CLINICAL STUDY

Determinants of serum T₄ and T₃ at the time of diagnosis in nosological types of thyrotoxicosis: a population-based study

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Abstract

Objective: To characterize thyroid hormone levels at the time of diagnosis in the nosological types of thyrotoxicosis diagnosed in the population and to analyze determinants for serum thyroxine (T₄) and tri-iodothyronine (T₃).

Design: Population-based study of thyrotoxicosis at disease onset.

Methods: In the period 1997–2000, we prospectively identified all patients diagnosed with incident primary overt thyrotoxicosis in a Danish population cohort and classified patients into ten well-defined nosological types of disease (*n*=1082). Untreated levels of serum T₃, T₄, and T₃:T₄ ratio were compared and related to sex, age, level of iodine deficiency, smoking status, alcohol intake, iodine supplement use, co-morbidity, and TSH receptor antibodies (TRAbs) in multivariate models.

Results: Graves' disease (GD) patients had much higher levels of T₃ and higher T₃:T₄ ratio at diagnosis compared with other thyrotoxic patients, but with a profound negative association between hormone levels and age. In GD, patients diagnosed in the area with more severe iodine deficiency had lower levels of T₃ and T₄. TRAb-negative GD patients had biochemically mild thyrotoxicosis. Higher age was also associated with lower degree of biochemical thyrotoxicosis in nodular toxic goiter. We found no association between serum T₃ and T₄ and sex, smoking habits, iodine supplements, alcohol intake, or co-morbidity in any type of thyrotoxicosis.

Conclusions: The study gives new insight into the hormonal presentation of thyrotoxicosis and showed that young age, positive TRAb levels, but also residency in the area with higher iodine intake was positively associated with biochemical disruption in GD.

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Introduction

Thyrotoxicosis is a common disease worldwide. We previously reported a lifetime risk for developing overt thyrotoxicosis to be around 10% in Danish women (1). Apart from excessive intake of thyroid hormone, and the rare case of extrathyroidal thyroid hormone production, thyrotoxicosis is due to either a hyperfunctioning thyroid gland (hyperthyroidism) or to hormonal leakage from thyroid follicles. The characteristic thyroid function tests in overt hyperthyroidism are a suppressed serum thyroid-stimulating hormone (TSH) and elevated tri-iodothyronine (T₃) and/or thyroxine (T₄).

The degree of hormonal abnormality in patients newly diagnosed with thyrotoxicosis has been presented in several studies, but no report has described in detail the levels of and balance between T₃ and T₄ observed at the time of diagnosis in the many nosological types of thyrotoxicosis. Thus, determinants for levels of serum T₃ and T₄ have not been studied in detail even if

previous studies reported on various details such as influence of age (2, 3), sex (3), iodine intake (4), and TSH receptor antibody (TRAb) concentration (5, 6). Limited patient characteristics were included in most of these studies, and often they did not take various possible confounders into account, such as smoking, known to aggravate iodine deficiency (7) and to modulate thyroid autoimmunity (8), alcohol intake that besides from influencing the risk of Graves' disease (GD) (9) also may supply flavonoids known to modulate the selenodeiodinase activity (10), intake of vitamin supplements often containing iodine, and co-morbidity. We used the population-based data collected as part of the The Danish Investigation on Iodine Intake and Thyroid Diseases (DanThyr) to identify and characterize determinants for hormonal levels in patients newly diagnosed with various nosological subtypes of thyrotoxicosis: GD, multinodular toxic goiter (MNTG), solitary toxic adenoma (STA), subacute thyroiditis (SAT), *post partum* thyroid dysfunction (PTPD),

amiodarone-associated thyrotoxicosis, post-radioiodine thyrotoxicosis, lithium-associated thyrotoxicosis, painless thyroiditis (PT), and thyroiditis due to surgical manipulation of the thyroid gland.

Subjects and methods

Identification, verification, and subtype classification of thyrotoxic patients

The study is part of DanThyr, which is an ongoing monitoring of the Danish iodine fortification program (11). This study deals with the period 1997–2000 just before the mandatory iodine fortification in Denmark, where we identified all new cases of primary overt thyrotoxicosis in two well-defined population cohorts (12). The two regions of inhabitancy were an area of mild iodine deficiency in Copenhagen in east Denmark ($n=227\ 632$, median urinary iodine excretion (UIE) in the population not taking vitamin supplements was $61\ \mu\text{g/l}$) and an area of moderate iodine deficiency around Aalborg in west Denmark ($n=311\ 102$, median UIE was $45\ \mu\text{g/l}$ (13)). The process of identification, verification, and subtype classification of cases with primary overt thyrotoxicosis has been described in detail (12, 14). In brief, patients were prospectively identified using a system monitoring the four diagnostic laboratories that performed all thyroid function tests requested by hospital and primary care institutions in the study areas: (i) Aalborg Hospital Laboratory; (ii) Bispebjerg Hospital Laboratory; (iii) Frederiksberg Hospital Laboratory; and (iv) Laboratory of General Practitioners in Copenhagen, (ii–iv) all in Copenhagen). Results of thyroid function tests were on a daily basis imported from the laboratory databases into a register database, and subjects with blood tests fulfilling the criteria of incident overt, biochemical thyrotoxicosis were identified for individual evaluation.

We subsequently gathered information from hospital medical records, general practitioners, additional laboratory tests performed, and thyroid scintigraphy, and used these data to verify and classify thyrotoxicosis ($n=1082$) into well-defined nosological types as previously defined in detail (15).

TSH, T_3 , and T_4 assays

All TSH, T_3 , and T_4 serum concentrations in this study were obtained at the time of diagnosis of thyrotoxicosis, i.e. before treatment was started (1). Aalborg Hospital Laboratory analyzed TSH by Immunoluminometric LUMitest from BRAHMS (Berlin, Germany), reference interval $0.3\text{--}4.5\ \text{mU/l}$; total T_4 and total T_3 by means of competitive RIA Amerlex-m T_4 and T_3 RIA kit by Ortho-Clinical Diagnostics (part of Johnson-Johnson, New Brunswick, NJ, USA), T_4 reference interval $60\text{--}140\ \text{nmol/l}$, and T_3 reference interval $1.2\text{--}2.7\ \text{nmol/l}$.

Bispebjerg Hospital Laboratory analyzed TSH by Micro-particle Enzyme Immunoassay, AxSYM Ultrasensitive hTSH II by Abbott Laboratories, reference interval $0.15\text{--}5.0\ \text{mU/l}$; T_4 by Fluorescence Polarization Immunoassay, AxSYM by Abbott Laboratories, reference interval $60\text{--}140\ \text{mU/l}$; and T_3 by Microparticle Enzyme Immunoassay, AxSYM T_3 by Abbott Laboratories, reference interval $1.2\text{--}2.3\ \text{nmol/l}$. Frederiksberg Hospital Laboratory analyzed TSH by Time-resolved Fluoroimmunoassay based on direct sandwich technique, AutoDelfia hTSH Ultra Kit by Wallac (Turku, Finland), reference interval $0.15\text{--}4.5\ \text{mU/l}$; T_4 and T_3 by Fluoroimmunoassay based on competitive reaction, AutoDelfia T_4 and T_3 Kit by Wallac, T_4 reference interval $60\text{--}160\ \text{nmol/l}$, and T_3 reference interval $0.9\text{--}2.7\ \text{nmol/l}$. T_4 results above $300\ \text{nmol/l}$ from this laboratory were recorded as $>300\ \text{nmol/l}$ ($n=18$). For calculations, they were given the value $339\ \text{mU/l}$, which corresponds to the median of T_4 values $>300\ \text{nmol/l}$ from the other three laboratories providing quantified T_4 levels also in this range. T_3 levels above $10\ \text{nmol/l}$ from the same laboratory were recorded as $>10\ \text{nmol/l}$ ($n=5$), and in this study, they were given the value $11.89\ \text{nmol/l}$, which was the median value of $T_3 >10\ \text{nmol/l}$ from the other three laboratories. The Laboratory of General Practitioners in Copenhagen analyzed TSH by Two-sided Chemiluminometric Immunoassay, Ciba Corning Diagnostics (Medfield, MA, USA) ACS TSH by Ciba Corning Diagnostics, reference interval $0.2\text{--}5.0\ \text{mU/l}$; T_4 and T_3 were analyzed using Competitive Immunoassay (Ciba Corning ACS T_4 and T_3 by Ciba Corning Diagnostics), T_4 reference interval $60\text{--}140\ \text{nmol/l}$, and T_3 reference interval $1.1\text{--}2.6\ \text{nmol/l}$. A detailed assay description including comparison between the four diagnostic laboratories has been given previously (14, 15). Of the 1082 thyrotoxic patients in this study, 1060 were diagnosed based on a combination of suppressed serum TSH and elevated T_3 and/or T_4 . The remaining 22 patients were diagnosed solely on the basis of TSH and free thyroid hormones.

TRAb assay

TRAb levels were measured using DYNOTest TRAK human by BRAHMS. TRAb was measured within a short time after thyrotoxicosis was diagnosed (median (interquartile range defined as the 25–75% range): 51 (37–79) days). TRAb measurements $>1.0\ \text{IU/l}$ were regarded as antibody positive (TRAb+) (16). Patients with diffuse and good scintigraphic uptake and no measurable TRAb were classified as TRAb-negative GD.

Additional patient information

We obtained funding to perform comprehensive investigation of patients newly diagnosed with thyrotoxicosis during the study period 1997–2000. In periods where funding was available, patients with newly diagnosed

thyrotoxicosis ($n=685$) were invited, and 68.5% ($n=469$) joined a comprehensive investigation (GD, $n=288$; MNTG, $n=111$; STA, $n=34$; other subtypes, $n=36$). This included collection of information on lifetime smoking habits, alcohol consumption, and intake of vitamin supplements and estrogens (oral contraceptives (OC) or postmenopausal hormone replacement therapy (HRT)). Patients were asked whether they had suffered from non-thyroidal diseases, and co-morbidity was defined present if they had previously suffered from acute myocardial infarction, angina pectoris, cardiac arrhythmia, hypertension, cerebral stroke, epilepsy, diabetes mellitus, asthma, chronic obstructive pulmonary disease, or gastrointestinal ulcer.

Statistical analysis

We used Statistical Package for Social Sciences version 15.0 (SPSS) for the calculations. Comparisons between groups were performed using Mann–Whitney U test and Kruskal–Wallis test. Multivariate linear regression analyses including hormonal status ($\log T_3$, $\log T_4$, or T_3/T_4 , all normally distributed) as dependent variables were performed in two models. Model A included sex, age, and region as explanatory variables and was performed in all patients diagnosed with the three most common types of thyrotoxicosis: GD ($n=484$), MNTG ($n=405$), and STA ($n=71$). Model B, comprising those who attended the comprehensive investigational program and had filled out questionnaires (GD ($n=288$), MNTG ($n=111$), and STA ($n=34$)), included as explanatory variables also smoking habits, alcohol consumption, intake of vitamin supplements, co-morbidity, and TRAb concentrations. In models, we excluded variables that did not meet $P < 5\%$. In multivariate regression exploring eight variables, the P value to be significant was $< 0.64\%$ (Bonferroni's correction (17), $P = 1 - \sqrt[8]{0.95}$).

Ethical approval

This study was approved by the Regional Ethics Committee in North Jutland and Copenhagen. Registry permission was obtained from the Danish Data Protection Agency. All participants of the comprehensive investigational program gave their written informed consent. No conflicts of interest have occurred during implementation or completion of the study.

Results

The age and gender distributions of patients suffering from ten well-defined nosological subtypes of thyrotoxicosis are given in Table 1. Median age at diagnosis of the various types spanned from 30.7 years in women diagnosed with *post partum* thyrotoxicosis up to 69.6 years in patients suffering from MNTG. Most subtypes were more common in women (1).

Table 1 Number of patients, age, and sex in ten types of thyrotoxicosis.

Nosological subtype of thyrotoxicosis	<i>n</i>	Age (median and IQR ^a)	Sex (F/M, women %)
All types, combined	1082	54.2 (39.5–69.8)	890/192 (82.3)
GD	484	44.2 (32.8–56.5)	398/86 (82.2)
MNTG	405	69.6 (56.4–77.6)	350/55 (86.6)
STA	71	65.8 (51.9–72.4)	46/25 (64.8)
SAT	38	44.5 (37.7–49.5)	31/7 (81.6)
PPTD ^b	37	30.7 (25.5–33.4)	37/0 (100)
Amiodarone associated	14	56.1 (40.9–74.2)	3/11 (21.4)
Post radioiodine	12	64.0 (46.8–66.8)	10/2 (83.3)
Lithium associated	11	56.5 (40.2–70.7)	9/2 (81.8)
PT ^c	9	44.3 (32.0–78.6)	0/9 (0)
Manipulation thyroiditis ^d	1	53.5	1/0 (100)

GD, Graves' disease; MNTG, multinodular toxic goiter; PT, painless thyroiditis; STA, solitary toxic adenoma.

^aInterquartile range (IQR) depicting 25 and 75% percentiles.

^bFirst-time thyrotoxicosis within 1 year after delivery (PPTD, *post partum* thyroid dysfunction).

^cCriteria for subtype classification were thyroid scintigraphic picture with no uptake, no signs/symptoms suggesting subacute thyroiditis (SAT), no delivery within 1 year, no contrast media given within 1 year, and no recent neck surgery.

^dTransient thyrotoxicosis was diagnosed 2 days after surgery for a PTH-producing adenoma.

Serum T_3 and T_4 in subtypes of thyrotoxicosis

Ordered comparison of serum T_3 , serum T_4 , and $T_3:T_4$ ratio in each of the ten subtypes of thyrotoxicosis is depicted in Fig. 1. Serum T_3 concentrations were particularly high in GD patients (Fig. 1a), whereas patients with amiodarone-associated thyrotoxicosis had the highest T_4 concentrations (Fig. 1b). The highest $T_3:T_4$ ratios were found in patients with GD, whereas a relatively low serum $T_3:T_4$ ratio was present in manipulation thyroiditis, SAT, amiodarone-induced thyrotoxicosis, and PT (Fig. 1c).

The distributions of serum T_3 , serum T_4 , and $T_3:T_4$ ratio in patients suffering from the three main subtypes of thyrotoxicosis (GD, MNTG, and STA) are shown in Fig. 2. Evidently, patients with Graves' thyrotoxicosis had higher and more dispersed concentrations of serum T_3 (Fig. 2a) but also of serum T_4 (Fig. 2b). They also exhibited higher $T_3:T_4$ ratios than patients suffering from MNTG and STA (Fig. 2c) but with a considerable overlap. The hormone levels in MNTG and STA were comparable.

T_3 , T_4 , and T_3/T_4 in subgroups

The levels of T_3 , T_4 , and T_3/T_4 in GD, MNTG, and STA stratified on sex, age, region of inhabitancy, smoking habits, alcohol intake, iodine-containing vitamin supplements, co-morbidity, and TRAb (positive/negative) are depicted in Table 2. Young patients had high serum T_3 levels in both GD and MNTG, and $T_3:T_4$ ratios were highest in patients aged below 60 years.

Figure 1 Serum (a) T_3 , (b) T_4 , and (c) $T_3:T_4$ ratio in ten nosological types of newly diagnosed thyrotoxicosis ranked from high to low mean concentrations. Geometric means ($\pm$ S.E.M.) are depicted for T_3 and T_4 and means for $T_3:T_4$ ratios. $T_3:T_4$ ratios are expressed as molar ratios multiplied by 1000. STA, solitary toxic adenoma; PPTD, *post partum* thyroid dysfunction with thyrotoxicosis at the time of diagnosis; MNTG, multinodular toxic goiter; SAT, subacute thyroiditis with thyrotoxicosis at the time of diagnosis.

In GD, alcohol abstainers had somewhat lower T_3 and $T_3:T_4$ and marginally lower T_4 values. Finally, GD patients categorized as TRAb positive (91.8% of those who had TRAb measured) had higher T_3 and T_4 compared with those without measurable TRAb. We found no differences in hormone levels after stratification on sex, smoking habits, iodine supplementation, or co-morbidity.

Notably, patients diagnosed with PPTD had somewhat higher $T_3:T_4$ ratios compared with patients with

SAT or silent thyroiditis thyrotoxicosis. A putative mechanism might be that thyroglobulin contained a relatively higher T_3 than T_4 during the *post partum* period caused by worsening of iodine deficiency during pregnancy and lactation (18, 19). Another mechanism might be misclassification. Of all 1082 patients, 59 were diagnosed *post partum*, and 22 of these suffered from GD, leaving 37 patients with PPTD. Of those, 24 were classified with certainty to suffer from PPTD (spontaneous normalization of TSH, T_3 , and T_4 without treatment and/or no 99-Tcchnetium uptake on thyroid scintigraphy), whereas no information on TRAb, scintigraphy, or later thyroid function tests was available in 13 patients. However, we found no difference in T_3/T_4 levels between certain and possible PPTD patients (18.6 vs 20.9, $P=0.11$).

At the time of diagnosis, a few patients were pregnant (GD, $n=3$; MNTG, $n=1$; and no STA patients), and some received OC (GD, $n=49$; MNTG, $n=18$; and STA, $n=9$) or postmenopausal HRT (GD, $n=11$; MNTG, $n=9$; and STA, $n=4$). As might be expected, serum T_3 and T_4 levels were higher in these patients. In GD, use of OC was associated with higher serum T_4 levels (OC users vs non-OC users, 256 vs 202 nmol/l, $P<0.001$) and higher serum T_3 levels (7.20 vs 5.00, $P=0.02$). However, exclusion of patients who were pregnant or received estrogens did not alter the pattern of differences between types of disease or the association with the other variables investigated.

Determinants for hormonal levels in GD, MNTG, and STA

In the multivariate regression analyses, we explored which variables were associated with serum T_3 , T_4 , and $T_3:T_4$ ratio. Results are embedded in Table 2, and we confirmed in the models that high age was a strong determinant for low levels of thyroid hormone (especially serum T_3 , but also T_4) in GD, MNTG, and STA and that patients living in the region with higher iodine intake had higher biochemical degree of thyrotoxicosis. Positive TRAb was positively associated with level of serum T_3 in particular, but also with serum T_4 , and the $T_3:T_4$ ratio.

The interaction between age and type of disease on serum T_3 and T_4 concentrations is illustrated in Fig. 3. Young (<40 years old) patients with GD had a 1.8 times higher T_3 and a 1.5 times higher T_4 at diagnosis compared with those with nodular toxic disease (MNTG and STA combined). On the other hand, no hormonal difference between the two subtypes of disease was observed in the group of patients aged 80 years and above.

Discussion

Patients newly diagnosed with thyrotoxicosis present with a spectrum of biochemical abnormalities that may

Figure 2 Serum (a) T_3 , (b) T_4 , and (c) T_3/T_4 distributions in the three most common types of thyrotoxicosis: Graves' disease (GD), multinodular toxic goiter (MNTG), and solitary toxic adenoma (STA). $T_3:T_4$ ratios are expressed as molar ratios multiplied by 1000. Geometric mean of T_3 : GD vs MNTG+STA, 5.2 vs 3.0 nmol/l, $P<0.001$. Geometric mean of T_4 : GD vs MNTG+STA, 216 vs 162 nmol/l, $P<0.001$. $T_3:T_4$ ratio: GD vs MNTG+STA: 24.7 vs 18.7×10^{-3} , $P<0.001$.

depend on type of disease as well as various patient characteristics. Our study is the first to describe such diversity in a cohort of thyrotoxic patients diagnosed in a population.

Serum T_3 and T_4 in subtypes of thyrotoxicosis

Many studies have reported T_3 and T_4 levels in patients diagnosed with all types of thyrotoxicosis combined (20) or in a few (4, 21, 22, 23) or even up to four nosological subtypes of thyrotoxicosis (24). In this study, comparing ten nosological types of thyrotoxicosis, GD patients presented the highest T_3 level and $T_3:T_4$ ratio, which is in accordance with other studies (4, 22, 23, 25). Similarly, amiodarone-associated thyrotoxicosis was dominated by high serum T_4 levels as previously reported by Martino *et al.* (26). In general, our patients had somewhat lower serum T_3 and T_4 levels than in many other studies. A likely reason for this difference is the population-based nature of our study, whereas other studies have been based on patients referred from primary care (27). Other possible causes may be earlier detection of Graves' hyperthyroidism in Denmark, differential disease classification, different iodine intake levels, or different ages of patients at onset. Low $T_3:T_4$ ratios below 20 have been reported in thyrotoxic patients suffering from SAT (25, 28) and PT (21, 25). This is confirmed in our study where we calculated mean $T_3:T_4$ ratios to be 16.0 in SAT and 15.0 in PT. In addition, we found that GD patients had higher T_3 and T_4 levels compared with MNTG but not among those aged 80+ years. It must be emphasized that there is a considerable overlap in serum T_3 and T_4 in the different subtypes both in this and in other studies (5, 25, 29). However, a serum $T_3 \geq 5.0$ mmol/l (approximately twice the upper reference limit) was nearly only seen in patients with GD, where 52.6% had such severe abnormality compared with only 4.0/5.2% of patients newly diagnosed with MNTG/STA.

Determinants for hormonal levels in GD, MNTG, and STA

Age was a strong determinant for thyroid hormone levels in patients suffering from GD, MNTG, and STA. A few studies also found lower T_3 (30, 31) and T_4 (30) levels among the elderly thyrotoxic patients not classified into specific subtype of disorder. Aizawa *et al.* (2) studying 371 GD patients and Allahabadia *et al.* (3) reporting on 536 GD patients also found lower T_3 and T_4 concentrations among elderly patients.

Several studies have reported a connection between iodine intake and the prevalence of T_3 and T_4 toxicosis, the former being more prevalent in iodine-deficient areas (4, 32) and the latter dominating in areas with a higher iodine intake (33). We found that T_4 was higher in GD and MNTG patients diagnosed in the region with the highest iodine intake.

Table 2 Serum T_3 , serum T_4 , and molar $T_3:T_4$ ratio at the time of diagnosis in three common types of overt hyperthyroidism (GD, MNTG, and STA). Values of serum T_3 , serum T_4 , and molar $T_3:T_4$ ratio were stratified on sex, age, region, smoking status, alcohol intake, iodine supplements, co-morbidity, and TRAb.

	GD				MNTG				STA			
	n^a	Serum T_3^b Median (IQR)	Serum T_4^b Median (IQR)	$T_3/T_4^b \times 10^3$ Median (IQR)	n^a	Serum T_3^b Median (IQR)	Serum T_4^b Median (IQR)	$T_3/T_4^b \times 10^3$ Median (IQR)	n^a	Serum T_3^b Median (IQR)	Serum T_4^b Median (IQR)	$T_3/T_4^b \times 10^3$ Median (IQR)
Sex												
Women	398	5.20 (3.73–7.38)	217 (177–266)	23.9 (20.3–29.5)	350	3.00 (2.58–3.40)	159 (144–185)	18.8 (15.5–21.5)	46	2.96 (2.80–3.41)	145 (124–168)	22.2 (17.9–24.6)
Men	86	5.02 (3.20–7.14)	218 (171–281)	24.9 (19.6–29.1)	55	2.92 (2.40–3.60)	161 (140–178)	19.2 (14.3–24.4)	25	3.27 (2.97–3.78)	144 (131–162)	22.5 (20.6–25.5)
Multivariate testing (P)		NS	NS	NS		NS	NS	NS		NS	NS	NS
Age (years)												
0–40	204	6.30 (4.29–8.70)	237 (193–287)	26.4 (22.2–32.0)	13	3.30 (2.85–4.60)	161 (149–195)	20.9 (17.1–23.7)	7	3.30 (2.96–3.40)	125 (108–155)	23.2 (21.9–27.5)
40–60	182	4.93 (3.70–6.69)	203 (172–254)	23.8 (20.4–28.9)	111	3.15 (2.86–3.77)	157 (139–186)	20.3 (18.4–23.3)	20	3.32 (2.81–4.60)	145 (119–188)	24.6 (23.2–28.5)
Above 60	98	3.95 (2.96–5.53)	202 (163–228)	20.9 (16.7–25.1)	281	2.90 (2.30–3.27)	159 (144–185)	17.7 (13.9–21.0)	44	3.00 (2.78–3.32)	146 (134–162)	20.8 (17.3–23.8)
Multivariate testing (P)		<0.001	<0.001	<0.001		<0.001	NS	<0.001		0.028	NS	NS
Region ^c												
Moderate ID	293	4.95 (3.67–7.03)	205 (167–251)	23.9 (20.3–28.8)	252	3.07 (2.71–3.40)	155 (143–178)	19.3 (16.5–22.4)	22	3.05 (2.80–3.56)	141 (124–159)	23.0 (20.6–25.9)
Mild ID	191	5.85 (3.90–8.01)	230 (190–289)	24.8 (20.1–31.1)	153	2.90 (2.20–3.50)	168 (145–201)	17.2 (12.4–21.0)	19	3.10 (2.80–3.46)	160 (140–199)	24.4 (16.7–24.4)
Multivariate testing (P)		0.018	<0.001	NS		NS	<0.001	0.024		NS	NS	NS
Smoking status ^d												
Current smoker	112	5.00 (3.80–7.20)	212 (174–269)	24.2 (20.3–29.0)	47	3.10 (2.78–3.46)	145 (135–177)	21.2 (18.3–23.5)	15	3.39 (2.88–4.17)	145 (119–166)	24.6 (20.5–26.5)
Non-smoker	172	5.25 (3.70–7.40)	212 (170–270)	24.1 (21.4–29.5)	63	3.00 (2.78–3.43)	152 (140–174)	19.7 (17.2–22.7)	16	2.95 (2.78–3.49)	131 (125–158)	22.7 (19.3–28.5)
Multivariate testing (P)		NS	NS	NS		NS	NS	NS		NS	NS	NS
Alcohol intake ^e												
0 units/week	76	4.30 (3.33–6.35)	194 (168–250)	22.8 (19.2–26.8)	40	3.00 (2.80–3.61)	148 (136–176)	19.6 (16.5–23.2)	5	2.98 (2.44–4.28)	148 (119–185)	23.8 (16.1–27.3)
≥1 unit/week	207	5.60 (3.90–7.44)	219 (174–274)	24.8 (21.9–29.9)	61	3.04 (2.78–3.41)	153 (138–178)	20.5 (17.9–22.9)	26	2.98 (2.80–3.75)	138 (124–161)	24.1 (20.8–28.0)
Multivariate testing (P)		NS	NS	0.024		NS	NS	NS		NS	NS	NS
Iodine supplements ^f												
0 µg/day	155	5.40 (3.87–7.78)	218 (181–281)	24.6 (20.2–30.7)	66	3.01 (2.75–3.41)	148 (137–173)	19.9 (17.6–23.5)	16	2.96 (2.79–3.30)	129 (118–153)	25.2 (20.7–28.6)
1–149 µg/day	16	4.94 (3.70–8.90)	185 (158–277)	26.7 (22.6–32.1)	6	3.02 (2.30–3.76)	178 (155–173)	19.2 (12.9–21.8)	3	4.24 (4.10–4.80)	195 (166–230)	24.6 (17.8–25.5)
≥150 µg/day	69	4.93 (3.61–6.82)	204 (162–275)	23.5 (20.1–29.0)	21	3.06 (2.79–3.43)	157 (136–180)	19.8 (17.1–21.5)	10	3.10 (2.96–3.65)	146 (134–161)	23.6 (19.8–24.5)
Multivariate testing (P)		NS	NS	NS		NS	NS	NS		NS	NS	0.041
Co-morbidity ^g												
Yes	126	4.80 (3.50–7.15)	204 (166–251)	24.0 (20.3–29.0)	70	3.05 (2.80–3.56)	147 (136–176)	20.6 (17.7–23.5)	20	3.03 (2.78–3.88)	145 (129–164)	21.3 (18.6–26.3)
No	158	5.59 (3.88–7.60)	219 (177–276)	24.3 (21.8–29.4)	40	2.99 (2.70–3.25)	154 (137–176)	19.8 (17.8–21.8)	11	2.98 (2.80–3.60)	125 (108–158)	24.8 (22.8–28.2)
Multivariate testing (P)		NS	NS	NS		NS	NS	NS		NS	NS	NS
TRAb												
TRAb+ (≥1 kU/l)	259	5.35 (3.90–7.42)	212 (176–271)	24.4 (21.8–29.8)	— ^h	— ^h	— ^h	— ^h	— ^h	— ^h	— ^h	— ^h
TRAb –	23	2.90 (2.64–3.46)	158 (148–179)	18.8 (14.7–20.5)								
Multivariate testing (P)		<0.001	<0.001	<0.001								

^aInformation on sex, age, and region of inhabitancy was obtained for all patients diagnosed with Graves' disease (GD)/multinodular toxic goiter (MNTG)/solitary toxic adenoma (STA) ($n=484/405/71$). Information on remaining variables was obtained for those patients (GD/MNTG/STA, $n=288/111/26$) who attended the investigational program except for a few missing values.

^bMultivariate linear regression analysis including hormonal status ($\log T_3$, $\log T_4$, or T_3/T_4) as dependent variables, and sex, age, region, smoking habits, alcohol consumption, intake of vitamin supplements, co-morbidity, and TRAb concentrations as explanatory variables.

^cTwo regions represented moderate iodine deficiency (Aalborg: $n=311$; median urinary iodine excretion (UIE) in the population was 45 µg/l) and mild iodine deficiency (Copenhagen; $n=227$ 632; median UIE was 61 µg/l (1)) in people with no intake of vitamin supplements.

^dSmoking history was dichotomized into current and non-current smokers. Validity of smoking status has been verified in a previous DanThyr study (8).

^eAlcohol intake (units/week) of wine, beer, spirits, and liquor were added into a variable of total weekly alcohol consumption.

^fInformation on vitamin supplement intake was obtained from questionnaire. No patient diagnosed with GD, MNTG, or STA received iodine-containing medication.

^gCo-morbidity was defined present if patients had previously suffered from acute myocardial infarction, angina pectoris, cardiac arrhythmia, hypertension, cerebral stroke, epilepsy, diabetes mellitus, asthma, chronic obstructive pulmonary disease, or gastrointestinal ulcer.

^hAll patients with MNTG and STA were TRAb negative.

Figure 3 Age-stratified serum (a) T_3 and (b) T_4 concentrations in Graves' disease (GD; $n=484$) and nodular toxic goiter (multinodular toxic goiter and solitary toxic adenoma combined, $n=405+71$). Plots indicate geometric mean $\pm$ S.E.M. of total T_3 and T_4 concentrations. Patients diagnosed with GD compared with those with nodular toxic goiter: * $P < 0.01$ and ** $P < 0.001$.

This may possibly be caused by iodine being available for T_4 production with higher iodine intake. On the other hand, we found no association between iodine intake from vitamin supplements and the degree of biochemical thyrotoxicosis at disease onset. Finally, TRAb-negative GD patients had mild thyrotoxicosis, which is in accordance with most (34, 35, 36) but not all studies (5).

Based on our findings, two extremes of GD patients were identified: a group of women aged <40 years living in Copenhagen with only mild iodine deficiency and having measurable TRAb (high T_3 group), and at the other end of the spectrum a group of TRAb-negative patients aged ≥ 40 years who lived in and around Aalborg with moderate iodine-deficiency (low T_3 group). Comparison between these two groups revealed that the first group had 2.7 times higher serum T_3 (6.97 vs 2.61, $P < 0.001$, data not shown).

Pathophysiological mechanisms

In euthyroid subjects, around 20% of circulating T_3 is secreted by the thyroid gland, whereas 80% originates from peripheral deiodination from T_4 (37). However, in several pathological states, a relatively higher fraction of T_3 production takes place in the thyroid gland (18). The mechanisms are several, including a relatively high T_3 content in thyroglobulin (38, 39) and especially a very active deiodination of T_4 to T_3 by type I iodothyronine deiodinase in the hyperactive thyroid gland (23). Moreover, enhancement of intrathyroidal iodine deficiency caused by the high thyroidal hormone production and turnover may play a role (40). High T_3 content of thyroglobulin (41) and high thyroidal type I iodothyronine deiodinase activity (23) may also be the major mechanisms behind the relatively high serum T_3 in nodular thyroid disease.

Our study gives no insight into the mechanism behind the severe biochemical thyrotoxicosis often observed among young patients. It may be speculated that the higher serum T_3 in young patients may arise from a more responsive thyroid gland to stimulation, to differences in metabolism of thyroid hormones, or perhaps due to the higher number of thyroid function tests performed among elderly patients consulting their general practitioner for unspecific complaints. In GD, Vos *et al.* (6) illustrated higher TRAb levels in the young. We found some evidence for the latter explanation, as median TRAb levels were 9.6, 7.5 and 6.1 IU/l among GD patients aged <40 , 40–60 and 60+ years of age respectively ($P=0.038$, data not shown).

The low $T_3:T_4$ ratios found in SAT and silent thyroiditis may be due to the fact that T_4 leaving the damaged thyroid gland escapes the thyroidal deiodinases, but it may also be due to a relatively low activity of the deiodinases, as these enzymes are inhibited by interferon and interleukins (42).

Strengths and limitations

A major strength of this study is that all patients diagnosed with thyrotoxicosis in a defined population were included, both patients diagnosed in hospital and patients referred from primary care to hospital, but also the large fraction of patients entirely taken care of by the general practitioners. Most other studies were based on patients referred to hospital, and it may be difficult to compare studies, as the referral pattern may differ (27). This study identified patients who for some reason had thyroid testing performed. Thus, we missed patients who never had their thyroid function tested. A major limitation is that information on smoking habits, alcohol intake, iodine supplementation, use of estrogens, and co-morbidity was obtained only by questionnaires and in a limited subset of patients.

Another limitation is that we had only total T_3 and T_4 available, as changes in thyroid hormone binding

capacity may influence iodothyronine concentrations (43). Principal findings were unaltered when we excluded patients with high estrogen levels from pregnancy or estrogen intake.

We included a number of possible confounders as explanatory variables. However, cancer was not asked for in the questionnaires. Therefore, our co-morbidity index is not fully comparable with, e.g. Charlson's co-morbidity index.

One possible determinant for hormonal levels not investigated in this study is selenium. Selenium status has been proposed to have an effect on occurrence of thyroid disease (44), and in euthyroid subjects, a low T₃/T₄ had strong association with low selenium among the elderly (45).

Conclusion

This is the first study describing the phenomenology throughout the entire spectrum of thyrotoxic entities. We found that young patients suffering from GD and MNTG had a much higher degree of biochemical thyrotoxicosis compared with the elderly patients. This may challenge the diagnostic process in elderly subjects, who may only show subtle signs and symptoms suggestive of a thyrotoxic state.

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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Author contribution statement

Drs P Laurberg and A Carlé had full access to all data in the study and take full responsibility for the integrity of the data and the accuracy of all analyses. P Laurberg, H Perrild, N Knudsen, L Ovesen, L B Rasmussen, I B Pedersen, and A Carlé were involved in study concept and design. A Carlé, I B Pedersen, and N Knudsen involved in acquisition of data. A Carlé, P Laurberg, N Knudsen, I B Pedersen, L Ovesen, H Perrild, and L B Rasmussen were involved in analysis and interpretation of data. A Carlé drafted the manuscript. P Laurberg, H Perrild, N Knudsen, L Ovesen, L B Rasmussen, and I B Pedersen performed critical revision of the manuscript for important intellectual content and final approval. Statistical analyses were performed by A Carlé and P Laurberg. A Carlé, P Laurberg, H Perrild, N Knudsen, L Ovesen, L B Rasmussen, and I B Pedersen obtained funding.

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