ORIGINAL ARTICLE

Serum thyroglobulin as a biomarker of iodine deficiency in adult populations

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Summary

Objective To clarify which factors may influence the serum Tg level in an adult population and how this may affect Tg as a biomarker of iodine deficiency (ID).

Design and methods Two identical cross-sectional studies were performed before (C1a: 1997–98, $n = 4649$) and after (C2: 2004–05, $n = 3570$) the Danish mandatory iodine fortification (IF) of salt (2000). Additionally, a follow-up study of C1a was performed after IF (C1b: 2008–10, $n = 2465$). The studies took place in two regions with mild (Copenhagen) and moderate (Aalborg) ID before IF. Serum Tg was measured by immunoradiometric method and investigated as outcome variable in multivariate models.

Results Multiple factors were associated with serum Tg. Some were directly related to iodine intake (cohort, urinary iodine concentration (UIC) level and region), and some were likely mediators of iodine intake effects on Tg (thyroid nodularity, thyroid size and autonomy with low TSH). Others were caused by Tg assay interference (Tg-Ab positivity), aggravation of ID (childbirths and smoking) or TSH stimulation of the thyroid. Estimated 24-h urinary iodine excretion was a more sensitive predictor of Tg than UIC. Iodine supplement users had low median Tg values compared with nonusers both before and after IF.

Conclusions Multiple factors should be taken into consideration when evaluating Tg as a marker of ID in adult populations, and the Tg results may depend on the assay used. Still, Tg is a sensitive marker of ID. We suggest including a reference population with known sufficient iodine intake when Tg is used to evaluate ID.

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Introduction

Iodine is essential for the synthesis of thyroid hormones, and iodine deficiency (ID)-related disorders[1] have affected billions of people.[2]

Several studies have found a sensitive inverse association between iodine intake and serum thyroglobulin (Tg),[3–7] and Tg is recommended by the Wold Health Organization (WHO), United Nations Children's Fund (UNICEF) and the Iodine Global Network (IGN) as an effect indicator when monitoring the iodine status of a population.[8] However, various challenges exist when using Tg as a biomarker of ID. One is that large intermethod differences exist and another that results can be hampered by circulating thyroglobulin autoantibodies (Tg-Ab) especially in adults where such autoantibodies are commonly present.[9] Both assay differences and autoantibodies are known to be of major importance when serum Tg is used in the monitoring of patients with differentiated thyroid carcinoma.[9]

Recently, a fixed cut-off value for Tg indicating ID has been discussed and proposed, mainly based on data from schoolchildren using dried blood spot samples,[10,11] but detailed studies on the factors that may influence serum Tg in an adult population are needed.

In Denmark, a population monitoring programme (DanThyr) was initiated before implementation of the mandatory iodine fortification (IF) of salt in year 2000. As a part of this monitoring programme, adults from two regions with different iodine intake were examined in details before and after the mandatory IF. This study includes data from the cohorts investigated in DanThyr with the aim of illuminating which factors determine the serum Tg level of an adult population and clarifying the usefulness of Tg as a biomarker of ID.

Subjects and methods

Design and study population

The Danish mandatory IF programme commenced in the year 2000. Iodine (13 ppm) was added to household salt and to salt

used for commercial bread production.[12] Before IF was implemented, a monitoring programme (DanThyr) was initiated to improve knowledge on how to evaluate the iodine status of a population and to monitor the iodine intake of the Danish population.[13] As a part of DanThyr a cross-sectional study, Cohort 1a was performed before IF in 1997–1998. The investigation took place in two regions of Denmark with different iodine intake due to differences in the iodine content of the groundwater[14]: Copenhagen with mild ID and Aalborg with moderate ID.[15] Participants were randomly chosen in specific age and sex groups (women 18–22, 25–30, 40–45 and 60–65 years and men 60–65 years) from the national civil registration system, where all inhabitants of Denmark are registered by a unique 10-digit number. As depicted in Fig. 1, a total of 4649 subjects participated in the investigation, 2429 in Copenhagen (median urinary iodine concentration (UIC) was 68 μg/l) and 2220 in Aalborg (median UIC was 53 μg/l).[16]

Cohort 1a was designed for at later follow-up after IF, and in 2008–2010, a reinvestigation of the same participants was completed: Cohort 1b. All participants from C1a were identified, 403 had deceased and 72 had emigrated out of the country leaving 4174 to be invited for the follow-up investigation of which 2465 subjects participated, 1236 in Copenhagen (median UIC was 84 μg/l) and 1229 in Aalborg (median UIC was 83 μg/l) (Fig. 1).[17]

In addition, a second cross-sectional study was performed in 2004–2005, 4–5 years after IF: Cohort 2 (Fig. 1). Participants of the Cohort 2 investigation were randomly selected in the same regions and within the same age and sex groups as in the Cohort 1a study, making the two cross-sectional studies directly comparable.[18] There was no overlap between participants of Cohort 1a and Cohort 2. A total of 3570 subjects participated, 1785 in Copenhagen (median UIC was 108 μg/l) and 1785 in Aalborg (median UIC was 93 μg/l).

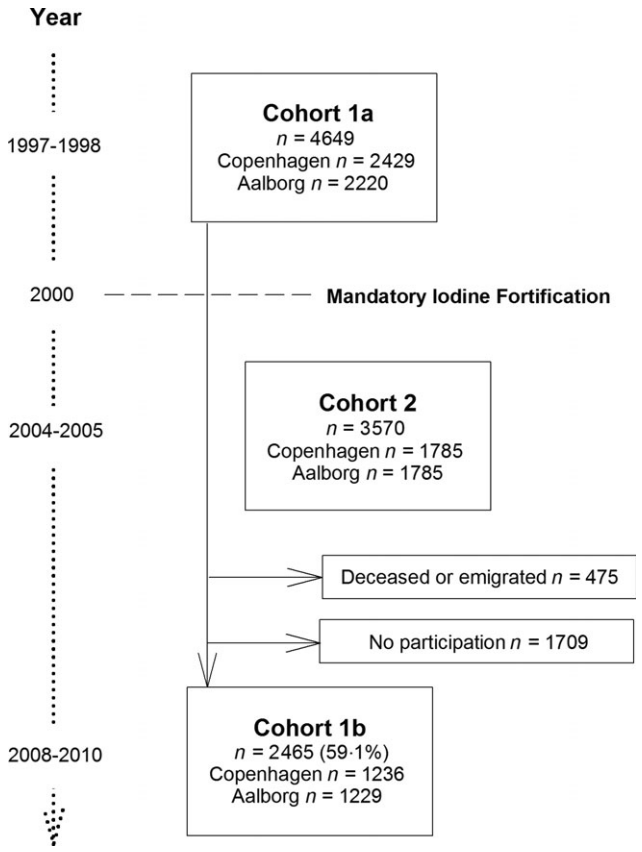

Fig. 1 Flow chart illustrating participants of the DanThyr cohorts.

The participants of the DanThyr cohorts were examined at 'The Centre for Prevention of Goitre and Thyroid Diseases' at either Bispebjerg University Hospital, in the region of Copenhagen, or at Aalborg University Hospital. Each centre had a physician and a sonographer performing the examinations. Participants answered health questionnaires, gave blood and urine samples, had a thyroid ultrasonography (US) performed and were interviewed where their use of iodine supplements was registered.

Owing to the prospective design of the monitoring programme, all procedures were similar in Cohort 1a, Cohort 1b and Cohort 2.

Ultrasonography

All US examinations were performed using the same US apparatus (Sonoline Versa Pro 7·5 MHz 70 mm linear transducer, Siemens, Germany) by the same two sonographers. The two sonographers' comparability was continuously studied, and the monitoring showed good correlation.[18–20]

Thyroid volume was calculated as maximal length*depth*width*π/6 for each lobe,[19] and thyroid enlargement was defined as a total thyroid volume >18 ml for women and >25 ml for men corresponding to the mean thyroid volume +3SD in an iodine sufficient population.[21]

Thyroid nodules >5 mm were registered, and multinodularity was defined as ≥2 thyroid nodules present, each with a maximal diameter >10 mm.

Laboratory procedures

Nonfasting blood and nonfasting spot urine samples were collected between 8·00 a.m. and 5·30 p.m. Serum and urine samples were kept frozen (−20 °C) until study end where they were analysed in random order.

In Cohort 1a, serum Tg was analysed with immunoluminometric assays (LUMITEST, BRAHMS Diagnostica GmbH, Berlin, Germany) using a Stratec autoanalyzer (STRATEC Biomedical Systems AG, Birkenfeld, Germany). The effective working range of the assay was 1–500 μg/l. In 12 consecutive assays, the interassay coefficients of variation (CVs) for samples measured with average Tg concentrations of 8·1, 45 and 154 μg/l were 6·8, 4·5 and 3·3%, respectively.

In Cohort 2 and Cohort 1b, serum Tg was measured using an immunoflourescent assay (hTg KRYPTOR BRAHMS) with a functional sensitivity <0·8 ng/ml (information from manufacturer). In 115 consecutive assays, the interassay CVs for samples measured with average Tg concentrations of 3·3 and 50·5 μg/l were 5·6 and 2·8%, respectively. KRYPTOR hTg can be

calibrated to CRM 457 with a factor 1·92 (1 μg/l in KRYPTOR hTg corresponded to 1·92 (95% CI 1·75–2·09) μg/l hTg CRM 457).

A total of 101 random antibody-negative serum samples from Cohort 1a was kept frozen and reanalysed with the second assay used in the Cohort 2 and in the Cohort 1b study. Disagreement was found between the two Tg methods and a linear regression model (TG(C2&C1b) = 1·487 + 0·693xTg(C1a)) was used to adjust serum Tg from the Cohort 1a study to the second assay.[22]

Tg-Ab was analysed using RIA (DYNOtest, BRAHMS) with a functional sensitivity at 20 kU/l in the Cohort 1a study. In Cohort 2 and Cohort 1b, Tg-Ab was analysed using an immunoflourescent assay (anti-Tgn KRYPTOR, BRAHMS). We reanalysed Tg-Ab in 201 serum samples kept frozen from Cohort 1a with the new assay and found a high level of agreement between the two methods. Thus, a cut-off of 20 KU/l was also used to indicate Tg-Ab positivity in Cohort 2 and Cohort 1b.

In Cohort 1a, TSH was analysed using LUMItest assays (BRAHMS, Berlin, Germany), and in Cohort 2 and Cohort 1b, TSH was measured with the Roche Modular E system by electrochemical luminescence using ELECSYS. A subsample (201 blood samples) from Cohort 1a remeasured with the method used in Cohort 2 and Cohort 1b showed a good agreement between the two methods.[23]

UIC (μg/l) was analysed using the Ce^{4+}/As^{3+} method after alkaline ashing.[24,25] The analytical sensitivity was 2 μg/l. The iodine laboratory is certified by the U.S. Centre for Disease 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 Mann–Whitney's *U*-test for medians of continuous variables, and a two-sided $P < 0{\cdot}05$ was considered statistically significant.

Participants treated for thyroid disease (current or previous treatment with medicine, surgery or radioactive iodine therapy) in Cohort 1a ($n = 228$), Cohort 2 ($n = 192$) and Cohort 1b ($n = 228$) were excluded from the primary analyses. Participants with Tg-Ab >20 kU/l in Cohort 1a ($n = 599$), Cohort 2 ($n = 640$) and Cohort 1b ($n = 649$) were excluded from the analyses of Fig. 3.

Urinary iodine excretion was expressed as spot UIC (μg/l) and as estimated 24-h urinary iodine excretion (UIE) (μg/24 h) calculated from the reported 24-h urinary creatinine excretion in a Danish population study.[26]

Multivariate linear regression models were used to investigate how various factors associated with serum Tg. The primary model included only women and a separate model limited to men and women aged 60–65 years was used to investigate the possible influence of gender on Tg. The models used ln-transformed Tg concentration as the dependent variable and included: Age, region, cohort, UIC, TSH, thyroid nodularity, thyroid enlargement, daily smoking, parity and Tg-Ab positivity as independent variables. Interactions between relevant variables were investigated, and significant interactions between region and cohort ($P = 0{\cdot}001$), region and UIC ($P = 0{\cdot}001$), TSH and thyroid enlargement ($P < 0{\cdot}001$) and TSH and parity ($P = 0{\cdot}01$) were observed. In an additional model, est. 24-h UIE was added to the primary model to compare the predictive value of est. 24-h UIE vs. UIC on serum Tg.

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

The Study populations

Participant characteristics differed between the cohorts (Table 1), as Cohort 1b was a follow-up investigation of Cohort 1a. At the time of investigation participants of Cohort 1b were on average 11 years older than participants of Cohort 1a and Cohort 2. Table 2 shows median serum Tg concentrations according to various participant characteristics.

Determinants of serum Tg

In a multivariate linear regression model, multiple factors were related to serum Tg (Fig. 2). Some factors were directly related to iodine intake, where both time of investigation in relation to IF (cohort), UIC level and region of residence were significant predictors of Tg. Other predictors were thyroid nodularity, thyroid size and thyroid autonomy (low TSH) as likely mediators of iodine intake effects on Tg or were caused by high thyroid activity secondary to high TSH. In the multivariate analyses, high TSH values predicted Tg only when Tg-Ab were adjusted for. Other factors could be explained by assay interference seen in Tg-Ab-positive participants or by aggravation of ID in relation to childbirth and smoking.

In a separate analysis, we replaced UIC with est. 24-h UIE in the multivariate regression model and found that est. 24-h UIE was a better predictor of serum Tg than UIC as illustrated in Fig. 3.

Reference population taking iodine supplements

As shown in Table 1, approximately one-third of participants in each cohort took iodine supplements and we investigated the feasibility of using this group of participants as an iodine sufficient reference population. Before IF (cohort 1a), both iodine supplement users and nonusers of iodine supplements had insufficient iodine intake with median UIC below 100 μg/l (Fig. 4a). After IF, iodine supplement users had a sufficient

Table 1. Characteristics of participants in the DanThyr cohorts, *n* (%)

	Cohort 1a *n* = 4649	Cohort 2 *n* = 3570	Cohort 1b *n* = 2465
Age groups* (years)			
Women, 18–22	835 (22·2)	556 (20·4)	303 (19·5)
Women, 25–30	779 (20·7)	555 (20·4)	314 (20·2)
Women, 40–45	729 (19·4)	526 (19·3)	407 (26·2)
Women, 60–65	582 (15·5)	465 (17·1)	202 (13·0)
Men, 60–65	834 (22·2)	621 (22·8)	327 (21·1)
Region			
Copenhagen	1980 (52·7)	1383 (50·8)	827 (53·3)
Aalborg	1779 (47·3)	1340 (49·2)	726 (46·7)
Daily smokers	1454 (38·7)	757 (27·9)	315 (20·8)
Parity†			
Nulliparous	1534 (52·4)	1120 (53·5)	218 (21·4)
Parous	1391 (47·6)	974 (46·5)	799 (78·6)
Tg-Ab ≥20 kU/l	599 (13·7)	640 (19·0)	649 (29·5)
TSH (mU/l)			
<0·4	214 (4·9)	73 (2·2)	77 (3·5)
0·4–3·6	3956 (90·8)	3101 (92·2)	2007 (91·1)
>3·6	188 (4·3)	189 (5·6)	118 (5·4)
Urinary iodine concentration (μg/l)			
<50	1717 (39·7)	689 (20·6)	598 (27·3)
50–99	1486 (34·3)	972 (29·0)	738 (33·7)
≥100	1125 (26·0)	1690 (50·4)	853 (39·0)
Est. 24-h urinary iodine excretion (μg/24 h)			
<50	729 (17·0)	98 (2·9)	75 (3·5)
50–99	1539 (35·8)	736 (22·1)	591 (27·4)
≥100	2032 (47·3)	2502 (75·0)	1491 (69·1)
Use of iodine supplements	1500 (34·4)	970 (28·8)	788 (35·8)
Thyroid enlargement (>18/25 ml‡)	764 (17·6)	362 (10·8)	337 (15·4)
Multinodularity	660 (15·2)	467 (14·0)	421 (19·3)

*Note that participants in Cohort 1b were on average 11·2 years older at the time of study.
†Only women included in the analyses.
‡>18 ml for women and >25 ml for men by ultrasonography.

Table 2. Median Tg (μg/l) according to characteristics of participants in the DanThyr cohorts (25th–75th percentiles)

	Cohort 1a *n* = 4649	Cohort 2 *n* = 3570	Cohort 1b *n* = 2465
Age groups* (years)			
Women, 18–22	8·5 (5·4–13·8)	7·6 (5·0–11·2)	7·4 (4·3–12·0)
Women, 25–30	9·3 (5·6–15·9)	7·6 (4·8–12·5)	7·7 (5·0–12·9)
Women, 40–45	11·4 (6·3–20·3)	9·5 (5·7–16·3)	9·9 (5·3–17·4)
Women, 60–65	12·8 (6·6–24·1)	11·0 (6·1–18·5)	10·1 (5·8–18·4)
Men, 60–65	10·0 (5·7–17·5)	9·3 (5·7–15·1)	7·6 (4·4–13·6)
Region			
Copenhagen	9·0 (5·4–15·0)	8·6 (5·3–13·4)	8·2 (4·8–14·6)
Aalborg	11·6 (6·4–21·3)	8·9 (5·5–15·2)	8·7 (4·8–15·0)
Daily smokers			
No	8·7 (5·1–15·2)	8·0 (5·0–12·7)	7·6 (4·5–13·3)
Yes	13·0 (7·7–22·5)	11·9 (7·0–18·0)	12·0 (7·1–19·5)
Parity†			
Nulliparous	8·9 (5·4–15·1)	7·8 (5·0–12·0)	7·7 (4·7–13·3)
Parous	11·5 (6·5–21·2)	10·0 (5·8–16·6)	8·4 (5·0–14·6)
Tg-Ab ≥20 kU/l			
No	10·4 (6·1–18·0)	9·1 (5·8–14·5)	8·9 (5·3–15·1)
Yes	7·2 (3·3–15·0)	7·0 (3·9–12·9)	7·5 (3·8–14·1)
TSH (mU/l)			
<0·4	16·4 (8·0–37·4)	13·8 (7·7–26·1)	20·1 (8·9–40·0)
0·4–3·6	10·0 (5·8–17·0)	8·6 (5·4–14·0)	8·2 (4·8–14·2)
>3·6	8·7 (3·2–20·7)	9·3 (5·6–15·4)	7·6 (3·7–20·3)
Urinary iodine concentration (μg/l)			
<50	11·6 (6·5–20·9)	9·2 (5·3–15·4)	9·3 (5·4–14·7)
50–99	9·9 (5·9–17·3)	9·3 (6·0–15·0)	8·4 (4·8–15·6)
≥100	8·2 (4·9–14·2)	8·3 (5·1–13·4)	8·0 (4·6–13·9)
Est. 24-h urinary iodine excretion (μg/24 h)			
<50	13·6 (8·0–24·5)	11·6 (6·8–17·9)	7·7 (4·7–12·9)
50–99	10·4 (5·9–17·7)	9·0 (5·7–14·9)	9·4 (5·5–15·5)
≥100	8·7 (5·3–15·1)	8·6 (5·3–13·8)	8·1 (4·7–14·3)
Use of iodine supplements			
No	11·0 (6·3–19·3)	9·2 (5·6–14·7)	9·1 (5·0–15·5)
Yes	8·4 (5·1–14·9)	7·9 (5·0–12·9)	7·6 (4·7–13·3)
Thyroid enlargement (>18/25 ml‡)			
No	9·1 (5·5–15·2)	8·3 (5·2–13·2)	7·6 (4·5–12·5)
Yes	19·2 (10·2–33·9)	15·8 (9·0–27·9)	16·1 (9·1–27·7)
Multinodularity			
No	9·3 (5·5–15·9)	8·2 (5·2–13·2)	7·6 (4·5–12·7)
Yes	16·8 (9·2–29·9)	13·4 (8·3–24·8)	14·3 (7·6–25·4)

*Note that participants in Cohort 1b were on average 11·2 years older at the time of study.
†Only women included in the analyses.
‡>18 ml for women and >25 ml for men by ultrasonography.

iodine intake with UIC at or above 100 μg/l whereas low UIC levels were evident for nonusers of iodine supplements in Cohort 2 and Cohort 1b. Correspondingly, high median Tg values were apparent for nonusers of iodine supplements compared with supplement users both before and after IF, and median Tg was 8 μg/l in the group of participants with sufficient iodine intake (Fig. 4b).

Sensitivity analysis

Cohort 1b was a follow-up investigation of participants in Cohort 1a. Thus, Cohort 1b and Cohort 1a were not independent of each other, and participants of Cohorts 1b were at the time of investigation on average 11 years older than participants of Cohort 1a and Cohort 2. In a sensitivity analysis, we excluded participants of Cohort 1b and repeated the multivariate linear regression model. This showed similar results for all investigated factors except for parity, which no longer showed a borderline statistically significant relation to serum Tg ($P = 0{\cdot}152$). Additional analysis was made without age in the multivariate regression model. Again, this showed similar result except for parity, which was now a strong determinant of the serum Tg level ($P < 0{\cdot}001$).

Discussion

Principal findings

In this population-based study of Danish adults, we found that multiple factors were related to serum Tg. Some were directly

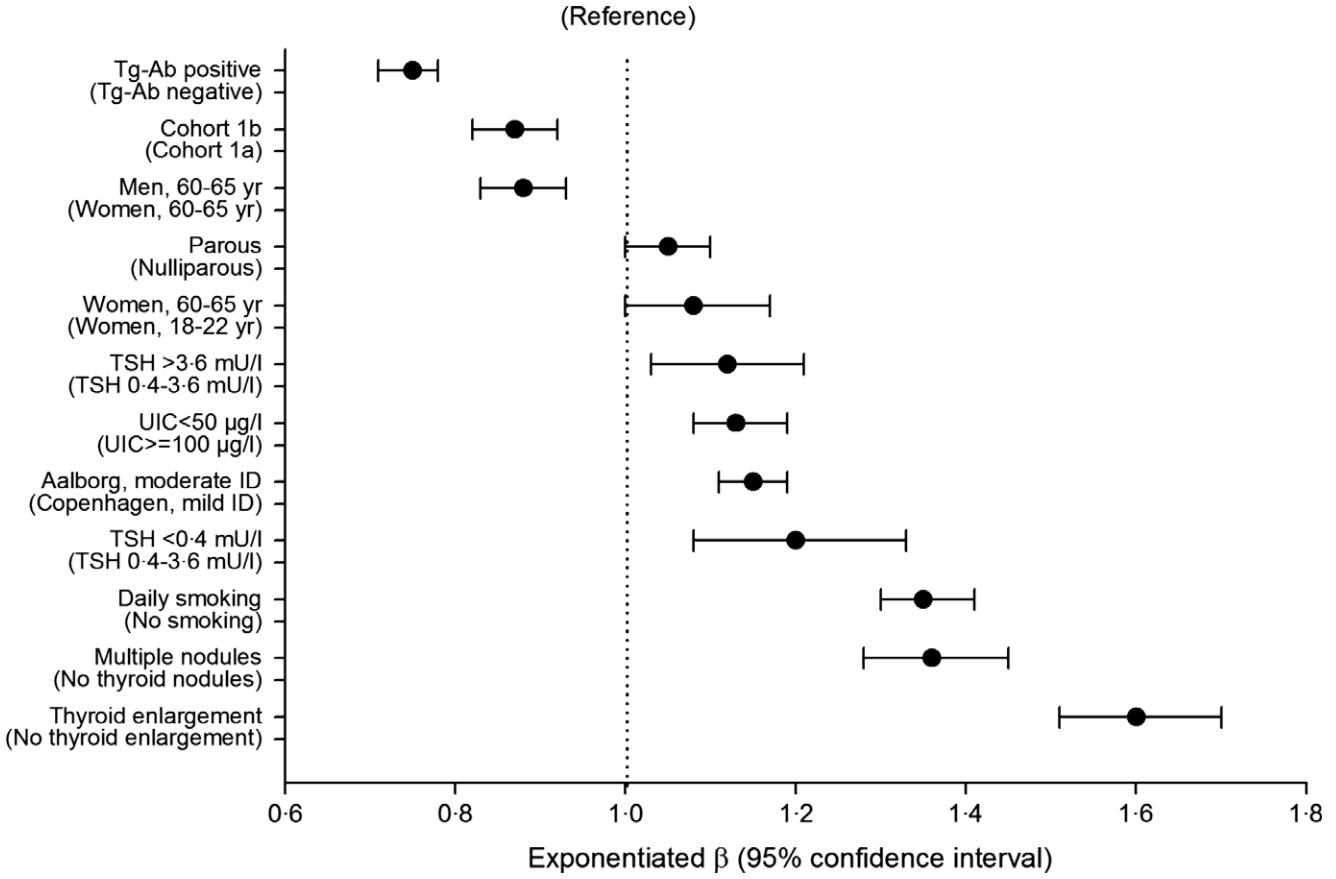

Fig. 2 Multivariate linear regression model with ln serum Tg concentration as dependent variable. The figure illustrates the ranked associations between various participant characteristics and serum Tg. Results are exponentiated β and 95% confidence interval. The reference groups are indicated in brackets.

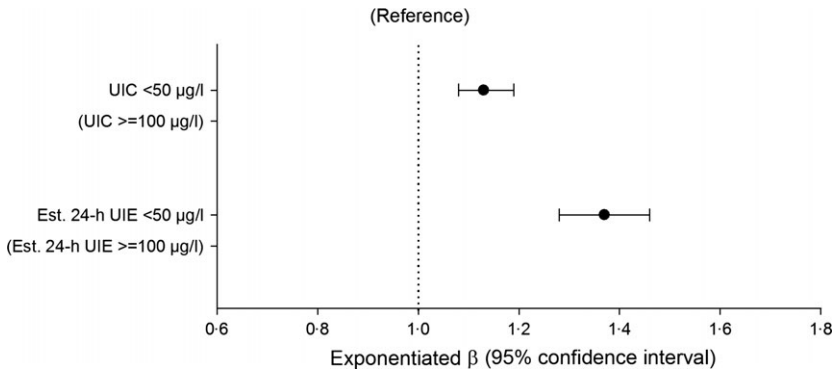

Fig. 3 Multivariate linear regression model with ln serum Tg concentration as dependent variable. The figure illustrates the associations between UIC as well as est. 24-h UIE and serum Tg. Results are exponentiated β and 95% confidence interval. The reference groups are indicated in brackets.

related to iodine intake (cohort, UIC level and region), and some were likely mediators of iodine intake effects on Tg (thyroid nodularity, thyroid size and thyroid autonomy (low TSH)) or caused by high thyroid activity secondary to high TSH. Others were caused by assay interference (Tg-Ab positivity) or aggravation of ID (childbirths and smoking). In addition, the est. 24-h UIE was a better predictor of serum Tg than UIC. The majority of factors were directly or indirectly associated with iodine intake making Tg a sensitive marker of the iodine status in a population.

We compared participants having a low UIC with a reference population taking iodine supplements and having a median UIC within the recommended level.

Determinants of serum thyroglobulin

Serum Tg is a known tumour marker for monitoring patients after treatment for differentiated thyroid carcinoma and analytical challenges such as circulating Tg-autoantibodies are well known.[27] In addition, intermethod differences are high and the

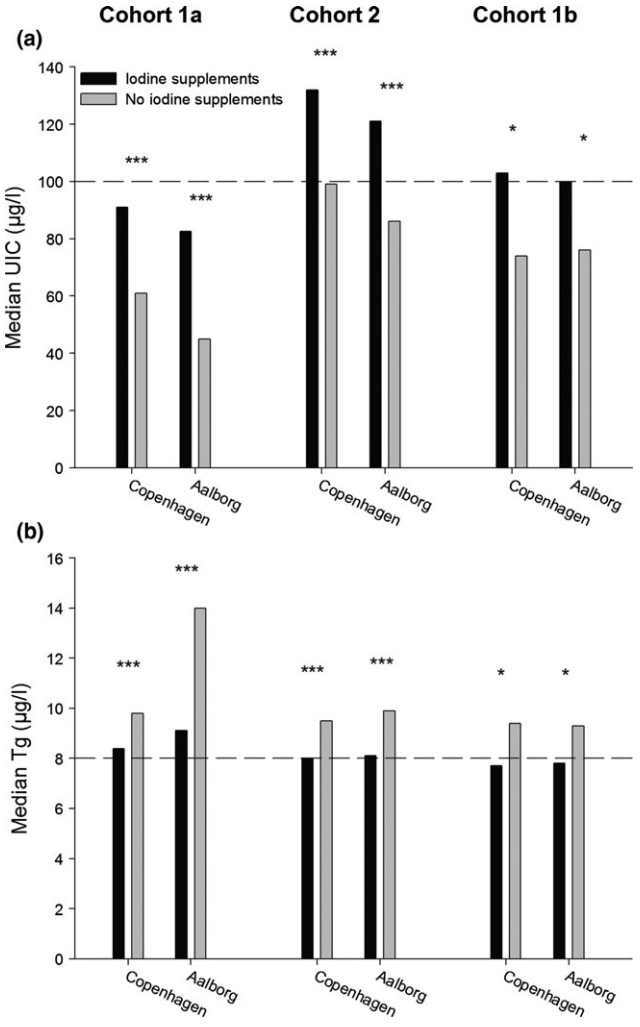

Fig. 4 (a) Median UIC (µg/l) according to individual intake of iodine supplements in participants of the DanThyr cohorts. Nearly, all supplements contained 150 µg iodine. Participants with Tg-Ab >20 kU/l in Cohort 1a (n = 599), Cohort 2 (n = 640) and Cohort 1b (n = 649) were excluded from the analyses. To assist visual comparison, a horizontal stippled line has been added corresponding to the lowest value of UIC indicating iodine sufficiency (median UIC = 100 µg/l). (b) Median Tg (µg/l) according to individual intake of iodine supplements in participants of the DanThyr cohorts. Participants with Tg-Ab >20 kU/l were excluded from the analyses. A horizontal stippled line has been added corresponding to the median Tg value of the group with the highest median UIC (Cohort 2 (after IF) participants from Copenhagen taking iodine supplements (median Tg = 8 µg/l)). $^{*}P < 0{\cdot}05$, $^{***}P < 0{\cdot}001$ between groups.

influence of Tg-Ab positivity depends on the method used. In a Tg-Ab-positive individual, the immunometric assay will tend to underestimate the total serum Tg level whereas the radioimmunoassay typically will overestimate the total serum Tg level.[27] In accordance, our study found that Tg-Ab positivity predicted a lower measured Tg level. However, results from a previous study by Vejbjerg *et al.*[7] suggested that exclusion of Tg-Ab-positive participants may not be essential for the interpretations of population-based data as results showed the same trends and did not alter the overall conclusions. Although, Tg-Ab may influence the level of Tg, and inclusion of an iodine sufficient reference population as suggested by the present study (see below) may be essential.

Both time of investigation in relation to IF (cohort) and region of habitation, as proxy variables of iodine intake, were significant predictors of Tg. In accordance with previous studies, we found that a low iodine intake level was associated with a high Tg level.[4,5,7,28] Furthermore, gender played an important role, where female sex predicted higher Tg levels. The mechanism behind this result may be the higher prevalence of thyroid diseases among women compared with men, even though both thyroid nodules and thyroid enlargement at baseline were adjusted for in the multivariate model. Thus, residual confounding may be at play. A newly published study by Chong *et al.*[28] performed in three Chinese regions with different iodine intake levels corroborated these results. The authors also found a highly significant association between high age and high Tg level where we only found a borderline significant relation.

Abnormal TSH values were associated with higher Tg values which is also in accordance with the results from a multivariate model performed by Chong *et al.*[28] High TSH increases thyroid gland activity and therefore also the Tg level. The mechanism behind our finding of low TSH levels predicting high serum Tg may be the thyroid autonomy often seen in ID populations.[29]

In accordance with previous studies,[30–32] smoking predicted high Tg levels as a factor aggravating ID through thiocyanate. Thiocyanate is abundant in cigarette smokers and is a competitive inhibitor of iodine transport. Furthermore, pregnancy can also be classified as an aggravating factor of ID[33–35] and childbirth showed a borderline significant association with Tg.

The association between thyroid enlargement and Tg as well as that between thyroid nodules and Tg in an adult population is well established[4,5,7,36] and was confirmed in this study. Our results suggest that iodine influences the Tg level through several mechanisms. UIC is a direct measure of current iodine intake in a population, and in agreement with other studies, low UIC was associated with high Tg values. Because stratification according to median UIC has limitations[37] we also calculated and included est. 24-h UIE and as may be expected estimated 24-h UIE was a more sensitive predictor of the Tg level than UIC.

Iodine sufficient reference population

The sensitive association between iodine intake and Tg is further complicated by high intermethod variability, that is only marginally reduced by CRM-457 standardization.[38] A way to avoid this problem could be to include an iodine sufficient reference population in each study performed. In the present study, we used participants taking iodine supplements as a reference population and found that participants who were examined after IF and who did not take iodine supplements, had high median Tg values compared with iodine supplement users. This difference in Tg corresponded to the difference in UIC found in the two groups, and also to the results found in our follow-up investigation.[22]

Strength and limitations

In this study, we included data from two cross-sectional studies (C1a and C2) and one follow-up study (C1b), and considered these as independent cohorts. As C1b was a follow-up investigation of C1a, they were not independent which was a major limitation. Additionally, study results can be difficult to interpret because participants of C1b were 11 years older than participants of C1a and C2. However, sensitivity analysis revealed no major difference in results after excluding C1b or age from the regression model. A third cross-sectional investigation with participants from the same regions and within the same age and sex groups as in C1a and C2 may provide epidemiologically more accurate information.

Median UIC from spot urine samples can be used to determine the iodine status of a population. However, UIC is very imprecise when used in an individual, and stratification according to UIC level may cause a problem. Therefore, we also calculated and included the estimated 24-h UIE in our regression model. In the multivariate regression model, UIC depended on cohort and region and interactions were present, which may bias the results.

The prospective design of the monitoring programme enabled similar procedures in the cohorts investigated and sonographers as well as the ultrasonography apparatus were the same in C1a, C2 and C1b. Different assays were used for analysing serum Tg in C1a *vs* C2 and C1b and disagreement was found between the two Tg methods, but we adjusted the C1a serum Tg to account for the assay change.

The study population only included adults within specific age and sex groups; therefore, we cannot generalize our results to the entire population. We acquired information on the actual daily intake of iodine containing supplement, but had no information on the duration of such supplement intake. This may have caused an attenuation of the association between iodine supplementation and median Tg.

Conclusions

Tg was a sensitive marker of iodine intake. However, multiple factors were associated with Tg in an adult population, and some, but not all, were effect modifiers of ID. This should be taken into consideration when using Tg as a marker of ID in an adult population. The Tg results may depend on the analytical method, and we suggest to include a reference population with sufficient iodine intake when Tg is used to evaluate ID.

More studies using different assays in various populations are needed to clarify the usefulness of a specific cut-off value for median Tg to indicate ID in a population. An interesting approach when using Tg for monitoring is the use of dried blood spot for analyses.[39]

Declaration of interest

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

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References

1 Delange, F. (1994) The disorders induced by iodine deficiency. *Thyroid*, **4**, 107–128.
2 Kelly, F.C. & Snedden, W.W. (1960) Prevalence and distribution of endemic goitre. In: WHO ed. Endemic Goitre. WHO, Geneva, 227–233.
3 Kahaly, G., Dienes, H.P., Beyer, J. *et al.* (1997) Randomized, double blind, placebo-controlled trial of low dose iodide in endemic goiter. *The Journal of Clinical Endocrinology and Metabolism*, **82**, 4049–4053.
4 Laurberg, P., Pedersen, K.M., Hreidarsson, A. *et al.* (1998) Iodine intake and the pattern of thyroid disorders: a comparative epidemiological study of thyroid abnormalities in the elderly in Iceland and in Jutland, Denmark. *The Journal of Clinical Endocrinology and Metabolism*, **83**, 765–769.
5 Knudsen, N., Bulow, I., Jorgensen, T. *et al.* (2001) Serum Tg–a sensitive marker of thyroid abnormalities and iodine deficiency in epidemiological studies. *The Journal of Clinical Endocrinology and Metabolism*, **86**, 3599–3603.
6 Teng, W., Shan, Z., Teng, X. *et al.* (2006) Effect of iodine intake on thyroid diseases in China. *The New England Journal of Medicine*, **354**, 2783–2793.
7 Vejbjerg, P., Knudsen, N., Perrild, H. *et al.* (2009) Thyroglobulin as a marker of iodine nutrition status in the general population. *European Journal of Endocrinology*, **161**, 475–481.
8 WHO, UNICEF, ICCIDD (2007) Assessment of Iodine Deficiency Disorders and Monitoring their Elimination, 3rd edn. World Health Organization: Geneva.
9 Spencer, C.A., Bergoglio, L.M., Kazarosyan, M. *et al.* (2005) Clinical impact of thyroglobulin (Tg) and Tg autoantibody method differences on the management of patients with differentiated thyroid carcinomas. *The Journal of Clinical Endocrinology and Metabolism*, **90**, 5566–5575.
10 Zimmermann, M.B., Aeberli, I., Andersson, M. *et al.* (2013) Thyroglobulin is a sensitive measure of both deficient and excess iodine intakes in children and indicates no adverse effects on thyroid function in the UIC range of 100-299 mug/L: a UNICEF/ICCIDD study group report. *The Journal of Clinical Endocrinology and Metabolism*, **98**, 1271–1280.
11 Ma, Z.F. & Skeaff, S.A. (2014) Thyroglobulin as a biomarker of iodine deficiency: a review. *Thyroid*, **24**, 1195–1209.

12 Fødevareministeriets bekendtgørelse (29. juni 2000) Bekendtgørelse om tilsætning af jod til husholdningssalt og salt i brød og almindeligt bagværk m.v., nr. 627.
13 Laurberg, P., Jorgensen, T., Perrild, H. *et al.* (2006) The Danish investigation on iodine intake and thyroid disease, DanThyr: status and perspectives. *European Journal of Endocrinology*, **155**, 219–228.
14 Andersen, S., Pedersen, K.M., Iversen, F. *et al.* (2008) Naturally occurring iodine in humic substances in drinking water in Denmark is bioavailable and determines population iodine intake. *The British Journal of Nutrition*, **99**, 319–325.
15 Knudsen, N., Bulow, I., Jorgensen, T. *et al.* (2000) Comparative study of thyroid function and types of thyroid dysfunction in two areas in Denmark with slightly different iodine status. *European Journal of Endocrinology*, **143**, 485–491.
16 Knudsen, N., Bulow, I., Jorgensen, T. *et al.* (2000) Goitre prevalence and thyroid abnormalities at ultrasonography: a comparative epidemiological study in two regions with slightly different iodine status. *Clinical Endocrinology*, **53**, 479–485.
17 Rasmussen, L.B., Jorgensen, T., Perrild, H. *et al.* (2014) Mandatory iodine fortification of bread and salt increases iodine excretion in adults in Denmark - A 11-year follow-up study. *Clinical Nutrition*, **33**, 1033–1040.
18 Vejbjerg, P., Knudsen, N., Perrild, H. *et al.* (2007) Effect of a mandatory iodization program on thyroid gland volume based on individuals' age, gender, and preceding severity of dietary iodine deficiency: a prospective, population-based study. *The Journal of Clinical Endocrinology and Metabolism*, **92**, 1397–1401.
19 Knudsen, N., Bols, B., Bulow, I. *et al.* (1999) Validation of ultrasonography of the thyroid gland for epidemiological purposes. *Thyroid*, **9**, 1069–1074.
20 Krejbjerg, A., Bjergved, L., Pedersen, I.B. *et al.* (2014) Iodine fortification may influence the age-related change in thyroid volume: a longitudinal population-based study (DanThyr). *European Journal of Endocrinology*, **170**, 507–517.
21 Gutekunst, R., Becker, W., Hehrmann, R. *et al.* (1988) Ultrasonic diagnosis of the thyroid gland. *Deutsche Medizinische Wochenschrift (1946)*, **113**, 1109–1112.
22 Krejbjerg, A., Bjergved, L., Pedersen, I.B. *et al.* (2015) Serum thyroglobulin before and after iodization of salt: an 11-year DanThyr follow-up study. *European Journal of Endocrinology*, **173**, 573–581.
23 Bjergved, L., Jorgensen, T., Perrild, H. *et al.* (2012) Predictors of change in serum TSH after iodine fortification: an 11-year follow-up to the DanThyr study. *The Journal of Clinical Endocrinology and Metabolism*, **97**, 4022–4029.
24 Wilson, B. & Van Zyl, A. (1967) The estimation of iodine in thyroidal amino acids by alkaline ashing. *The South African Journal of Medical Sciences*, **32**, 70–82.
25 Laurberg, P. (1987) Thyroxine and 3,5,3′-triiodothyronine content of thyroglobulin in thyroid needle aspirates in hyperthyroidism and hypothyroidism. *The Journal of Clinical Endocrinology and Metabolism*, **64**, 969–974.
26 Toft, U., Cerqueira, C., Andreasen, A.H. *et al.* (2014) Estimating salt intake in a Caucasian population: can spot urine substitute 24-hour urine samples? *European Journal of Preventive Cardiology*, **21**, 1300–1307.
27 Spencer, C., Petrovic, I., Fatemi, S. *et al.* (2014) Serum thyroglobulin (Tg) monitoring of patients with differentiated thyroid cancer using sensitive (second-generation) immunometric assays can be disrupted by false-negative and false-positive serum thyroglobulin autoantibody misclassifications. *The Journal of Clinical Endocrinology and Metabolism*, **99**, 4589–4599.
28 Chong, W., Shi, X., Shan, Z. *et al.* (2015) Tg in adults as a sensitive biomarker of iodine status: a 5-year follow up population study in different levels of iodine intake regions. *PLoS ONE*, **10**, e0135553.
29 Stanbury, J.B., Ermans, A.E., Bourdoux, P. *et al.* (1998) Iodine-induced hyperthyroidism: occurrence and epidemiology. *Thyroid*, **8**, 83–100.
30 Christensen, S.B., Ericsson, U.B., Janzon, L. *et al.* (1984) Influence of cigarette smoking on goiter formation, thyroglobulin, and thyroid hormone levels in women. *The Journal of Clinical Endocrinology and Metabolism*, **58**, 615–618.
31 Knudsen, N., Bulow, I., Laurberg, P. *et al.* (2002) Association of tobacco smoking with goiter in a low-iodine-intake area. *Archives of Internal Medicine*, **162**, 439–443.
32 Andersen, S.L., Nohr, S.B., Wu, C.S. *et al.* (2013) Thyroglobulin in smoking mothers and their newborns at delivery suggests autoregulation of placental iodide transport overcoming thiocyanate inhibition. *European Journal of Endocrinology*, **168**, 723–731.
33 Pedersen, K.M., Borlum, K.G., Knudsen, P.R. *et al.* (1988) Urinary iodine excretion is low and serum thyroglobulin high in pregnant women in parts of Denmark. *Acta Obstetricia et Gynecologica Scandinavica*, **67**, 413–416.
34 Moreno-Reyes, R., Glinoer, D., Van Oyen, H. *et al.* (2013) High prevalence of thyroid disorders in pregnant women in a mildly iodine-deficient country: a population-based study. *The Journal of Clinical Endocrinology and Metabolism*, **98**, 3694–3701.
35 Andersen, S.L., Sorensen, L.K., Krejbjerg, A. *et al.* (2013) Iodine deficiency in Danish pregnant women. *Danish Medical Journal*, **60**, A4657.
36 Gutekunst, R., Smolarek, H., Hasenpusch, U. *et al.* (1986) Goitre epidemiology: thyroid volume, iodine excretion, thyroglobulin and thyrotropin in Germany and Sweden. *Acta Endocrinologica*, **112**, 494–501.
37 Vejbjerg, P., Knudsen, N., Perrild, H. *et al.* (2009) Estimation of iodine intake from various urinary iodine measurements in population studies. *Thyroid*, **19**, 1281–1286.
38 Lee, J.I., Kim, J.Y., Choi, J.Y. *et al.* (2010) Differences in serum thyroglobulin measurements by 3 commercial immunoradiometric assay kits and laboratory standardization using Certified Reference Material 457 (CRM-457). *Head and Neck*, **32**, 1161–1166.
39 Stinca, S., Andersson, M., Erhardt, J. *et al.* (2015) Development and validation of a new low-cost enzyme-linked immunoassay for serum and dried blood spot thyroglobulin. *Thyroid*, **25**, 1297–1305.