID and Aalborg (western Denmark) with moderate ID according to the World Health Organization criteria (1). 4,649 subjects (50.1% of those invited) participated in the baseline study, 2,429 in Copenhagen and 2,220 in Aalborg (10).

The follow-up investigation 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). All participants from the baseline study were identified, 4,174 subjects were invited for participation (72 subjects had emigrated and 403 subjects had died) and 2465 subjects participated (59.1%) (Figure 1). All examinations took place at the Centre for Prevention of Goitre and Thyroid Diseases at either Aalborg Hospital in Aalborg or Bispebjerg Hospital in the region of Copenhagen. Examinations were performed by two teams each including a physician and a sonographer. The participants answered health, food frequency and food supplements questionnaires and gave blood and urine samples. Furthermore, a thyroid ultrasonography (US), a physical examination and an interview was performed. Because of the planned follow-up design, all procedures were similar in the baseline and in the follow-up study.

At follow-up, Aalborg had changed iodine status from moderate to mild ID (median urinary iodine concentration had changed from 53 to 83 μg/l) whereas Copenhagen still had mild ID despite an increase in median iodine excretion (from 68 to 84 μg/l) (12).

Ultrasonography

All ultrasonography (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) at baseline and at follow-up. Interobserver agreement was studied before the baseline study (13) and a confirmatory interobservational study was performed before the follow-up study showing continued good correlation (thyroid volume, $r = 0.95$, $P < .001$).

Thyroid volume was calculated as maximal length*depth*width*Π/6 for each lobe (13). Thyroid nodules ≥ 5 mm were registered and in the event of more than three nodules in one lobe, only the three largest nodules were registered. The thyroid gland structure was defined as diffuse when no nodules were registered, solitary nodule when one nodule was registered and multinodular when more than one nodule was registered. In accordance with the protocol approved by the Ethics Committee participants with solitary nodules ≥ 20 mm was referred to a ^{99m}Tc-pertechnetate scintigraphy with test response to their general practitioner (GP), moreover these patients were advised to contact their GP for test response and further follow-up. Thus, no clinical follow-up with fine needle aspiration biopsy or other measures were performed by us. At the reinvestigation, none of these patients with solitary nodules ≥ 20 mm had subsequently undergone surgery.

Laboratory procedures

Blood and urine samples were collected in the time interval between 8.00 a.m. and 5.30 p.m. Samples were kept frozen (-20 C) and analyzed in random order. Iodine concentrations (μg/liter) were analyzed in the spot urine samples by the Ce^{4+}/As^{3+} method after digestion by alkaline ashing as described previously (14, 15). The analytical sensitivity was 2 μg/liter and the iodine laboratory is certified by the U.S. Center for Disease Control and Prevention's EQUIP Program. TSH was analyzed at baseline with LUMItest assays (BRAHMS, Berlin, Germany). Thyroid peroxidase antibody (TPO-Ab) was measured by radioimmunoassay (RIA) (RIA; Dynotest anti-TPO, BRAHMS Diagnostica, Berlin, Germany) (16).

Variable definitions

Thyroid nodules included partly cystic nodules and solid nodules, but not thin-walled cysts. Nodule size was defined as the largest diameter on longitudinal view by US, and in case of multiple nodules, the diameter of the largest nodule was used. Thyroid enlargement was defined as a thyroid volume exceeding 18 ml for women and 25 ml for men, corresponding to the mean +

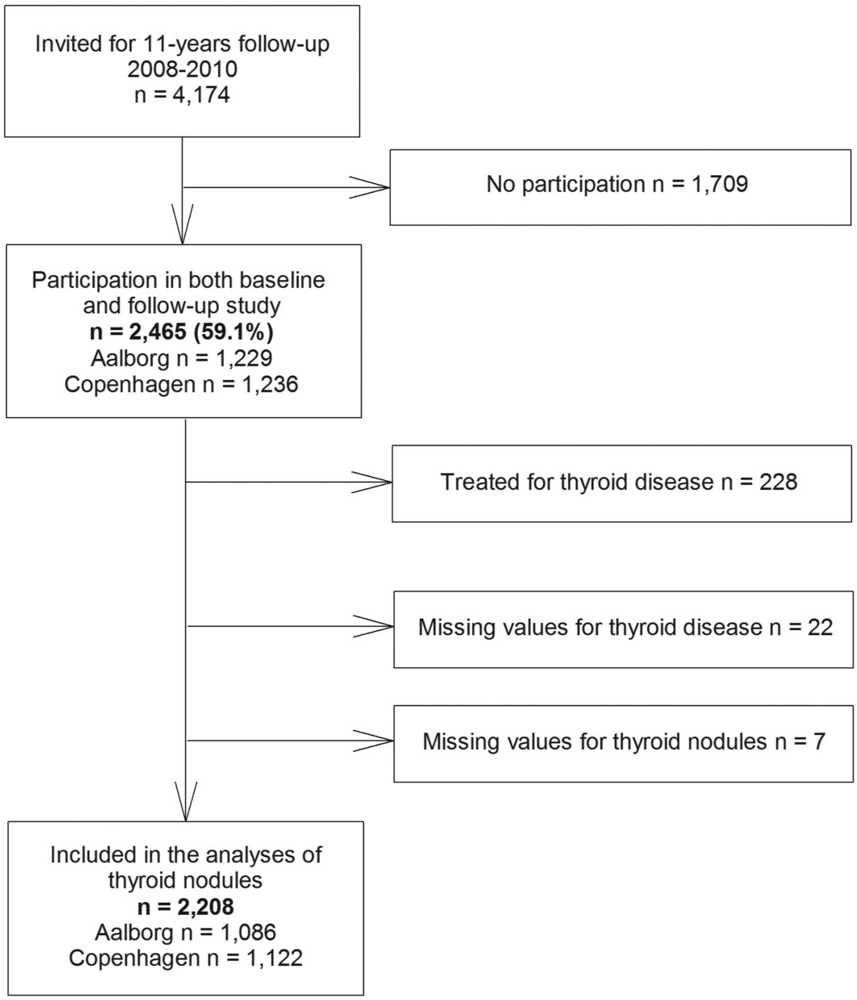

Figure 1. Flowchart explaining the number of participants included in the final study population.

3 SD in iodine sufficient populations (17). BMI was analyzed in three categories: I) BMI < 18.5 kg/m^2, II) 18.5 kg/m$^2 \leq$BMI < 25 kg/m^2 and III) BMI $\geq$ 25 kg/m^2. TPO-Ab $\geq$ 30 kU/liter was considered antibody positive (16). TSH was analyzed in three categories: 1) TSH $<$ 0.4 mU/liter, 2) 0.4 mU/liter$\leq$ TSH $\leq$ 3.6 mU/liter, 3) TSH $>$ 3.6 mU/liter (18).

Statistical Analysis

All data processing was done with the STATA version 11.0 (Stata Corp., College Station, Texas, USA). Comparisons were made using χ^2 test for categorical variables and McNemars or Adjusted Wald test for related categorical variables. Comparisons between two groups were made with Mann-Whitney´s U test for medians of continuous variables and Kruskal-Wallis test was used for comparisons between multiple groups. Two-sided $P < .05$ was considered significant.

Possible selection bias in follow-up was evaluated by a multivariate logistic regression model including participation of invited subjects as the dependent variable and central baseline characteristics as potential predictors. We computed inverse probability weights to account for missing at random, calculated weighted prevalence data and used these weights in our regression models.

Participants previously treated for thyroid disease (current or previous treatment with medicine, surgery or radioactive iodine therapy) at baseline or at follow-up (n = 228) and participants with no information on treatment for thyroid disease or thyroid nodules (n = 29), were excluded leaving 2208 subjects for the analysis (Figure 1).

For the analysis of age dependent prevalence of thyroid nodules before and after IF we considered the participants around 40 years of age at follow-up and at baseline as two independent cohorts and used χ^2 test to compare the prevalences.

To calculate the incidence rates of both solitary and multiple nodules, we excluded subjects with nodules ($>$5 mm) at baseline leaving 1,590 subjects for the analyses. Incidence rates (IR) per 1000 person years (py) with 95% confidence interval (CI) and IR ratios were calculated according to standard procedures (19). For calculation of normalization rates, we selected the participants with either solitary or multiple nodules ($>$5 mm) at baseline for further analyses (n = 618).

Multivariate logistic regression models were used to determine if age, region (proxy for iodine intake), nodule size, TSH or thyroid enlargement at baseline were predictors of nodule incidence or nodule disappearance. Only women were included in these analyses. Separate logistic regression analyses, limited to participants from the age of 60–65 years at inclusion, were made to decide whether sex was a predictor of thyroid nodule incidence or disappearance.

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.

Results

Study population

As depicted in Table 1, participation in the follow-up study was predicted by a few central baseline characteristics; age 40–45 years and male sex were predictors of participation, whereas daily smoking and high BMI predicted nonparticipation.

Thyroid nodule prevalence

The prevalences of thyroid nodules at baseline and at follow-up are presented in Table 2. Overall, the follow-up

Table 1. Predictors of participation in the follow-up study among invited subjects ($n = 4,174$). Multivariate logistic regression models, with participation in the follow-up study as dependent variable and with central baseline characteristics as independent variables.

		Participation	
	n	Odds ratio (95% CI)	p
Age groups			
Women, 18–22 yr	923	1.00 (ref.)	
Women, 25–30 yr	905	1.12 (0.93–1.36)	0.23
Women, 40–45 yr	894	2.46 (1.99–3.05)	<0.001
Women, 60–65 yr	747	0.89 (0.71–1.12)	0.33
Sex			
Women, 60–65 yr	747	1.00 (ref.)	
Men, 60–65 yr	705	1.49 (1.17–1.90)	0.001
Region			
Copenhagen	2,135	1.00 (ref.)	
Aalborg	2,039	1.08 (0.95–1.23)	0.25
Daily smokers			
Yes	1,461	0.65 (0.57–0.75)	<0.001
No	2,710	1.00 (ref.)	
Body mass index			
<18.5 kg/m^2	150	0.93 (0.66–1.31)	0.68
18.5–24.9 kg/m^2	2,386	1.00 (ref.)	
≥ 25 kg/m^2	1,625	0.85 (0.74–0.98)	0.03
Family history of thyroid disease			
Yes	816	1.13 (0.96–1.33)	0.14
No	3,358	1.00 (ref.)	
Treated for thyroid disease			
Yes	188	0.92 (0.67–1.28)	0.63
No	3,986	1.00 (ref.)	
TPO-Ab ≥ 30 kU/liter			
Yes	615	1.21 (1.00–1.46)	0.05
No	3,480	1.00 (ref.)	
Thyroid enlargement (>18/25 ml)			
Yes	924	1.12 (0.93–1.34)	0.23
No	3,243	1.00 (ref.)	
Thyroid nodularity			
No nodules	2,967	1.00 (ref.)	
Solitary nodule	568	1.07 (0.88–1.31)	0.49
Multiple nodules	632	0.98 (0.79–1.20)	0.83

period saw an increased prevalence of multiple nodules. This increase was mainly caused by an increased prevalence in Aalborg (previously moderate ID), present in all age groups except the youngest. Minor changes were observed among solitary nodules where only women aged 25–45 years at baseline had an increased prevalence during the eleven years. After stratifying by region, this increase was only significant in Aalborg among women aged 25–30 years (Table 2).

To illustrate the age-dependent changes in prevalence before and after IF, we depicted the occurrence of solitary and multiple nodules among women according to their age at examination (Figure 2). When we considered the participants aged 40 years at the first investigation (1997–1998) and those aged 40 years at the second investigation (2008–2010) as two independent cohorts, and compared the prevalences of nodules, both solitary ($P = .004$) and multiple nodules ($P < .001$) were more frequent at the first investigation compared to the second investigation. This difference appeared to continue in the older age groups of women with solitary nodules, but resolved in women with multiple nodules.

Thyroid nodule incidence

Of the 1,590 subjects with diffuse structure at baseline, a total of 132 (8.3%) had a new finding of a solitary nodule; incidence rate 7.4 (CI 6.2–8.7) per 1000 person years (py) and a total of 96 subjects (6.0%) had a new finding of multiple nodules; incidence rate 5.3 (CI 4.3–6.5) per 1000 py. Incidences were stratified by region and depicted in Figure 3. There was an overall difference in incidence rates between the two regions with higher incidences in Aalborg than in Copenhagen. However, this regional difference was only of borderline significance after stratifying by nodularity (Figure 3).

We used a multivariate logistic regression model to investigate possible predictors for the development of thyroid nodules and found high age (60–65 years: OR 5.27 (CI 2.76–10.05), $P < .001$ with 18–22 years as reference) as well as thyroid enlargement at baseline (OR 2.68 (CI 1.65–4.36), $P < .001$) to be predictors of nodule development. In similar analyses restricted to women and men aged 60–65 years old no significant association was found between sex and the development of thyroid nodules.

Table 2. Prevalence (%) of thyroid nodules (≥10 mm) in two areas with formerly mild (Copenhagen) and formerly moderate ID (Aalborg), at *baseline* (1997–1998) before mandatory IF (2000) and at *11-years follow-up* (2008–2010).

		All			
Group*	N	Solitary (%) *Baseline / follow-up*	*P*	Multinodular (%) *Baseline / follow-up*	*P*
Women					
18–22 yr	460	0.6/ 1.5	0.20	0.7 / 2.0	0.06
25–30 yr	480	1.9 / 4.8	0.01	2.3 / 6.5	<0.001
40–45 yr	580	9.1 / 5.9	0.01	15.9 / 21.9	<0.001
60–65 yr	286	10.4 / 6.9	0.07	23.3 / 29.5	0.02
Men, 60–65 yr	402	8.6 / 7.6	0.49	12.6 / 15.7	0.07
Total	2,208	5.6 / 5.1	0.34	9.8 / 13.8	<0.001
		Formerly *mild* ID (Copenhagen)			
Group*	n	Solitary (%) *Baseline / follow-up*	*P*	Multinodular (%) *Baseline / follow-up*	*P*
Women					
18–22 yr	236	1.2 / 1.0	0.68	1.2 / 2.0	0.51
25–30 yr	246	2.3 / 4.0	0.26	3.0 / 6.4	0.01
40–45 yr	310	7.8 / 5.0	0.08	18.6 / 22.8	0.07
60–65 yr	142	6.9 / 3.8	0.19	24.0 / 24.5	0.90
Men, 60–65 yr	188	8.4 / 7.6	0.73	11.6 / 11.0	0.81
Total	1,122	4.9 / 4.0	0.23	10.5 / 12.4	0.06
		Formerly *moderate* ID (Aalborg)			
Group*	n	Solitary (%) *Baseline / follow-up*	*P*	Multinodular (%) *Baseline / follow-up*	*P*
Women					
18–22 yr	224	0.0 / 2.2	0.06	0.0 / 2.1	0.06
25–30 yr	234	1.4 / 5.6	0.02	1.6 / 6.6	0.003
40–45 yr	270	10.5 / 6.9	0.05	12.8 / 20.8	<0.001
60–65 yr	144	14.0 / 10.2	0.20	22.6 / 34.6	<0.001
Men, 60–65 yr	214	8.9 / 7.5	0.54	13.6 / 20.1	0.01
Total	1,086	6.3 / 6.2	0.86	9.1 / 15.4	<0.001

Data represent prevalence in percent. Subjects treated for thyroid disease were excluded ($n = 228$). Data were missing for 29 subjects. Comparisons were made using Adjusted Wald test.

* Values are age at baseline. After IF participants were in average 11.2 yr older.

Change in baseline thyroid nodularity

A change from solitary or multinodular structure at baseline was common during the 11-year follow-up period (Table 3). Only one third of solitary nodules at baseline were identified as solitary at the follow-up examination, whereas two thirds of multinodular thyroid glands at baseline still were multinodular at follow-up.

Table 3 presents central characteristics according to change in thyroid nodularity during follow-up. In participants with a solitary nodule at baseline, small nodules in young participants were more likely to have disappeared, whereas thyroid volume and region of habitation were not important.

Among participants with multinodular glands at baseline, change in thyroid nodularity during the follow-up period was associated with region of habitation, baseline nodule size, baseline thyroid volume and thyroid volume at follow-up (Table 3).

Daily smoking and parity at baseline was not associated with changes in thyroid nodularity. Neither was baseline nodular structure among solitary nodules at baseline (data not shown).

Of the 618 subjects with thyroid nodules at baseline 23.8% had no evident nodule at follow-up ($n = 147$); corresponding to a normalization rate of 21.2 (CI 17.9–24.9) per 1000 person years (py). As illustrated in Figure 3 regional differences were present in the normalization rates, and the normalization rates tended to be opposite of incidence rates (higher incidence and lower normalization rates in Aalborg with the lowest preiodization urinary iodine excretion). Solitary nodules had a significantly higher normalization rate than multiple nodules; normalization rate ratio 0.47 (CI 0.32–0.67). The analyses were repeated for nodules > 10 mm and showed the same results (data not shown).

In a multivariate logistic regression model, region (OR 0.21 (CI 0.08–0.56), $P = .002$) with Copenhagen as reference) predicted disappearance of multiple nodules whereas none of the variables investigated, including baseline TSH, predicted disappearance of a solitary nodule. In a separate logistic regression analysis restricted to women and men aged 60–65 years, no significant association was found between sex and nodule disappearance (data not shown).

Discussion

Principal findings

We performed a planned 11-year follow-up investigation of thyroid nodules. The follow-up investigation took

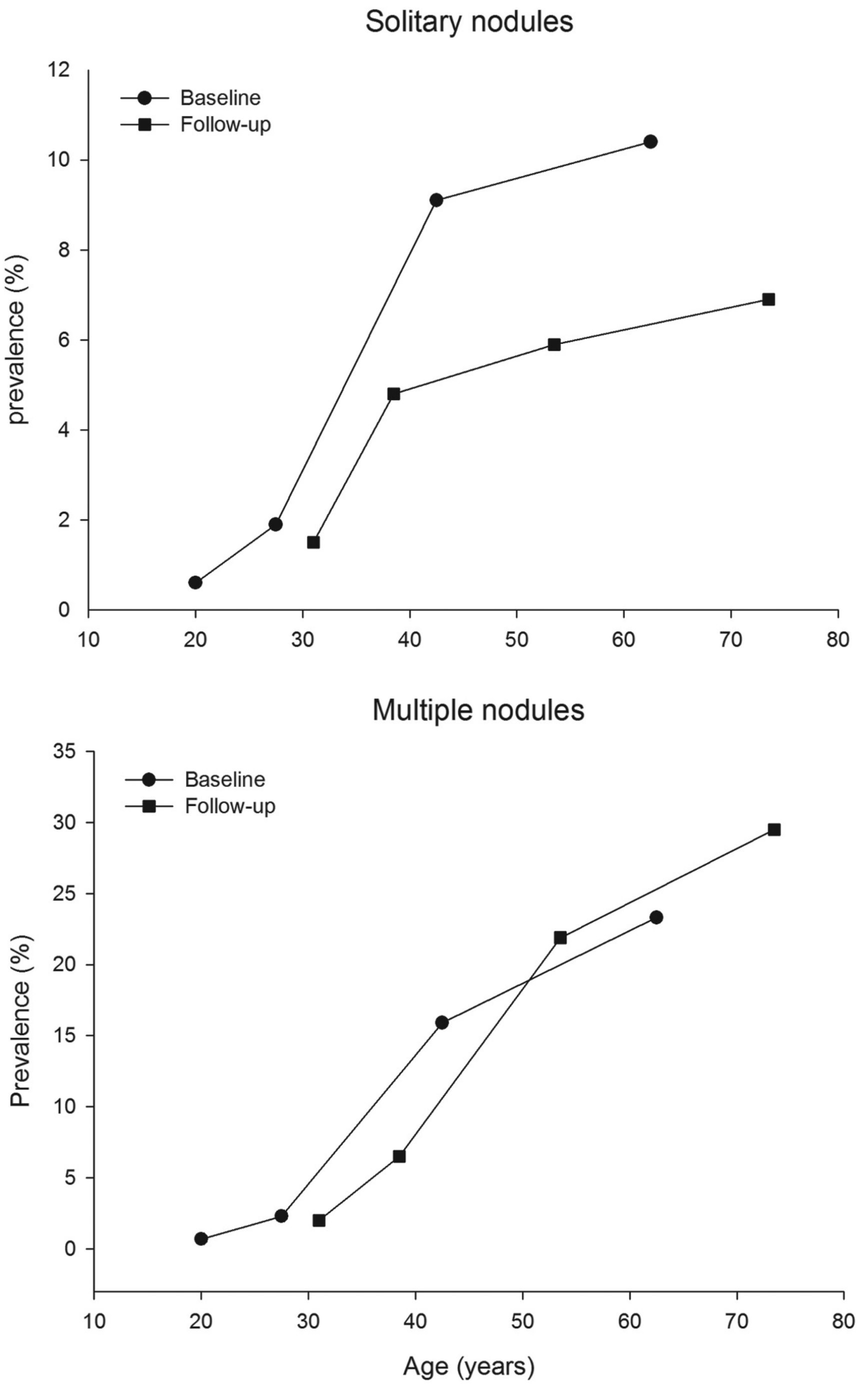

Figure 2. Illustration of the age dependent changes in thyroid nodule prevalence of solitary and multiple nodules before and after IF. Note that participants in the follow-up study were in average 11.2 years older. The analyses include only women and subjects treated for thyroid disease were excluded (n = 228). To assist visualization, graphical lines have been added between various age groups of women. Women aged 40 years at the first investigation had significantly lower prevalence of both solitary nodules (P = .004) and multiple nodules ($P <$ .001) than woman aged 40 years at the second investigation.

place 8.6 years after the mandatory iodine fortification of salt was introduced in Denmark. The 11-year follow-up period saw an increased prevalence of multinodularity, especially in Aalborg (previously moderate ID). However, when we compared woman aged 40 years at the first investigation (1997–1998) with woman aged 40 years at the second investigation (2008–2010) the prevalence of both solitary and multiple nodules were lower at the second investigation.

Changes in thyroid structure and disappearance of thyroid nodules during the 11 years were common. One third of solitary thyroid nodules identified at baseline had disappeared eleven years later, whereas one fifth of previously multinodular glands were diffuse at follow-up. Overall, the balance between normalization rates and incidence rates showed regional differences with higher incidence and lower normalization rates in Aalborg with the lowest preiodization urinary iodine excretion.

Thus, we observed very dynamic changes, with both appearance and disappearance of thyroid nodules, during follow-up in this Danish population study.

Thyroid nodule incidence

Two longitudinal population-based studies with follow-up information on thyroid structure are available (7, 8). Völzke et al (8) performed a 5-year follow-up study in previously iodine deficient Germany and reported, in accordance with our results, an overall thyroid nodule incidence rate of 16.6 per 1000 py. Furthermore, the follow-up period saw a higher incidence of thyroid nodules among women and an age-related incidence increase. This is in agreement with the results of our study, where we found female sex and aging to be predictors of thyroid nodule development.

In another 5-year follow-up study, Yu et al (7) investigated three regions in China with different iodine intake; mild iodine deficiency, more than adequate iodine intake and excessive iodine intake. They found cumulative incidences of solitary nodules between 4.0–5.6% and cumulative incidences of multiple nodules be-

Table 3. Changes in nodularity: Participants with thyroid nodules at baseline were stratified according to their thyroid nodularity at follow-up.

	Solitary nodule at baseline			
	n = 135			
	No nodules *(follow-up)* n = 40	Solitary nodule *(follow-up)* n = 46	Multinodular *(follow-up)* n = 49	p [a]
Baseline characteristics				
Nodule size, mm	11.9 (11.0–15.9)	15.2 (11.7–23.3)	14.2 (11.8–18.9)	0.01
Total thyroid volume, mL	15.9 (11.5–24.6)	14.7 (11.9–18.9)	18.1 (12.8–23.9)	0.20
Region				0.83
Aalborg	20 (27.8)	26 (36.1)	26 (36.1)	
Copenhagen	20 (31.7)	20 (31.7)	23 (36.5)	
Age groups				0.01
Women, 18–22 yr	3 (60.0)	2 (40.0)	0	
Women, 25–30 yr	6 (66.7)	1 (11.1)	2 (22.2)	
Women, 40–45 yr	8 (14.5)	19 (34.5)	28 (50.9)	
Women, 60–65 yr	8 (26.7)	10 (33.3)	12 (40.0)	
Men, 60–65 yr	15 (41.7)	14 (38.9)	7 (19.4)	
Follow-up characteristics				
Nodule size, mm	NA	16.7 (12.5–28.5)	16.5 (13.0–22.1)	1.00
Total thyroid volume, mL	13.9 (10.0–21.3)	14.0 (10.6–17.0)	16.3 (12.1–23.3)	0.24
	Multiple nodules at baseline			
	n = 222			
	No nodules *(follow-up)* n = 50	Solitary nodule *(follow-up)* n = 15	Multinodular *(follow-up)* n = 157	p [a]
Baseline characteristics				
Largest nodule size, mm	11.7 (10.7–14.2)	15.9 (11.6–40.1)	16.8 (12.9–21.0)	<0.001
Total thyroid volume, mL	16.9 (12.6–21.2)	23.5 (14.5–35.9)	22.2 (17.2–29.7)	<0.001
Region				0.001
Aalborg	39 (30.5)	6 (4.7)	83 (64.8)	
Copenhagen	11 (11.7)	9 (9.6)	74 (78.7)	
Age groups				0.30
Women, 18–22 yr	2 (66.7)	1 (33.3)	0	
Women, 25–30 yr	2 (16.7)	1 (8.3)	9 (75.0)	
Women, 40–45 yr	18 (20.0)	6 (6.7)	66 (73.3)	
Women, 60–65 yr	15 (22.4)	3 (4.5)	49 (73.1)	
Men, 60–65 yr	13 (26.0)	4 (8.0)	33 (66.0)	
Follow-up characteristics				
Nodule size, mm	NA	17.4 (12.5–36.0)	17.6 (14.8–23.0)	0.99
Total thyroid volume, mL	16.2 (11.8–19.9)	20.2 (14.5–39.2)	23.2 (16.3–31.7)	<0.001

Data represent median (25^{th}-75^{th} percentiles) and n (%). Nodules ≥ 10 mm were included in the analyses.

[a]Comparisons between the three (or two when applicable) outcome groups were made using Kruskal-Wallis rank test or Mann-Whitney's *U* test for medians of continuous variables and χ^2 test for categorical variables.

tween 0.4–1.2% in the three regions, with no regional differences. These cumulative incidences are lower than the numbers reported in our study and in addition Yu et al found similar incidences between men and women in all three regions. There are several disparities between the studies that may explain the different results: different follow-up periods, ethnic origin and difference in iodine intake. The different ethnicity of the study populations may influence the results and the iodine intake in the Chinese study population was high. In our study IF had led to an increase in the iodine intake level, but still both regions were classified as mildly iodine deficient at follow-up. It may be hypothesized that a higher level of IF in Denmark might have reduced the incidence of nodules in our study, then again the iodine intake level in the German study population (8) was higher than ours, and still results were similar.

Thyroid nodule disappearance

We have no knowledge of other studies with systematic follow-up information on thyroid nodules before and after IF. However, the natural history of thyroid nodules detected by US has been investigated by some other studies with follow-up periods between one and five years (7, 8, 20–24). Most these studies described changes in thyroid structure to be common, in accordance with our results, but absolute normalization rates varied considerably between studies.

In a 5-year follow up study, Völzke et al (8) found an overall normalization rate of 30.5 per 1000 py which was even higher than the normalization rate presented in our study. Likewise, Brander et al (21) performed a 5-year follow-up and found that 24% of the investigated nodules had disappeared in the follow-up period. In contrast, an Italian study by Rago et al (22) described that none of the examined nodules had disappeared but 30% of the examined subjects with thyroid nodules at baseline had more nodules during the 3-year follow-up period. Erdogan et al (24) did a retrospective study on thyroid nodules with a mean observation time of 3 years and found that 4% of the nodules disappeared.

Iodine intake has varied considerably during the past decades in many parts of the world (25–28). We speculate if the differences in normalization rates could be due to differences in actual iodine intake or in the timing in relation to a previous increase in iodine intake. A strong association between iodine intake and thyroid nodularity has been described by numerous investigators (6). In a Chinese study, Yu et al (7) found that 48.5% of baseline solitary nodules had disappeared during the 5 years of follow-up, with no regional differences, whereas 60.6% of multiple nodules had disappeared with a significantly higher frequency in the area with excessive iodine intake compared to the two other regions.

In accordance with the results of Yu et al (7) we found high normalization rates of solitary as well as multiple nodules, although higher among solitary than multiple nodules. Correspondingly, we observed a regional difference in the normalization of multiple nodules, but not solitary nodules, with the highest normalization rate in the region with the highest iodine intake. This was confirmed in a multivariate logistic regression model, where region (proxy for iodine intake) was a predictor for normalization of multiple nodules but not solitary nodules. Thus, iodine seems to play an important role in the structure normalization of thyroid glands harboring multiple thyroid nodules. This notion is supported by a prospective randomized and placebo-controlled trial of patients with thyroid nodules undergoing therapy (29); patients treated with iodine alone had a significant reduction in nodular volume compared to placebo.

During the 11-year follow-up median urinary iodine excretion had increased in our study participants owing to the mandatory Danish IF in 2000, and at follow-up both areas were only mildly iodine deficient. Compared to Copenhagen, Aalborg had the largest increase in iodine excretion during the follow-up period (12), and despite this increase, Aalborg had a lower normalization rate of multiple nodules than Copenhagen. This may suggest that the former longstanding level of iodine deficiency had a major impact on the normalization of thyroid nodules, and that it will take several years before all benefits from an iodization program are in effect. A certain degree of "iodine memory" may be in action. Indeed, our observation suggests that thyroid abnormalities in a population reflect not only the actual iodine intake, but also previous iodine intake of the population (4).

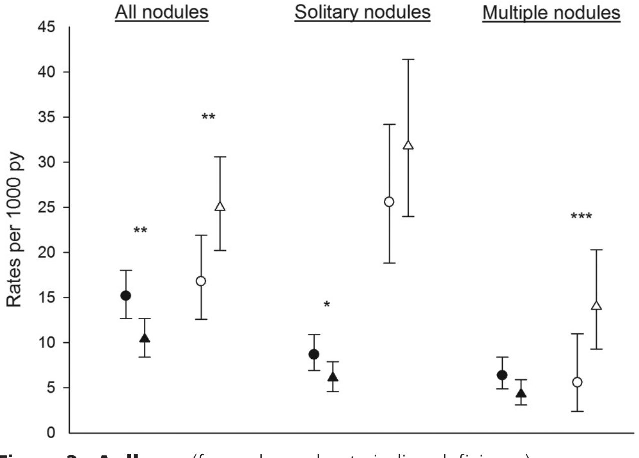

Figure 3. Aalborg (formerly moderate iodine deficiency): Incidence rates ○ Normalization rates **Copenhagen** (formerly mild iodine deficiency): ▲Incidence rates: △Normalization rates Incidence and normalization rates per 1000 person years (py) of thyroid nodules according to region with 95% confidence intervals. Included in the analyses for incidents rates were subjects with diffuse structure at baseline (n = 1,590) where a total of 228 nodules had occurred during follow-up (132 solitary nodules and 96 multiple nodule). Normalization rates included subjects with thyroid nodules at baseline (n = 618, 314 solitary nodules and 304 multiple nodules) where a total of 147 nodules had disappeared during follow-up (101 solitary nodules and 46 multiple nodules). P values are results of normalization rate ratios between the regions for each nodule group. Subjects treated for thyroid disease were excluded (n = 228). * $P < .05$ ** $P < .01$ *** $P < .001$

Strength and limitations

The relatively low participation rate of 59.1% could introduce selection bias, and participants did differ from nonparticipants on a few central variables. However, none of the variables describing baseline thyroid disease, thyroid enlargement or thyroid nodularity were predictors of participation and we used inverse probability weights to account for missing at random. The sample size became low in the stratified analyses. This leads to a relatively low statistical power and allowed only a restricted number of possible predictors in our multivariate regression model. We compared the prevalence of thyroid nodules in women around 40 years of age before and after IF. In these analyses we considered the group at baseline and the group at follow-up as independent, and even though these women were different, they were not entirely independent from each other, which might bias our results.

This study has no control group (no group without IF) and therefore no conclusions regarding causal relationships between IF and nodule disappearance can be drawn from our study. A complicating factor in the interpretation of data is that all participants in the follow-up study had become 11 years older. Consequently, it is difficult to say to what degree our results were due to the influence of iodine or participants aging. Previous studies, including the baseline study of our investigation (10), have shown that the prevalence of thyroid nodules increases with age.

The strength of our study was the prospectively planned follow-up design where all procedures in both the baseline and the follow-up study were similar, and where all US examinations were carried out by the same US apparatus and by the same two sonographers after controlling performances.

Although we have follow-up data on a relatively large cohort, generalization of our results to the entire population is not possible since the study cohort primarily consisted of women in specific age strata.

Conclusions

In a follow-up study after the Danish iodization program, one third of solitary thyroid nodules identified at baseline had disappeared eleven years later, whereas one fifth of previous multinodular glands were now diffuse. Thus, thyroid nodularity is a dynamic and not a necessarily irreversible abnormality.

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