Serum thyroglobulin before and after iodization of salt: an 11-year DanThyr follow-up study

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Abstract

Objective: Our objective was to investigate individual serum thyroglobulin (Tg) changes in relation to iodine fortification (IF) and to clarify possible predictors of these changes.

Design: We performed a longitudinal population-based study (DanThyr) in two regions with different iodine intake at baseline: Aalborg (moderate iodine deficiency (ID)) and Copenhagen (mild ID). Participants were examined at baseline (1997) before the mandatory IF of salt (2000) and again at follow-up (2008) after IF.

Methods: We examined 2465 adults and a total of 1417 participants with no previous thyroid disease and without Tg-autoantibodies were included in the analyses. Serum Tg was measured by immunoradiometric method. We registered participants with a daily intake of iodine from supplements in addition to IF.

Results: Overall, the follow-up period saw no change in median Tg in Copenhagen (9.1/9.1 µg/l, $P=0.67$) while Tg decreased significantly in Aalborg (11.4/9.0 µg/l, $P<0.001$). Regional differences were evident before IF (Copenhagen/Aalborg, 9.1/11.4 µg/l, $P<0.001$), whereas no differences existed after IF (9.1/9.0 µg/l, $P=1.00$). Living in Aalborg ($P<0.001$) and not using iodine supplements at baseline ($P=0.001$) predicted a decrease in Tg whereas baseline thyroid enlargement ($P=0.02$) and multinodularity ($P=0.01$) were associated with an individual increase in Tg during follow-up.

Conclusions: After IF we observed a decrease in median Tg in Aalborg and the previously observed regional differences between Aalborg and Copenhagen had levelled out. Likewise, living in Aalborg was a strong predictor of an individual decrease in serum Tg. Thus, even small differences in iodine intake at baseline were very important for the individual response to IF.

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Introduction

Iodine is an essential part of thyroid hormones and therefore iodine is needed for normal metabolism, growth and development (1, 2, 3). Low iodine intake causes iodine deficiency (ID)-related disorders that have affected billions of people worldwide (4, 5).

Thyroglobulin (Tg) is a 660 kDa protein exclusively synthesized by thyroid epithelial cells organized in follicular structures. Tg plays an important role as a matrix

in the synthesis and storage of the thyroid hormone in the follicular lumen (6). In the 1960s a new sensitive RIA method detected Tg in monkey and human serum, challenging the belief that Tg did not leave the thyroid gland (7, 8). These observations lead to further studies investigating changes in circulating Tg during the 1970s (9, 10) where Van Herle *et al.* (10) described a high mean serum Tg among residents of an endemic goitre region.

In 1985 these observations were confirmed and extended by Fenzi *et al.* (11) who investigated residents of a moderate endemic goitre area. Since then RIAs and screening for Tg autoantibodies (Tg-Ab) were improved (12) and several studies found an inverse association between iodine intake and serum Tg (13, 14, 15, 16, 17). Therefore, it has been suggested that serum Tg values in a population is a sensitive marker of iodine intake, and that serum Tg can be used to monitor the iodine status of a population. However, only few studies have investigated serum Tg in relation to iodine fortification (IF) (18, 19, 20, 21), and no previous longitudinal study gave information on serum Tg both before and after IF.

Our study is a prospectively planned 11-year follow-up study performed in two Danish regions with different iodine intake at baseline. Baseline information on serum Tg was obtained before the mandatory IF initiated in year 2000 and the follow-up study was performed 8.6 years after IF. The main goal of our study was to investigate individual serum Tg changes in relation to IF and to clarify possible predictors of these changes. In addition, we wanted to elucidate serum Tg levels in the Danish population using both our longitudinal data and data from a previous cross-sectional study performed 4 years after IF.

Subjects and methods

Study population and design

In 1997–1998 a cross-sectional study (Cohort 1a (C1a)) was performed in two regions of Denmark with mild (Copenhagen) and moderate (Aalborg) ID. The study was a part of the DanThyr program monitoring the mandatory Danish nationwide IF of salt. The IF was initiated in year 2000 and consisted of adding 13 µg iodine/g salt in household salt and into salt for the production of bread. The program was designed to increase the average daily iodine intake among adult Danes by 50 µg (22). Participants were chosen at random within specific age and sex groups using the Danish civil registration system: women aged 18–22 years, 25–30 years, 40–45 years and 60–65 years and men aged 60–65 years. A total of 4649 subjects participated (50.1% of the invited): 2429 participants in Copenhagen and 2220 participants in Aalborg. Median urinary iodine concentration (UIC) in Copenhagen was 68 µg/l (61 µg/l in participants not taking iodine supplements) and in Aalborg median UIC was 53 µg/l (45 µg/l). The study of this cohort was described in detail previously (18).

As a part of the monitoring program, a second cross-sectional study (Cohort 2 (C2)) was performed in 2004–2005 after IF in year 2000. The C2 study comprised participants selected in the same regions and within the same age and sex groups as in C1a, thus making the two cohorts directly comparable. A total of 3570 subjects participated (46.6% of those invited). Median UIC in Copenhagen was 108 µg/l (99 µg/l in non-supplement-users) and median UIC in Aalborg was 93 µg/l (86 µg/l) classifying Copenhagen as iodine sufficient and Aalborg as mildly iodine deficient according to WHO (23). Details of the study have previously been published (21).

From February 2008 to February 2010, we conducted a follow-up investigation (Cohort 1b (C1b)) of the first cross-sectional study (C1a). Of the 4649 participants in C1a, 72 subjects had emigrated (out of the country) and 403 subjects deceased during follow-up, allowing 4174 subjects to be invited for participation in C1b. The mean follow-up time was 11.2 years (range: 10.1–12.8 years) and 2465 subjects participated, corresponding to 59.1% of the invited (Fig. 1). The examinations were performed at the Centre for Prevention of Goitre and Thyroid Diseases at

Figure 1

Flowchart illustrates participants included in the final study population of the follow-up Cohort 1b.

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either Aalborg University Hospital or Bispebjerg University Hospital in the region of Copenhagen. At each centre a team including a physician and a sonographer performed the examinations. The participants answered questionnaires (health, food frequency and food supplements), gave blood and urine samples, underwent a physical examination, had a thyroid ultrasonography performed and were interviewed.

Participants were asked to bring with them all dietary supplements taken, and daily intake of iodine from supplements was registered. Owing to the planned follow-up design, all procedures were kept similar in the baseline and in the follow-up study. Median UIC in Copenhagen at follow-up was 84 µg/l (76 µg/l for participants not taking iodine supplementation) and in Aalborg median iodine concentration was 83 µg/l (73 µg/l), classifying both Copenhagen and Aalborg as mildly iodine deficient (24).

Participants differed from non-participants of the follow-up study on baseline smoking status, BMI and presence of TPO-Ab (25). The thyroid ultrasonography examinations were performed as described in detail previously (25).

Laboratory procedures

Non-fasting blood samples and non-fasting spot urine samples were collected between 0800 h and 1730 h. Serum and urine samples were kept frozen (−20 °C) and analysed in random order at the study end.

In the baseline study (C1a), serum Tg was analysed with immunoluminometric assays (LUMITEST, BRAHMS Diagnostica GmbH, Berlin, Germany) by a Stratec auto-analyzer (STRATEC Biomedical Systems AG, Birkenfeld, Germany). The effective working range of the assay was 1–500 µg/l. In 12 consecutive assays the inter-assay coefficients of variation (CV) for samples measured with average Tg concentrations of 8.1, 45 and 154 µg/l were 6.8, 4.5 and 3.3%.

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

To allow direct comparison between baseline and follow-up Tg values, we measured Tg in 101 random antibody-negative serum samples kept frozen from the baseline study with the new assay. There was a high

correlation between the two methods ($r_s=0.98$) but a Bland–Altman plot showed differences in the level of measurement results. A linear regression model showed Tg (follow-up) = $1.487 + 0.693 \times \text{Tg (baseline)}$. This equation was used to adjust Tg measured at baseline to the assay used at follow-up and adjusted baseline Tg was used in all data analyses.

In the C1a cohort, Tg-Ab were measured using RIA (DYNOTest, BRAHMS) with functional assay sensitivity at 20 kU/l. In C2 and C1b Tg-Ab were analysed with an immunofluorescent assay (anti-Tgn KRYPTOR, BRAHMS). We re-measured Tg-Ab in 201 sera (106 Tg-Ab positive) kept frozen from the baseline study with the new assay. Correlation was high ($r_s=0.94$) and a Bland–Altman plot showed a high level of agreement between the two methods. Thus, we used a cut-off of 20 kU/l to indicate Tg-Ab positivity in both C1a, C2 and C1b.

Iodine concentrations (µg/l) were measured in the non-fasting spot urine samples by the $\text{Ce}^{4+}/\text{As}^{3+}$ method after alkaline ashing as previously described (26, 27). The analytical sensitivity was 2 µg/l and the iodine laboratory is certified by the US Center for Disease Control and Prevention's EQUIP Program.

Statistical analysis

All data processing was done with the STATA version 11.0 (Stata Corp., College Station, TX, USA). Comparisons were made using the χ^2 test for categorical variables and Mann–Whitney's *U* test for medians of continuous variables. Comparisons between related continuous variables were made with Wilcoxon Signed Rank test. Two-sided $P < 0.05$ was considered statistically significant.

Participants treated for thyroid disease (current or previous treatment with medicine, surgery or radioactive iodine therapy) at baseline or at follow-up ($n=228$), participants with missing values on treatment for thyroid disease or serum Tg concentration ($n=60$) and participants with Tg-Ab > 20 kU/l ($n=760$), were excluded from primary analyses, leaving 1417 participants for the analyses (Fig. 1).

Multiple linear regression models were used to investigate possible baseline predictors of individual changes in Tg. The primary model included only women and a separate model restricted to men and women aged 60–65 years was used to investigate if sex was associated with individual changes in Tg. The models used individual changes in Tg as outcome variable and included: age, region and at baseline: usage of iodine supplements, thyroid enlargement, multinodularity, daily smoking,

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alcohol consumption and childbirths as possible predictors. Interactions between relevant variables were investigated and a significant interaction between region and smoking was observed ($P=0.001$).

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 performed in accordance with the Declaration of Helsinki and all participants gave written informed consent.

Results

Study population

As depicted in Table 1, median Tg at inclusion in 1997–1998 did not differ between participants and non-participants of the 2008–2010 follow-up study C1b.

Thyroglobulin

When we compared median serum Tg before and after IF by paired analyses of our follow-up cohort ($n=1417$) no significant changes in Tg were observed in Copenhagen whereas median Tg had decreased in all age and sex groups in Aalborg (Table 2). Before IF, regional differences

in median Tg were evident in all groups except 60–65-year-old women. In contrast, no regional differences in Tg were observed after IF.

Predictors of Tg change

Iodine intake level at baseline (higher in Copenhagen than Aalborg, see Methods) was a strong predictor of individual change in Tg after IF. Thus, participants from Aalborg (formerly moderate ID) were more likely to have a decrease in serum Tg compared with participants living in Copenhagen (formerly mild ID) (Table 3). In addition, no use of iodine containing supplements at baseline was a predictor of a decrease in serum Tg during follow-up compared with iodine supplement users at baseline.

Baseline thyroid enlargement and multinodularity predicted an individual increase in serum Tg during the 11-year follow-up period, whereas daily smoking, alcohol consumption and parity at baseline did not predict changes in Tg during the follow-up period (Table 3).

In additional analysis, we found a larger decrease in serum Tg among participants who stopped smoking during follow-up ($n=178$) compared with participants without changes in their smoking habits ($n=515$) (median individual Tg change $-2.5/-0.8 \mu\text{g/l}$, $P<0.001$). No difference in Tg change was found between the few participants ($n=20$) who started smoking and participants without any change in their smoking habits ($1.0/-0.8 \mu\text{g/l}$, $P=0.35$).

Table 1 Median thyroglobulin at inclusion according to baseline characteristics of participants and non-participants in the follow-up study C1b. Comparisons between participants and nonparticipants were made using Mann–Witney's test.

Baseline characteristics	Participants ($n=2465$)		Non-participants ($n=1709$)		P^a
	n (%)	Median Tg (25th–75th percentiles)	n (%)	Median Tg (25th–75th percentiles)	
Age groups					
Women, 18–22 years	489 (19.8)	9.6 (5.1–18.1)	434 (25.4)	10.7 (5.9–17.8)	0.10
Women, 25–30 years	514 (20.9)	11.7 (6.1–21.2)	391 (22.9)	11.2 (6.0–21.4)	0.98
Women, 40–45 years	657 (26.7)	14.4 (6.9–27.3)	237 (13.9)	13.7 (7.2–27.1)	0.78
Women, 60–65 years	381 (15.5)	14.0 (5.2–30.2)	366 (21.4)	16.4 (7.1–32.2)	0.11
Men, 60–65 years	424 (17.2)	11.6 (5.5–21.5)	281 (16.4)	12.8 (6.1–24.1)	0.07
Region					
Copenhagen	1236 (50.1)	10.6 (5.3–19.3)	899 (52.6)	10.6 (5.7–19.3)	0.57
Aalborg	1229 (49.9)	13.9 (6.6–27.0)	810 (47.4)	15.1 (7.9–30.0)	0.06
Daily smokers	793 (32.2)	16.8 (8.9–28.9)	668 (39.1)	15.6 (8.5–30.4)	0.54
Family history of thyroid disease	507 (20.6)	13.2 (6.5–23.5)	309 (18.1)	13.1 (6.3–27.2)	0.76
Treated for thyroid disease	104 (4.2)	17.3 (2.6–47.3)	84 (4.9)	13.0 (4.2–25.9)	0.33
Thyroid enlargement ($> 18/25$ ml)	468 (19.0)	24.9 (11.5–48.9)	282 (16.6)	26.3 (13.4–48.1)	0.22
Thyroid nodularity					
Solitary nodule	353 (14.3)	15.8 (6.6–29.2)	215 (12.6)	17.0 (8.1–33.4)	0.17
Multiple nodules	374 (15.2)	22.7 (11.1–45.5)	258 (15.1)	21.3 (10.9–39.2)	0.52

^a P values for comparison between median Tg among participants and non-participants.

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Table 2 Median thyroglobulin level in two areas with formerly mild (Copenhagen) and formerly moderate ID (Aalborg). All participants were investigated both before (1997–1998) IF (2000) and at 11 years follow-up (2008–2010). Data represent median thyroglobulin level in $\mu\text{g/litre}$ (25th–75th percentiles). Only values from participants of both studies (C1a and C1b) were included. Subjects treated for thyroid disease ($n=228$) and participants with Tg-Ab >20 kU/l at either baseline or follow-up ($n=760$) were excluded. Data on thyroglobulin were missing for 38 subjects. Comparisons were made using Wilcoxon Sign Rank and Mann–Whitney's test.

Group ^a	Formerly mild ID (Copenhagen)			Formerly moderate ID (Aalborg)			Copenhagen vs Aalborg, before iodization, P	Copenhagen vs Aalborg, after iodization, P
	Before iodization $n=750$	After iodization $n=750$	P	Before iodization $n=667$	After iodization $n=667$	P		
Women								
18–22 years	7.2 (5.2–11.9)	7.3 (4.9–12.1)	0.21	10.2 (6.0–16.5)	8.4 (5.1–13.0)	<0.001	0.001	0.29
25–30 years	8.9 (5.6–13.6)	8.2 (5.4–12.7)	0.19	10.3 (6.5–17.8)	8.6 (5.4–12.9)	<0.001	0.01	0.91
40–45 years	10.7 (6.1–17.7)	10.9 (6.6–17.7)	0.58	15.4 (7.8–24.1)	10.9 (6.1–19.0)	<0.001	<0.001	0.76
60–65 years	11.4 (6.7–16.4)	12.0 (7.6–18.4)	0.09	12.6 (7.4–22.9)	9.9 (6.3–17.3)	0.01	0.34	0.28
Men, 60–65 years	8.3 (4.6–14.2)	8.3 (4.6–13.9)	0.51	10.2 (6.1–17.8)	7.7 (4.7–12.4)	<0.001	0.01	1.00
Total	9.1 (5.5–14.9)	9.1 (5.5–15.3)	0.67	11.4 (6.4–19.3)	9.0 (5.4–14.9)	<0.001	<0.001	1.00

^aAge at baseline. After IF participants were in average 11.2 years older.

Individual changes in serum Tg were not different among participants who changed their alcohol habits compared to those with the same alcohol consumption at both baseline and follow-up ($n=735$) (decreased alcohol consumption ($n=254$): $-0.5/-0.7$ $\mu\text{g/l}$, $P=0.88$; increased alcohol consumption ($n=102$): $-0.4/-0.7$ $\mu\text{g/l}$, $P=0.33$).

Median serum Tg at baseline was higher among parous women than nulliparous women (11.4/8.8 $\mu\text{g/l}$, $P<0.001$). At follow-up more women were parous, and no difference in median Tg was found between the two groups (9.1/8.1 $\mu\text{g/l}$, $P=0.22$). Furthermore, the individual changes in serum Tg were not different in women who gave birth during follow-up ($n=537$) compared with those who did not ($n=388$) ($-0.9/-1.0$ $\mu\text{g/l}$, $P=0.79$).

Iodine supplements and Tg

At baseline, 475 participants (33.5%) took iodine-containing supplements and at follow-up 252 had stopped and 266 had started taking supplements. This resulted in 489 participants (34.5%) taking iodine supplements at the follow-up investigation. Before IF, median Tg was significantly lower among iodine supplement users than non-users (8.1/11.2 $\mu\text{g/l}$, $P<0.001$) in both regions (Fig. 2). Furthermore, a regional difference in median Tg was evident for those participants not taking iodine supplements ($P<0.001$), whereas no statistically significant regional difference in Tg was found for iodine supplement users ($P=0.25$).

After IF, median Tg was still significantly lower among iodine supplement users than among non-users

(7.9/9.4 $\mu\text{g/l}$, $P=0.001$) in both regions (Fig. 2). Median Tg for iodine supplement users was at the same level as before IF, whereas median Tg for non-users had decreased in Aalborg after IF and the regional difference among non-users had disappeared ($P=0.99$).

Participants not taking iodine supplements at baseline before IF had a significant decrease in median Tg during the follow-up period (non-users at both baseline and follow-up ($n=676$): 11.5/9.6 $\mu\text{g/l}$, $P<0.001$; non-users who started taking supplements ($n=266$): 10.2/7.6 $\mu\text{g/l}$, $P<0.001$). No change in median Tg was found among participants taking iodine supplements at both baseline and follow-up ($n=223$) (8.1/8.8 $\mu\text{g/l}$, $P=0.81$). Users at baseline who stopped taking supplements during follow-up ($n=252$) had a borderline increase in Tg (8.3/9.1 $\mu\text{g/l}$, $P=0.06$).

Tg trends in the DanThyr cohorts

Median values of Tg in the cohorts investigated in DanThyr are shown in Fig. 3 and for this we used data from all participants investigated. Before IF (C1a) median Tg was higher in Aalborg (moderate ID) than Copenhagen (mild ID) in all age and sex groups. Furthermore, median Tg was higher in women 60–65 years old than in men 60–65 years old ($P<0.001$) and there was an age-dependent increase in median Tg among women.

After IF, similar patterns were seen for C2 investigated in 2004–2005 and for C1b investigated in 2008–2010: median Tg had become lower in all age and sex groups in Aalborg and the regional difference in median Tg had disappeared in all age and sex groups. The age-dependent

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Table 3 Predictors of Thyroglobulin change at 11-year follow-up. Multiple linear regression model with change in serum Tg as outcome variable and baseline characteristics as predictor variables. The estimate defines the number of units of change in serum Tg (y) in the specific class of the predictor (x) compared with the reference group (ref.). Participants treated for thyroid disease ($n=228$) and participants with Tg-Ab >20 kU/l at either baseline or follow-up were excluded from the analysis ($n=760$). The primary model included Tg-Ab negative women ($n=1110$), sex as a predictor of change in serum Tg was analysed in a separate model restricted to women and men aged 60–65 years ($n=480$).

Baseline characteristics	n	Estimate	P
Age ^a			
Women, 18–22 years	283	Ref.	
Women, 25–30 years	285	−0.679	0.52
Women, 40–45 years	369	−0.710	0.58
Women, 60–65 years	173	2.773	0.06
Sex			
Women, 60–65 years	173	Ref.	
Men, 60–65 years	307	2.408	0.101
Region			
Copenhagen	596	Ref.	
Aalborg	514	−3.268	<0.001
Iodine supplements at baseline			
Yes	382	Ref.	
No	728	−2.553	0.001
Thyroid enlargement at baseline (> 18/25 ml by US)			
No	940	Ref.	
Yes	169	2.562	0.02
Multinodularity (≥ 2 thyroid nodules)			
No	661	Ref.	
Yes	148	2.902	0.01
Daily smoking at baseline			
No	717	Ref.	
Yes	393	−0.823	0.29
Alcohol consumption at baseline			
< 1 drink/week	142	Ref.	
1–7 drinks/week	705	−0.275	0.80
8–28 drinks/week	249	−0.520	0.68
> 28 drinks/week	12	−1.427	0.69
Parity at baseline			
Nulliparous	441	Ref.	
Parous	669	−1.286	0.20

^aAge at baseline. At follow-up participants were on average 11.2 years older.

increase in median Tg was still evident but had levelled out. The sex-dependent difference in median Tg had decreased, but Tg was still significantly lower among men than women ($P<0.001$). Participants investigated in 2008–2010 (C1b) were selected among participants of C1a and were on average 11.2 years older than participants of C1a and C2. To take age differences into account we compared median Tg in 40–41-year-old women in C1a, C2 and C1b, and found a significantly higher median Tg in

C1a compared with C2 ($17.1/8.5$ $\mu\text{g/l}$, $P<0.001$) and compared with C1b ($17.1/7.7$ $\mu\text{g/l}$, $P<0.001$). We found no difference in median Tg among 40–41-year-old women between C2 and C1b ($8.5/7.7$ $\mu\text{g/l}$, $P=0.15$).

Discussion

Principal findings

We performed an 11-year follow-up investigation where participants were examined 8.6 years after the Danish mandatory IF of salt (13 $\mu\text{g/g}$) in two regions with different iodine intakes at baseline: Aalborg (moderate ID) and Copenhagen (mild ID). During the follow-up period, no change in median Tg was observed in Copenhagen while Aalborg had a decrease in median Tg in all age and sex groups. Additionally, regional differences were evident in all groups except the oldest group of women before IF, whereas no regional differences existed after IF. Living in Aalborg and not using iodine supplements at baseline were strong predictors of a decrease in serum Tg. Thus, degree of ID at baseline was the dominating predictor of a change in serum Tg. Furthermore, baseline thyroid enlargement and multinodularity were associated with

Figure 2

Median serum thyroglobulin ($\mu\text{g/l}$) by individual intake of iodine supplements in subjects who participated in both C1a (1997–98) and C1b (2008–10) according to region ($n=1417$). Nearly all supplements contained 150 μg iodine. To assist visual comparison, a line (horizontal stippled) has been added corresponding to the lowest value of median Tg found after IF in C1b among participants taking iodine supplements in Copenhagen ($\text{Tg}=7.7$ $\mu\text{g/l}$). $*P<0.05$, $**P<0.01$, $***P<0.001$ in between intake of iodine supplement analyses.

Figure 3

Median serum thyroglobulin ($\mu\text{g/l}$) by region in the DanThyr cohorts: C1a ($n=4649$), C2 ($n=3570$) and C1b ($n=2465$) according to age and sex groups. Note that participants in C1b were on average 11.2 years older. Subjects treated for thyroid disease (C1a: $n=228$, C2: $n=192$ and C1b: $n=228$) and subjects with Tg-Ab >20 kU/l (C1a: $n=599$, C2: $n=640$ and C1b: $n=649$)

were excluded from the analyses. To assist visual comparison, a line (horizontal stippled) has been added corresponding to the lowest value of median Tg found after IF in C1b among 29–33 year old women in Copenhagen ($\text{Tg}=7.3 \mu\text{g/l}$). *** $P<0.001$ in between region analyses.

an individual increase in serum Tg during follow-up whereas age *per se* had no predictive value.

In additional analyses, we found a higher median Tg among non-users of iodine containing supplements than among users both before and after IF. Regional differences in median Tg was only evident for non-users before IF.

Previous studies

Previous studies have investigated the relation between serum Tg and iodine intake in adult populations living in regions with different iodine intakes (13, 17, 18, 19, 20, 21). In accordance with our results, these studies found an inverse relation between serum Tg and iodine intake even in regions with small differences in iodine intake. Several studies concluded that even if serum Tg concentration is a non-specific marker of thyroid disease in the individual and even if Tg-Ab may influence measurements, Tg is a good marker of iodine intake in a population and that it is a useful tool in monitoring the iodine status (14, 18, 21, 28, 29). However, only one previous longitudinal population-based cohort study has investigated serum Tg in relation to iodine intake. This 5-year follow-up study was performed by Teng *et al.* (20) in three regions of China with different iodine intakes at both baseline and follow-up (mild ID, adequate iodine intake and excessive iodine intake). They found a significant difference in serum Tg between the three regions with a higher median Tg in the region with mild ID. The study commenced 3 years after IF and the study population had a stable iodine intake during

the follow-up period. Individual changes in serum Tg during follow-up were not presented.

Other studies have investigated the relation between iodine intake and Tg in randomised trials (15, 16). Pedersen *et al.* (15) investigated Danish pregnant women in an area with moderate ID, whereas Kahaly *et al.* (16) studied German adult patients with clinical symptoms of endemic goitre in a moderate ID area. Both studies found a continuous decrease in serum Tg compared with the control group when iodine supplements were administered. When iodine supplementation was stopped, Kahaly *et al.* (16) reported an increase in serum Tg to the level before supplementation was initiated. This supports the notion that serum Tg is a good and sensitive marker of changes in iodine intake and it is in accordance with our findings.

However, measuring serum Tg is challenging (30) and different analysing methods as well as interference from circulating autoantibodies can hamper interpretation and make comparisons between studies difficult. Therefore we chose to exclude Tg-Ab-positive participants from our analysis although a previous investigation (21) showed that the exclusion of Tg-Ab-positive participants may not influence the interpretation of population-based data.

During the follow-up period, median UIC had increased to a level that classified both Aalborg and Copenhagen as mildly iodine deficient at follow-up (24). However, at follow-up, UIC were lower than observed in C2 examined 4–5 years after IF. A major cause for the observed decrease in iodine concentration may be a reduction in the iodine content of common milk products (31).

Corresponding to the higher iodine intake in 2008–2010 compared with 1997–1998, serum Tg had decreased. The most prominent change occurred in Aalborg with the largest increase in median UIC. Likewise, multivariate regression analysis found living in Aalborg as well as no baseline usage of iodine supplements to be predictors of an individual decrease in serum Tg. Thus, the degree of baseline ID was the dominating predictor of a change in serum Tg even though the baseline differences in UIC between Aalborg and Copenhagen were small (53 µg/l vs 68 µg/l, and in participants not taking iodine supplements: 45 µg/l vs 61 µg/l). The difference in UIC is caused by differences in groundwater iodine content being around 5 µg/l in Aalborg and 20 µg/l in Copenhagen (32).

Despite the general decrease in serum Tg, median Tg among non-users of iodine supplements was still higher than median Tg among iodine supplement users after IF. The iodization of salt initiated in 2000 (13 µg/g) was cautious in order to minimize side effects (22) and the results of the present study raise the question if a moderate increase in the level of iodine added to the salt, bringing median urinary iodine values to a level around 100 µg/l as found in 2004–2005 (21) could be beneficial for the Danish population. This conclusion is in accordance with the results of urinary iodine measurements, indicating that the C1b cohort investigated in 2008–2010 was in general suffering from mild ID (24). A cross-sectional study with participants from the same regions and in the same age and sex groups as in the two cross-sectional studies C1a and C2 would give epidemiologically more precise information on the iodine status of the Danish population.

Strengths and limitations

Our follow-up study had a relatively low participation rate of 59.1%, which could lead to selection bias. However, baseline median Tg did not differ among participants and non-participants. The study population only included participants in specific age and sex groups, and although we have a relatively large study cohort, we cannot generalize our results to the entire population.

The strength of our study was the prospectively planned longitudinal design with investigation of participants both before and after IF, using identical procedures. Different assays were used for measuring serum Tg in C1a vs C2 and C1b, but we adjusted C1a serum Tg to account for assay change. Part of the study results can be difficult to interpret because all participants of the follow-up study were 11 years older than in the baseline study. In the analyses of Fig. 3 we included data from the cross-sectional

study C2 and considered C1a, C2 and C1b as three independent cohorts. This has limitations since C1a and C1b were not independent. Moreover, participants of C1b were 11 years older.

We obtained information on daily intake of iodine supplements, but no information on the duration of iodine supplementation including seasonal variations was registered. This might influence our results and cause an attenuation of the association between median Tg and iodine supplement intake.

Conclusions

After the mandatory IF of salt, we observed a decrease in median serum Tg in Aalborg and the regional differences in serum Tg observed before IF had levelled out. Likewise, living in Aalborg was a strong predictor of a decrease in serum Tg. Thus, even small differences in iodine intake at baseline were very important predictors of the response to IF.

After IF median Tg among non-users of iodine supplements was still higher than median Tg among iodine supplement users and these results may raise the question if a moderate increase in the level of iodine added to the salt could be beneficial for the population in the study.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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