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Antibodies to Thyroid Peroxidase and Thyroglobulin in Iodine Deficiencies

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Abstract

Autoimmune thyroiditis (AITD) is one of the most common autoimmune disorders. The etiology is unknown, but both genetic and environmental factors may be involved, including iodine intake. Nearly all patients with chronic AITD have high concentrations of thyroglobulin antibodies (Tg-Ab) and thyroperoxidase antibodies (TPO-Ab) in serum. Circulating thyroid autoantibodies are also common in population studies of apparently healthy subjects. Epidemiological studies on thyroid autoimmunity are difficult to compare, due to differences in the kinds of biochemical and epidemiological methods applied. The assays for measuring TPO-Ab and Tg-Ab have improved recently, but no reference ranges exist and different cutoff values are often chosen. A sudden increase in iodine intake by an iodine-deficient population may induce enhanced thyroid autoimmunity, although the findings are ambiguous. The exact mechanism behind such an increase in autoimmunity is unknown, but damage to thyroid tissue by free radicals and enhanced antigenicity of thyroglobulin may be involved. TPO-Ab and Tg-Ab are common both in populations with a stable high iodine intake and those with mild and moderate iodine deficiency (ID). The mechanisms behind the development of thyroid antibodies in ID may be different from the mechanisms involved in high iodine intake. It is possible that antibodies in some cases may develop secondarily to goiter formation, with exposure or release of Tg and other antigens from the thyroid. Further studies are needed to obtain information on the interplay between iodine intake and thyroid autoimmunity, but adjustment of population iodine intake to around the recommended level may be optimal for the prevention of thyroid disease.

Abbreviations

AITD	Autoimmune thyroiditis
ELISA	Enzyme-linked immunosorbent assays
ICCIDD	International Council for Control of Iodine Deficiency Disorders
ID	Iodine deficiency
IRMA	Immunoradiometric assays
Mic	Microsomal
MRC	Medical Research Council
RIA	Radioimmunoassays
Tg	Thyroglobulin
Tg-Ab	Thyroglobulin antibody
TPO	Thyroid peroxidase
TPO-Ab	Thyroid peroxidase antibody
UNICEF	United Nations Children's Fund
WHO	World Health Organization

Introduction

Autoimmune diseases are a poorly-understood group of disorders, which have been defined as clinical syndromes caused by activation of T or B lymphocytes or both, in the absence of ongoing infections or other discernible causes (Davidson and Daimond, 2001). Regulatory malfunction of the immune system is supposed to be secondary to a genetic predisposition currently thought to be multigenetic (Davidson and Daimond, 2001). However, even in a genetically predisposed person, a triggering event is probably required for frank autoreactivity, although knowledge of the nature of this trigger is often limited or unknown (Safran *et al.*, 1987; Tomer and Davies, 1993).

Autoimmune thyroiditis (AITD) is one of the most common autoimmune disorders. The humoral immune response is dominant in Graves' hyperthyroidism, whereas cellular immune response is more dominant in hypothyroidism caused by chronic AITD (Marcocci and Chiovato, 2000). Thyroid autoantibodies are proteins manufactured by the immune system that are directed against proteins in the thyroid. Although nearly all patients with chronic

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AITD have high concentrations of circulating thyroid autoantibodies (Arai *et al.*, 2000; Carle *et al.*, 2006; Feldt-Rasmussen, 1996), for the most part the disorder appears to be the consequence of tissue damage initiated by T lymphocytes (Weetman and McGregor, 1994). Measurement of autoantibodies against thyroid peroxidase (TPO-Ab) and thyroglobulin (Tg-Ab) has for many years been a major tool in the diagnosis of autoimmune thyroid diseases, such as Hashimoto's thyroiditis, primary myxedema and postpartum thyroiditis (Feldt-Rasmussen *et al.*, 1991; Feldt-Rasmussen, 1996).

Among the many environmental factors that have been suggested to take part in the development of thyroid autoimmunity, iodine intake may be the most important (Prummel *et al.*, 2004).

Thyroglobulin and thyroglobulin antibody

Thyroglobulin (Tg) is a large 660 kDa dimeric glycoprotein composed of two identical polypeptide chains, and is unique in its content of iodinated amino acids. Most iodinated amino acids in Tg are iodotyrosines, which serve as precursors of the biologically active thyroid hormones, thyroxine and triiodothyronine (Tomer, 1997). Tg is produced by the thyroid follicular cells and secreted into the follicular lumen, where it is stored as colloid. Small amounts of Tg are present in the circulation, which is primarily of clinical importance in diagnosing the persistence or recurrence of thyroid cancer after ablative therapy (Spencer *et al.*, 1999). However, Tg in serum is increased in almost all kinds of thyroid disease, including goiter (Knudsen *et al.*, 2001) and subacute thyroiditis (Hidaka *et al.*, 1994), the concentrations overlapping with those in healthy individuals.

Human Tg is one of the main autoantigenes in thyroid disease caused by AITD (Salvi *et al.*, 1988), but antibodies against Tg are also frequently measurable in serum from apparently healthy subjects from the population (Hollowell *et al.*, 1998; Pedersen *et al.*, 2003). Iodination of Tg may induce major changes in its stereochemical configuration (Dunn *et al.*, 1983), which may change its immunoreactivity and be important in the generation of thyroid autoantibodies (Saboori *et al.*, 1998).

Methods for the measurement of Tg-Ab

Tg-Ab was initially measured by passive tanned red cell hemagglutination, and the results were reported simply as positive or negative or as the dilution of serum giving a positive response (titer).

Subsequently, much more sensitive methods including radioimmunoassays (RIA), immunoradiometric assays (IRMA), enzyme-linked immunosorbent assays (ELISA), and chemiluminescence assays have been developed. The latter methods are now used in clinical practice, and the results are usually given quantitatively, in some cases in international units calibrated against the Medical Research Council (MRC) 65/93 Feldt-Rasmussen (1996). Several studies have found large differences in the performance of different assays for measuring Tg-Ab (Arai *et al.*, 2000; Feldt-Rasmussen, 1996; Lindberg *et al.*, 2001).

Figure 60.1 illustrates the discrepancy between the results obtained by different generations of assays. A new assay based on precipitation of ^{125}I Tg bound to Tg-Ab by the use of polyethylene glycol was compared with a commercially-available passive hemagglutination test (Laurberg and Pedersen, 1988). Twelve of 60 normal subjects and nearly all of 72 patients with various thyroid disorders had Tg-Ab detected by the new assay. On the other hand, none of the normal subjects and very few of the patients had Tg-Ab measured by the hemagglutination assay. The hemagglutination assay had not only a much lower sensitivity, but also a lower specificity, as some of the sera with very high concentrations of Tg-Ab did not give detectable agglutination.

Thyroid peroxidase and thyroid peroxidase antibody

Thyroid peroxidase (TPO) is a 100-kDa poorly glycosylated membrane-bound enzyme containing a heme prosthetic group. The enzyme is expressed on the apical membrane of thyrocytes, facing the colloid, where it is

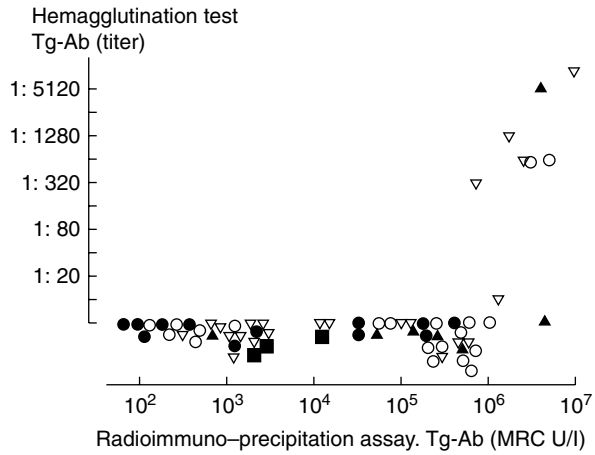

Figure 60.1 Tg-Ab concentrations in sera measured with both a newly-developed radioimmunoprecipitation assay and a commercially available passive hemagglutination assay (Wellcome, UK). (●) Normal subjects (12 out of 60 had Tg-Ab with the new assay); (▽) patients after treatment for Graves' disease (23 out of 25 had Tg-Ab); (○) patients after treatment for multinodular toxic goiter or with atoxic multinodular goiter (20 out of 37 had Tg-Ab); (▲) patients after treatment of spontaneously developed hypothyroidism (n = 7, all had Tg-Ab); (■) patients after previously subacute thyroiditis, (n = 3, all had Tg-Ab). Data from Laurberg and Pedersen (1988) with permission.

responsible for catalyzing iodine oxidation, iodination of tyrosine residues and coupling of iodotyrosines to generate thyroid hormones (McLachlan and Rapoport, 1992). TPO is identical with the previously defined thyroid microsomal antigen (Marcocci and Chiovato, 2000), and measurement of TPO-Ab has replaced measurements of antibodies against the microsomal antigen (Mic-Ab).

TPO, together with Tg, is the main autoantigen in AITD. Like Tg-Ab, TPO-Ab is found in the majority of patients with autoimmune thyroid diseases, and is also commonly measurable in apparently healthy subjects without symptoms or signs of thyroid disease (Hollowell *et al.*, 1998; Pedersen *et al.*, 2003). However, in the population, there is a considerably stronger association between elevated serum TSH, as a sign of impending thyroid failure, and the presence of TPO-Ab, than between TSH and Tg-Ab (**Figure 60.2**) (Bülow Pedersen, *et al.*, 2005).

Methods for measurement of TPO-Ab

Several methods are available for measurement of TPO-Ab in serum. Most early studies were done with assays based on immunoflourescence or passive tanned erythrocyte hemagglutination using crude thyroid microsomes as antigen, and the results were reported as positive or negative or as an antibody titer. After identification of TPO as the microsomal antigen, more sensitive methods for detecting TPO-Ab have been established, including RIA, IRMA, ELISA and chemiluminescence methods (Dherbomez *et al.*, 2000; Feldt-Rasmussen, 1996). Most TPO-Ab assays provide the antibody results in international units by using the standard MRC 66/387 with a calibration factor (Feldt-Rasmussen, 1996).

Measurement of antimicrosomal antibody has been associated with a low specificity due to interfering factors, such as the presence of Tg in the "purified" microsomes or other antibodies also reacting with the thyroid microsomal fraction (Feldt-Rasmussen *et al.*, 1991). The higher specificity and sensitivity of the newer assays have been shown in a number of studies (Arai *et al.*, 2000; Kasagi *et al.*, 1996; Feldt-Rasmussen *et al.*, 1983; Lindberg *et al.*, 2001; Roti *et al.*, 1992).

Detection of Tg-Ab and TPO-Ab in healthy individuals

Most authors define TPO-Ab and Tg-Ab positivity as the detection of antibodies above a certain assay detection limit (Hollowell *et al.*, 1998; Laurberg *et al.*, 1998; Pedersen *et al.*, 2003). However, it has also been suggested that antibodies are present at low concentrations in nearly all subjects (Jensen *et al.*, 2006; Zophel *et al.*, 2003). Zophel *et al.* (2003) found TPO-Ab in 1277 out of 1295 healthy subjects without signs of thyroid abnormalities. In the low measurable range below 1.1 IU/ml, TPO-Ab values followed a normal distribution that was independent of age and sex, and the authors concluded that a reference range independent of the population investigated

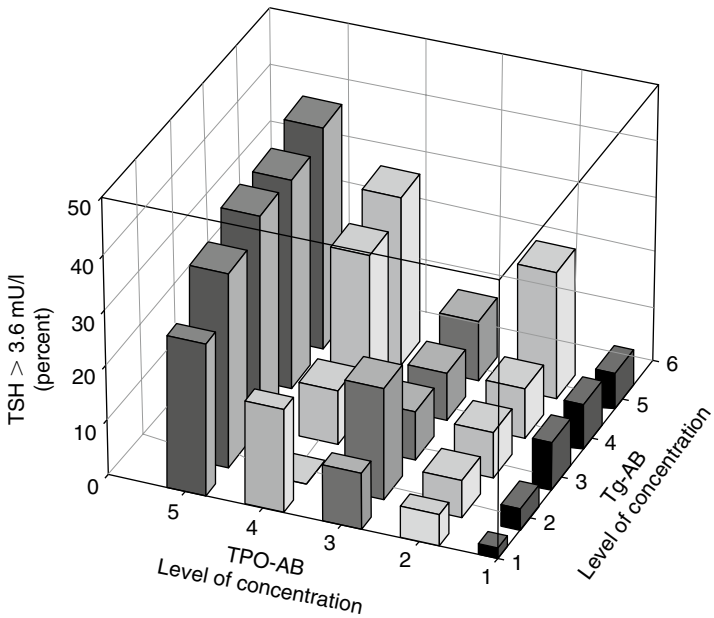

Figure 60.2 Prevalence rates (%) of elevated serum TSH at different concentrations of TPO-Ab and Tg-Ab (level 1: no antibodies, level 2–5: increasing antibody concentrations, i.e., quartiles of antibody positive participants). The prevalence of elevated TSH increased with increasing level of TPO-Ab (p for trend < 0.005). A less prominent but significant TPO-Ab-independent association between TSH and Tg-Ab was observed. The trend was statistically significant both in participants with Tg-Ab measured alone (TPO-Ab level 1, p for trend < 0.001) and in participants with TPO-Ab present (>30 U/l), (p for trend < 0.001). Data from Bülow Pedersen *et al.* (2005) with permission.

could be defined. It remains, however, to be finally proven that the low signals given by the majority of normal sera really reflect antibodies specifically directed against thyroid antigens, and not just "background" noise.

Thyroid autoantibodies and thyroid autoimmunity

A good correlation has been found between the presence of lymphocytic infiltration of the thyroid and the presence of thyroid antibodies in serum (Kasagi *et al.*, 1996; Roth *et al.*, 1997; Yoshida *et al.*, 1978), and between the severity of histological thyroiditis and the level of antibody in serum (Arai *et al.*, 2000). Currently, it is not known whether TPO-Ab and Tg-Ab are directly involved in the pathogenesis of chronic AITD or if one or both are nonpathogenic antibodies generated secondary to the tissue damage.

Unfortunately, most studies comparing lymphocytic infiltration in the thyroid and circulating autoantibodies in serum have been performed in areas with sufficient or excess iodine intake, and knowledge of the relationship between thyroid antibodies and histological findings in the thyroid in areas with low iodine intake is limited.

Is it possible to compare the prevalence rates of TPO-Ab and Tg-Ab in different studies?

There are many problems associated with the comparison of results obtained from different epidemiological studies on thyroid autoantibodies. As described above, one of the major problems is the differences in the use of biochemical/immunological methods, including absence of standardization of the assays, use of assays with different sensitivity and specificity, and possible differences in other technical details of the assays applied. Cut-off values for TPO-Ab and Tg-Ab were not always well-documented and varied largely (Hollowell *et al.*, 1998; Laurberg *et al.*, 1998; Pedersen *et al.*, 2003). In some studies, the cut-off values corresponded to the functional sensitivity given by the manufacturer, whereas rather high cut-off values were chosen by other investigators (Teng *et al.*, 2006).

The other problem is the differences in the epidemiological methods applied. It is well-established that thyroid autoantibodies are more commonly measurable in females than in males (Hollowell *et al.*, 1998; Laurberg *et al.*, 1998; Prentice *et al.*, 1990; Tunbridge *et al.*, 1977), and that the prevalence of antibodies increases with age, at least in females (Pedersen *et al.*, 2003). It is possible that this increase is present only up to a certain age, after which there may be a plateau (Aghini-Lombardi *et al.*, 1999; Hawkins *et al.*, 1980; Tunbridge *et al.*, 1977). The results obtained from the population studies should therefore be age and sex standardized before comparison. Some studies included highly selective participants, such as subjects above 70 years (Brochmann *et al.*, 1988), hospitalized geriatric patients (Szabolcs *et al.*, 1995), or elderly ambulatory women (Martinez-Weber *et al.*, 1993), whereas other investigators included randomly selected subjects from the population. A racial difference in susceptibility to thyroid autoimmunity has been shown in autopsy studies (Okayasu *et al.*, 1991, 1994) and in epidemiological studies (Hollowell *et al.*, 1998). Likewise, many other differences in genetic background and environmental factors, such as smoking habits, influence the results (Prummel *et al.*, 2004).

Comparison of epidemiological studies on the prevalence of circulating thyroid autoantibodies in areas with different iodine intakes is therefore difficult, unless the studies are designed as comparatives with exactly the same methods applied in two or more regions.

Intervention Studies on Iodine Intake

In areas where iodine deficiency (ID) was prevalent but disappeared after iodine prophylaxis, histological studies of thyroid glands surgically removed because of cancer or goiter have shown a higher prevalence of lymphocyte infiltration after the increased iodine intake (Harach and Williams, 1995). Clinical studies have confirmed an increase in the production of thyroid antibodies in association with an increase in iodine intake (Kahaly *et al.*, 1998; Koutras *et al.*, 1990; Zimmermann *et al.*, 2003). Epidemiological data to some degree support such an enhancing effect of an increased iodine intake on thyroid autoimmunity. It seems as if a sudden increase in iodine intake may be more important for the development of thyroiditis and generation of thyroid antibodies than exposure to a constant but high iodine level (Kahaly *et al.*, 1998; Laurberg *et al.*, 1998).

Intervention studies in animals

Experimental animal studies have shown that excessive iodine intake can precipitate thyroiditis with lymphocytic infiltration in the thyroid in genetically predisposed strains of rats (Allen and Braverman, 1990; Cohen and Weetman, 1988; Ruwhof and Drexhage, 2001), mice (Rose *et al.*, 1999) and chickens (Safran *et al.*, 1987; Sundick *et al.*, 1992). The opposite phenomenon, with increased thyroid autoimmunity in iodine-deficient animals has also been observed, albeit in genetically different strains of rats (Ruwhof and Drexhage, 2001). In the nonautoimmune prone Wistar rat, a low dietary iodine intake led to not only goiter formation and hypothyroidism, but also an intrathyroidal accumulation of dendritic cells and raised production of Tg-Ab (Mooij *et al.*, 1994; Ruwhof and Drexhage, 2001). It was suggested that this accumulation of dendritic cells could have a physiological function of regulating the growth and function of the thyrocytes

deprived of iodine. Furthermore, the generated Tg-Ab could have the physiological role of clearing the excess Tg, which is released from the thyroid in ID (Ruwhof and Drexhage, 2001). In iodine excess, the autoimmune reactivity of the nonautoimmune prone Wistar rat was depressed and a relatively high dietary iodine intake seemed optimal to keep thyroid autoreactivity at a minimum (Ruwhof and Drexhage, 2001).

The autoimmune prone BB-DP rat, on the other hand, more easily developed AITD under the influence of a diet rich in iodine, depending on the previous state of the thyroid (Ruwhof and Drexhage, 2001). In severe ID, those animals had less severe lymphocytic thyroiditis and the production of Tg-Ab was depressed, probably as part of a general lowering of thyroid autoimmunity induced by severe ID. The results obtained with these different strains of rats may be viewed as a model for the individual heterogeneity in response to changes in iodine intake.

Intervention studies in humans

Most intervention studies performed in humans have been in areas with ID. In a number of studies, administration of iodine in varying doses has been followed by an increase in thyroid autoimmunity (Kahaly *et al.*, 1998; Koutras *et al.*, 1990); however, this has not been a consistent finding (Benmiloud *et al.*, 1994; Liesenkotter *et al.*, 1996; Simescu *et al.*, 2002).

In a double-blind trial, 500 μg/day iodine orally or 0.125 mg/day L-tyroxine was given to 62 patients with ID (Kahaly *et al.*, 1998). After 6 months, high MicAb and Tg-Ab developed in 6 of the 31 patients receiving iodine compared to none in the tyroxine group. Fine needle biopsy revealed marked lymphocyte infiltration in all six antibody-positive subjects. After the withdrawal of iodine intake, antibody titer and lymphocyte infiltration decreased, and after 3 years, four of the six patients were again antibody negative.

In 30 goitrous subjects treated with 300 μg/day iodine as oral potassium iodide, 9 individuals (30%) developed elevated thyroid antibody titers during 6 months of follow-up. The induction of thyroid autoantibodies tended to be dose dependent, as only 12% of goitrous subjects receiving 0.15 mg/day iodine became antibody positive (Koutras *et al.*, 1990).

In a randomized study from Germany, 40 out of 83 euthyroid patients with AITD were treated with 250 μg/day potassium iodide for a mean period of 4 months. The diagnosis was based on positive TPO-Ab combined with moderate-to-severe hypoechogenic pattern of the thyroid by ultrasound. Urinary iodine excretion increased from a baseline mean of 72±38 μg/g to 268±173 μg/g creatinine. Seven patients in the iodine-treated group and one patient in the control group developed some degree of hypothyroidism, but no significant change in TPO-Ab level was observed. Unfortunately, Tg-Ab was not reported (Reinhardt *et al.*, 1998).

In concordance with that study, no thyroid autoantibodies appeared in 114 schoolchildren from an iodine-deficient area in Romania within 1 year after a single oral administration of iodized oil containing 200 mg iodine (Simescu *et al.*, 2002). Similarly, no changes were seen in the frequency of TPO-Ab in the early postpartum period in a group of 38 women from an iodine-deficient area who had received 300 μg potassium iodide/day during pregnancy from 10 to 12 weeks of gestation (Liesenkotter *et al.*, 1996). Nor did iodine supplementation (150 μg/day) during pregnancy and the postpartum period to TPO-Ab positive women in an area with mild-to-moderate ID induce or worsen postpartum thyroiditis (Nøhr *et al.*, 2000).

Intervention by Iodine Fortification of Salt in the Population

Iodine fortification governed by national health care agencies and guided by international organizations such as the Internal Council for the Control of Iodine Deficiency Disorders (ICCIDD), the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF), has successfully eliminated the risk of ID disorders in most countries throughout the world (WHO, 2001). It has been recommended that iodine fortification programs should be followed by monitoring of thyroid diseases to evaluate the effectiveness of the fortification, and to detect and counteract the unintended effects of iodine enrichment such as an increase in hyper- or hypothyroidism (WHO, 2001). In some areas, an increase in thyroid autoimmunity has been observed after iodine fortification (Harach and Williams, 1995), which can be regarded as an iodine intervention study at the population level, albeit with no parallel control.

In the goitrous region of Salta, Argentina, the prevalence of thyroiditis in females was studied before and after iodine prophylaxis was introduced in 1963 by registering lymphocytic infiltration in goiters that had been surgically removed because of thyroid cancer or adenoma (Harach and Williams, 1995). Urinary iodine excretion in schoolchildren increased from 9.3 μg/g creatinine in 1963 to 152 μg/g creatinine in 1975 and 110 μg/g creatinine in 1983. The frequency of lymphocytic infiltrate of the thyroid rose from 8% before iodine supplementation to 28% in those operated on within 10 years after iodine prophylaxis was introduced, and 23% in those operated on more than 10 years later. It can be argued that one of the causes for these findings may be that surgery was primarily performed for ID goiter and not autoimmune goiter before, and that ID goiter became less common after, the increase in iodine intake.

In a severely iodine-deficient area of Morocco, TPO-Ab and Tg-Ab were measured in 323 schoolchildren before

and up to 1 year after the distribution of iodized salt. The median urinary iodine excretion was 17 μg/l at baseline, which increased to 150–200 μg/l after iodine fortification. A transient increase in the prevalence of detectable TPO-Ab and Tg-Ab was seen, with levels returning to baseline within 1 year (Zimmermann *et al.*, 2003).

Although an iodine prophylaxis program has never been officially implemented in Greece, nutritional iodine intake has improved in recent years largely because of the commercial availability of iodized salt. Better socioeconomic conditions, in general, have also contributed to the improved iodine nutrition of Greek schoolchildren (Zois *et al.*, 2003). When a group of Greek schoolchildren was studied in 1994, the median urinary iodine excretion was 84 μg/l, and the prevalence of AITD diagnosed from the presence of thyroid autoantibodies combined with a characteristic pattern of thyroiditis at ultrasonography of the thyroid was 3.3% (Zois *et al.*, 2003). A new status on iodine intake and thyroid autoimmunity in schoolchildren was made in 2001 at a time when the median iodine excretion had increased to 202 μg/l. Sensitive but different antibody assays were used in the two studies. The prevalence of AITD had increased significantly to 9.6% (Zois *et al.*, 2003). Further, the prevalence of autoimmune stigmata in fine needle aspiration smears from thyroid nodules had increased from 5.9% to 13.9% (Doufas *et al.*, 1999).

Five years after the introduction of iodized salt in Sri Lanka, the prevalence of Tg-Ab was high among schoolchildren. The interpretation of the authors was that this was the result of the increased iodine intake (Premawardhana *et al.*, 2000). Three years later, when the status was re-evaluated, the prevalence of Tg-Ab had decreased significantly (Mazziotti *et al.*, 2003). Unfortunately, no pre-iodine data on thyroid autoantibodies were available.

Comparative Epidemiological Studies on Thyroid Autoantibodies

As described previously, it is difficult to compare the results from different studies on prevalence rates of circulation thyroid autoantibodies. Unfortunately, there are relatively few comparative studies of cohorts with different iodine intake in which sensitive assays have been applied in the analysis of thyroid autoantibodies.

In a comparative epidemiological study performed in Iceland, with a stable high iodine intake (median urinary iodine excretion about 300 μg/l), and Jutland, Denmark with a long-standing low iodine intake (median urinary iodine excretion about 40–60 μg/l), a randomly selected sample of elderly females and males aged 66–70 years were examined (Laurberg *et al.*, 1998). Except for the different level of iodine intake, the two groups were comparable. Clinical goiter was much more prevalent in Jutland than in Iceland (12.2% vs. 3.2%). The dominating type of hyperthyroidism in Jutland was multinodular toxic goiter, which was infrequent in Iceland. On the contrary, Graves' disease represented the main proportion of the cases of hyperthyroidism in Iceland. Somewhat unexpectedly, the prevalence rates of both TPO-Ab and Tg-Ab were nearly twice as high in Jutland, compared to Iceland with the highest iodine intake (Figure 60.3).

The DanThyr cohort was studied immediately before iodine fortification of salt was implemented in Denmark (Knudsen *et al.*, 2000). It comprised 4649 subjects living in two areas of Denmark with mild and moderate ID, respectively (Copenhagen, mild ID, median urinary iodine 61 μg/l; Aalborg, moderate ID, median iodine intake: 45 μg/l). The participants were randomly selected from the sex and age groups, females aged 18–22, 25–30, 40–45 and 60–65 years, and males aged 60–65 years. The sex and age distribution was equal in the two subcohorts. Assays with high sensitivity were used. The overall prevalence of antibodies (TPO-Ab and/or Tg-Ab) was 18.8% with higher frequencies in women and with age (Pedersen *et al.*, 2003). Within all age and sex groups, the prevalence rates of TPO-Ab and Tg-Ab were similar (overall TPO-Ab: 13.1%, Tg-Ab: 13.0%). The overall prevalence rate of thyroid antibodies (TPO-Ab and/or Tg-Ab) was the same in the two subcohorts. However, in the age group 60–65 years, a consistent pattern of more thyroid antibodies in moderate than in mild ID was found: 22.1 vs. 17.3%, $P = 0.02$. The prevalence of goiter was highest in the area with the lowest iodine intake due to a high presence of multinodular goiter (Knudsen *et al.*, 2000) as was the incidence rate of hyperthyroidism, whereas the incidence rate

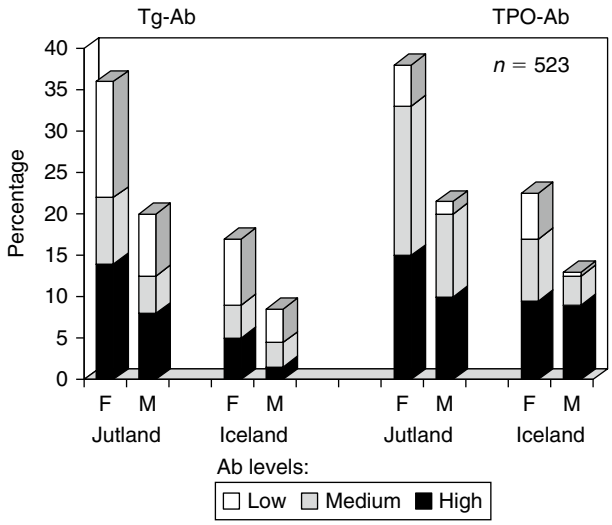

Figure 60.3 Prevalence rates (%) of Tg-Ab and TPO-Ab in elderly subjects from Jutland, Denmark, with-mild-to-moderate ID, and from Iceland with a stable high iodine intake. Different levels of antibody concentration are shown in each bar. Samples from the two areas were measured in random order in the same assays. Data from Laurberg *et al.* (1998).

Table 60.1 Thyroid autoantibodies in population studies from areas with different iodine intake

Author [country]	*Iodine status*	*Antibody assays*	*Cutoff*	*Age (years)*	*Prevalence of antibody (%)*
Laurberg *et al.*, (1998) [Denmark]	Moderate ID	Tg-Ab: Radioimmunoprecipitation	Detection limits	68	Tg-Ab: 31
		TPO-Ab: Enzyme-linked immunosorbent assay			TPO-Ab: 29
Pedersen *et al.*, (2003) [Denmark]	Moderate and mild ID	Tg-Ab: Radioimmunoassay	Detection limits	18–65	Tg-Ab: 13.0
		TPO-Ab: Radioimmunoassay			TPO-Ab: 13.1
Hintze *et al.*, (1991) [Germany]	Mild ID	Tg-Ab: Radioimmunoassay Mic-Ab: Radioimmunoassay	>Detection limits	60+	Tg-Ab: 10.1 Mic-Ab: 23.2
Knudsen *et al.*, (1999) [Denmark]	Mild ID	TPO-Ab: Enzyme-linked immunosorbent assay	>100 U/ml; Detection limits not given	41 and 71 years	TPO-Ab: 22.8
Aghini-Lombardi *et al.*, (1999) [Italy]	Mild ID	Tg-Ab: Agglutination	≥1:100; Detection limits not given	1+	Tg-Ab and/or TPO-Ab: 12.6
		TPO-Ab: Agglutination			
Teng *et al.*, (2006) [Panshan, China]	Mild ID	Tg-Ab: Chemiluminescence	>Detection limits	36 ± 13 (SD)	Tg-Ab: 9.0
		TPO-Ab: Chemiluminescence			TPO-Ab: 9.2
Fenzi *et al.*, (1986) [Italy]	Mild ID	Tg-Ab: Agglutination	≥1:100; Detection limits not given	Young adults	Tg-Ab and/or Mic-Ab: 14.4
		Mic-Ab: Agglutination			
Hollowell *et al.*, (1998) [United States]	Iodine sufficient	Tg-Ab: Radioimmunoassay	Detection limits	12+	Tg-Ab: 11.5
		TPO-Ab: Radioimmunoassay			TPO-Ab: 13.0
Bjøro *et al.*, (2000) [Norway]	Iodine sufficient	TPO-Ab: Luminoimmunoassay	>200 U/ml; Detection limits not given	40+	TPO-Ab: 9.7
Tunbridge *et al.*, (1977) [UK]	Iodine sufficient	Tg-Ab: Tanned red cell technique	Tg-Ab: ≥1:20	18+	Tg-Ab and/or Mic-Ab: 7.3
		Mic-Ab: Microhemagglutination	Mic-Ab: ≥1:100		
Bryhni *et al.*, (1996) [Norway]	Iodine sufficient	Tg-Ab: Agglutination	1:10 and 1:100; Detection limits not given	34 ± 8.4 (SD)	Tg-Ab: 2.8
		Mic-Ab: Agglutination			Mic-Ab: 6.1
Laurberg *et al.*, (1998) [Iceland]	Iodine sufficient	Tg-Ab: Radioimmunoprecipitation	Detection limits	66–70	Tg-Ab: 13
		TPO-Ab: Enzyme-linked immunosorbent			TPO-Ab: 18
Teng *et al.*, (2006) [Zhangwu, China]	More than adequate	Tg-Ab: Chemiluminescence	>Detection limits	36 ± 13 (SD)	Tg-Ab: 9.0
		TPO-Ab: Chemiluminescence			TPO-Ab: 9.8

Note: Prevalence rates of TPO-Ab and/or Tg-Ab in populations with different iodine intake. All studies included both females and males.

of hypothyroidism was highest in the area with only mild ID (Bülow Pedersen *et al.*, 2002).

In a follow-up study, 3761 randomly selected subjects, above 13 years, living in one of three regions in China with mild ID (median urinary iodine excretion: 84 μg/l), more than adequate iodine intake (urinary iodine: 243 μg/l), and excessive iodine intake (urinary iodine: 651 μg/l) were included (Teng *et al.*, 2006). At baseline, the prevalence rates of high level of TPO-Ab (TPO-Ab ≥ 50 IU/ml) and Tg-Ab (Tg-Ab ≥ 40 IU/ml) were measured using assays with high sensitivity. TPO-Ab was found in 9.2, 9.8 and 10.5%, and Tg-Ab in 9.0, 9.0 and 9.4%, with no statistically significant difference between the subcohorts. After 5 years of follow-up, there were still no differences in the rates of high levels of TPO-Ab and Tg-Ab in the three cohorts.

In Hungary, Szabolcs *et al.* (1997) screened elderly nursing home residents for thyroid disease. The three subcohorts

were comparable with respect to age and sex distribution. They were living in the same geographical and ethnographical region, but in areas with varying levels of iodine intake (median iodine excretion 72, 100 and 513 μg/g creatinine, respectively). The overall prevalence rates of positive thyroid antibodies (TPO-Ab and/or Tg-Ab > 100 U/ml) were similar in the three subcohorts. Further, the prevalence of high TPO-Ab (TPO-Ab > 1000 U/ml) was similar.

Fenzi studied the prevalence of thyroid antibodies in a moderate endemic goiter area of Italy (Fenzi *et al.*, 1986). Schoolchildren ($n = 142$) and their parents ($n = 159$) were included. The overall frequency of Mic-Ab and Tg-Ab in the adult population was 14.4%, which was significantly higher than that in the sex- and age-matched control group living in an iodine-sufficient area. It was observed that antibodies were more common in subjects with goiter compared to subjects without goiter.

In Turkey, mandatory iodization of salt was introduced in 1999. Two years later, a comparative cross-sectional study including 1733 adolescents from two areas with different iodine intakes was performed (Bastemir *et al.*, 2006). ID was still present in one of the two subcohorts (median urinary iodine excretion 61 μg/l, $n = 740$), whereas the other subcohort was iodine replete (median urinary excretion 139 μg/l, $n = 993$). The prevalence rate of thyroid autoantibodies (TPO-Ab and/or Tg-Ab) was significantly higher in the iodine-replete subcohort (18.5% vs. 6.6%). Considering the low age of the study population (14–18 years) and compared to other studies, the prevalence rates of both TPO-Ab and Tg-Ab were rather high in the iodine-replete subcohort. It is possible that the high values were caused by the sudden increase in iodine intake, and that a decrease in thyroid autoimmunity would occur over the following years (Zimmermann *et al.*, 2003).

Thyroid Autoantibodies in Iodine-Deficient Areas

A number of studies on the epidemiology of thyroid autoantibodies have been performed as descriptive studies in iodine-deficient areas. Results from some population-based studies from both iodine-deficient and iodine-sufficient areas are shown in Table 60.1. The results are ambiguous.

In an Italian survey, 1411 subjects representing the entire population of a small city, Pescopagano, was included (Aghini-Lombardi *et al.*, 1999). The area was an endemic goiter area with long-established, stable, mild ID with a median urinary iodine excretion of 55 μg/l. The prevalence of goiter was reported to be 16% in children and 59.8% in adults. Thyroid autoantibodies (TPO-Ab and/or Tg-Ab) were measurable in 12.6% of the entire population (females: 17.3%, males: 7%), increasing from 2.4% in children to 21.7% in the age group 46–55 years, with little change in older subjects. Antibodies were more frequent in subjects with goiter than without goiter. The antibody assays used for measuring TPO-Ab and Tg-Ab were both based on agglutination. It could therefore be speculated that the given prevalence rates were minimum rates, due to the relatively low sensitivity of such assays.

In a cross-sectional study from a borderline iodine-deficient area in Denmark (Copenhagen), Knudsen *et al.* included 2656 randomly selected subjects aged 41–71 years. The prevalence rate of TPO-Ab, measured by ELISA, was high at 22.8% (Knudsen *et al.*, 1999).

In a moderate ID area of Germany, Mic-Ab and TPO-Ab were measured in 466 randomly selected elderly subjects aged 60–98 years from the population (Hintze *et al.*, 1991). The prevalence rate of elevated Mic-Ab (>500 U/ml) was high at 23.2%, whereas Tg-Ab was significantly elevated (>200 U/ml) at 10%. The values were relatively high considering the low sensitivity of the Mic-Ab assay and the relatively high cut-off values.

In the Sardinian autoimmunity study, 8040 children living in 29 communities with borderline ID or mild-to-moderate ID were included (Loviselli *et al.*, 2001). Thyroid autoantibodies (Mic-Ab by passive hemagglutination technique ($n = 1670$) and TPO-Ab by RIA ($n = 6370$); Tg-Ab by RIA) were measurable in 2.9% of the participants, ranging between 0% and 7.3% without any geographical correlation to goiter prevalence and urinary iodine excretion. Thyroid autoantibodies were more often present in children with goiter, compared to children without goiter. The study may suggest that, at least in the iodine intake level range corresponding to moderate ID to low normal iodine intake, iodine intake does not affect the prevalence of thyroid autoantibodies in children.

Thyroid Autoantibodies in Iodine-Replete Areas

In the NHANES III study, TPO-Ab and Tg-Ab were measured in 15 592 randomly selected subjects, representative of the US population (Hollowell *et al.*, 1998). The antibodies were measured with highly sensitive assays based on RIA. Approximately 18% of the disease-free population had Tg-Ab and/or TPO-Ab above the detection limit (TPO-Ab ≥ 0.5 IU/ml, Tg-Ab ≥ 0.1 IU/ml). TPO-Ab was measurable in 13% and Tg-Ab in 11.5% of the population.

In the original Wickham study, thyroid autoantibodies were measured in 2779 subjects from the small city, Wickham, in the UK (Tunbridge *et al.*, 1977). Both Tg-Ab and Mic-Ab were measured with old assays with relatively low sensitivity. The prevalence of one or both antibodies was 11.2% in females and 2.8% in males. At the follow-up 20 years later, 26.4% of the participants were antibody positive (Vanderpump *et al.*, 1996). Possible explanations

for this huge increase in the prevalence of antibody-positive participants could be that the participants were 20 years older at follow-up and that newer and better assays had been used.

In Norway, the iodine intake is generally considered to have been sufficient for many years (Bjøro *et al.*, 2000). In two population-based Norwegian studies, Mic-Ab and Tg-Ab were detected with passive hemagglutination in elderly subjects aged >70 years (Brochmann *et al.*, 1988) and in younger subjects aged around 34 years (Bryhni *et al.*, 1996). Mic-Ab was detected in 15.7% vs. 6.1% of the subjects, respectively, and Tg-Ab in 11.7% vs. 2.8%, respectively. In another population-based Norwegian study, TPO-Ab was measured with a more sensitive luminoimmunoassay in randomly selected subjects above 40 years; 9.7% of the participants had TPO-Ab (females 13.9; males 2.8%).

In South Wales, UK, 414 asymptomatic randomly selected elderly subjects above 70 years were screened for autoimmune thyroid disease (Lazarus *et al.*, 1984): 18.6% of the participants had elevated levels of Mic-Ab and/or Tg-Ab (Mic-Ab 15.4%, Tg-Ab 13.3%, both antibodies present 8.5%).

The Paradox of Iodine Intake and Thyroid Autoimmunity

In spite of the difficulties in interpreting and comparing results from epidemiological studies on thyroid autoimmunity, there are certain tendencies in the relationship between thyroid autoimmunity and iodine intake.

A sudden increase in iodine intake in an iodine-deficient population may induce enhanced thyroid autoimmunity (Harach and Williams, 1995). Both cellular immune response with histological signs of thyroiditis and humoral immune response with circulating thyroid autoantibodies may be increased. It seems as if at least a part of this autoimmunity is reversible, and that the prevalence of antibodies will decrease to a lower level over time if the higher iodine intake is continued (Mazziotti *et al.*, 2003; Zimmermann *et al.*, 2003), or decrease in a relatively short time period if the iodine intake is reduced to the baseline level (Kahaly *et al.*, 1998).

"hypothyroidism" almost always defined as high TSH, which can be caused by the higher iodine levels!

Thyroid autoimmunity with lymphocytic infiltration in the thyroid and circulating thyroid autoantibodies seems to be common in populations with a stable high iodine intake. Subclinical and overt hypothyroidism is prevalent and more common than hyperthyroidism (Hollowell *et al.*, 2002; Vanderpump *et al.*, 1995). It is plausible that the impaired thyroid function associated with high iodine intake is caused by destruction of the thyroid by autoimmune processes in some cases, but not in all. In a population study from the coastal regions of Japan with excessive iodine intake, the authors found a high prevalence of subclinical hypothyroidism in antibody-negative subjects. There was a significant correlation between high iodine intake and hypothyroidism, which was not found in antibody-positive subjects (Suzuki *et al.*, 1965). It is possible that hypothyroidism in the antibody-negative subjects was secondary to the general inhibitory effect of a high iodine load on many thyroidal processes. Such autoregulation has probably been developed to protect against hyperfunction (Pisarev and Gärtner, 2000).

In mild and moderate ID, the prevalence rate of circulating thyroid autoantibodies in the population is also high (Laurberg *et al.*, 1998; Pedersen *et al.*, 2003). In such areas, nontoxic and toxic multinodular goiters are prevalent and overall hyperthyroidism is more common than hypothyroidism (Laurberg *et al.*, 1999). Results from areas with severe ID are limited and might, in some cases, be influenced by the general immunosuppressive effect of malnutrition, which may occur simultaneously (Salabe *et al.*, 1982).

Speculations on Possible Mechanisms behind Iodine Intake and Development of Antibodies

A number of mechanisms have been suggested to explain the association between thyroid autoimmunity and the level of iodine intake. A sudden shift from very low to high iodine intake may induce damage to the thyroid tissue by free radicals (Li and Boyages, 1994). Also, enhancement of the autoimmunogenic properties of thyroglobulin by increased iodination may play a role (Saboori *et al.*, 1998; Sundick *et al.*, 1987). Apart from these two mechanisms, no other model has been put forward that satisfactorily explains the association between excessive iodine intake and the generation of thyroid autoimmunity.

how about low selenium, Zn, B vits, A O3 oils and inositol?!

In iodine-deficient areas, the mechanism behind the development of thyroid autoantibodies may be different. It has been speculated that the development of antibodies in some cases may be secondary to goiter formation with exposure in the thyroid or release of antigens from the thyroid. Tg release from the thyroid is common in iodine-deficient areas with nodular goiter (Knudsen *et al.*, 2001); TPO may also be released from the thyroid. It is possible that mild and moderate ID over the years gradually induces circulating antibodies similar to the gradual induction of formation and growth of nodules. In accordance with such a mechanism, a higher prevalence of antibodies was found in subjects with goiter compared to subjects without goiter in areas with ID (Aghini-Lombardi *et al.*, 1999; Fenzi *et al.*, 1986; Bülow Pedersen *et al.*, 2005).

Autoimmunity, abnormal iodine supply and neoplasia are the three common mechanisms behind thyroid diseases in a population. Further studies are needed to obtain information on the interplay between iodine intake and thyroid autoimmunity. This may allow optimal prevention of thyroid disease by adjusting iodine intake in the individual and in the population.

Summary Points

- AITD is one of the most common autoimmune disorders.
- Nearly all patients with chronic AITD have high concentrations of TPO-Ab and Tg-Ab.
- A sudden increase in iodine intake may enhance thyroid autoimmunity.
- TPO-Ab and Tg-Ab are common in apparently healthy subjects with sufficient or excessive iodine intake.
- TPO-Ab and Tg-Ab are also common in populations with mild and moderate ID.
- The mechanisms behind the development of thyroid autoantibodies in iodine-sufficient and iodine-deficient populations may be different.
- In ID, thyroid autoantibodies may be secondary to goiter formation.
- Further studies are needed to obtain information on the interplay between iodine intake and thyroid autoimmunity.

References

Aghini-Lombardi, F., Antonangeli, L., Martino, E., Vitti, P., Maccherini, D., Leoli, F., Rago, T., Grasso, L., Valeriano, R., Balestrieri, A. and Pinchera, A. (1999). J. Clin. Endocrinol. Metab. 84, 561–566.

Allen, E.M. and Braverman, L.E. (1990). Endocrinology 127, 1613–1616.

Arai, T., Kurashima, C., Utsuyama, M., Sawabe, M. and Ito, H. (2000). Endocr. J. 47, 575–582.

Bastemir, M., Emral, R., Erdogan, G. and Gullu, S. (2006). Thyroid 16, 1265–1271.

Benmiloud, M., Chaouki, M.L., Gutekunst, R., Teichert, H.M., Wood, W.G. and Dunn, J.T. (1994). J. Clin. Endocrinol. Metab. 79, 20–24.

Bjøro, T., Holmen, J., Kruger, O., Midthjell, K., Hunstad, K., Schreiner, T., Sandnes, L. and Brochmann, H. (2000). Eur. J. Endocrinol. 143, 639–647.

Brochmann, H., Bjøro, T., Gaarder, P.I., Hanson, F. and Frey, H.M. (1988). Acta Endocrinol. (Copenh.) 117, 7–12.

Bryhni, B., Aanderud, S., Sundsfjord, J., Rekvig, O.P. and Jorde, R. (1996). J. Intern. Med. 239, 517–523.

Bülow Pedersen, I., Knudsen, N., Jorgensen, T., Perrild, H., Ovesen, L. and Laurberg, P. (2002). J. Clin. Endocrinol. Metab. 87, 4462–4469.

Bülow Pedersen, I., Laurberg, P., Knudsen, N., Jorgensen, T., Perrild, H., Ovesen, L. and Rasmussen, L.B. (2005). Clin. Endocrinol. 62, 713–720.

Carle, A., Laurberg, P., Knudsen, N., Perrild, H., Ovesen, L., Rasmussen, L.B., Jorgensen, T. and Pedersen, I.B. (2006). Autoimmunity 39, 497–503.

Cohen, S.B. and Weetman, A.P. (1988). J. Endocrinol. Invest. 11, 625–627.

Davidson, A. and Daimond, B. (2001). N. Engl. J. Med. 345, 340–350.

Dherbomez, M., Sapin, R., Gasser, F., Schlienger, J.L. and Wemeau, J.L. (2000). Clin. Chem. Lab. Med. 38, 561–566.

Doufas, A.G., Mastorakos, G., Chatziioannou, S., Tseleni-Balafouta, S., Piperingos, G., Boukis, M.A., Mantzos, E., Caraiskos, C.S., Mantzos, J., Alevizaki, M. and Koutras, D.A. (1999). Eur. J. Endocrinol. 140, 505–511.

Dunn, J.T., Kim, P.S., Dunn, A.D., Heppner, D.G. and Moore, R.C. (1983). J. Biol. Chem. 258, 9093–9099.

Feldt-Rasmussen, U. (1996). Clin. Chem. 42, 160–163.

Feldt-Rasmussen, U., Hoier-Madsen, M., Bech, K., Blichert-Toft, M., Bliddal, H., Date, J., Danneskiold-Samsoe, B., Hegedus, L., Hippe, E. and Hornnes, P.J. (1991). Autoimmunity 9, 245–254.

Feldt-Rasmussen, U., Perrild, H., Bech, K., Bliddal, H., Date, J., Madsen, M.H., Nordfang, O. and Ryder, L.P. (1983). Allergy 38, 49–56.

Fenzi, G.F., Giani, C., Ceccarelli, P., Bartalena, L., Macchia, E., Aghini-Lombardi, F., Vitti, P., Lari, R., Ceccarelli, C. and Baschieri, L. (1986). Endocrinol. Invest. 9, 161–164.

Harach, H.R. and Williams, E.D. (1995). Clin. Endocrinol. 43, 701–706.

Hawkins, B.R., Cheah, P.S., Dawkins, R.L., Whittingham, S., Burger, H.G., Patel, Y., Mackay, I.R. and Welborn, T.A. (1980). Lancet 15, 1057–1059.

Hidaka, Y., Nishi, I., Tamaki, H., Takeoka, K., Tada, H., Mitsuda, N. and Amino, N. (1994). Thyroid 4, 275–278.

Hintze, G., Burghardt, U., Baumert, J., Windeler, J. and Kobberling, J. (1991). Aging (Milano) 3, 325–331.

Hollowell, J.G., Staehling, N.W., Hannon, W.H., Flanders, D.W., Gunter, E.W., Maberly, G.F., Braverman, L.E., Pino, S., Miller, D.T., Garbe, P.L., DeLozier, D.M. and Jackson, R.J. (1998). J. Clin. Endocrinol. Metab. 83, 3401–3408.

Jensen, E.A., Petersen, P.H., Blaabjerg, O., Hansen, P.S., Brix, T.H. and Hegedus, L. (2006). Clin. Chem. Lab. Med. 44, 991–998.

Kahaly, G.J., Dienes, H.P., Beyer, J. and Hommel, G. (1998). Eur. J. Endocrinol. 139, 290–297.

Kasagi, K., Kousaka, T., Higuchi, K., Iida, Y., Misaki, T., Alam, M.S., Miyamoto, S., Yamabe, H. and Konishi, J. (1996). Thyroid 6, 445–450.

Knudsen, N., Bulow, I., Jorgensen, T., Laurberg, P., Ovesen, L. and Perrild, H. (2000). Clin. Endocrinol. (Oxf.) 53, 479–485.

Knudsen, N., Bulow, I., Jorgensen, T., Perrild, H., Ovesen, L. and Laurberg, P. (2001). J. Clin. Endocrinol. Metab. 86, 3599–3603.

Knudsen, N., Jorgensen, T., Rasmussen, S., Christiansen, E. and Perrild, H. (1999). Clin. Endocrinol. (Oxf.) 51, 361–367.

Koutras, D.A., Piperingos, G.D., Pallas, D., Katsouyanni, K., Karaiskos, K.S., Marafelia, P., Makriyannis, D., Kitsopanides, J., Sfontouris, J. and Mantzos, J. (1990). Thyroidology 2, 81–88.

Laurberg, P., Bulow Pedersen, I., Pedersen, K.M. and Vestergaard, H. (1999). Thyroid 9, 33–38.

Laurberg, P. and Pedersen, K.M. (1988). Scand. J. Clin. Lab. Invest. 48, 137–140.

Laurberg, P., Pedersen, K.M., Hreidarsson, A., Sigfusson, N., Iversen, E. and Knudsen, P.R. (1998). J. Clin. Endocrinol. Metab. 83, 765–769.

Lazarus, J.H., Burr, M.L., McGregor, A.M., Weetman, A.P., Ludgate, M., Woodhead, J.S. and Hall, R. (1984). Acta Endocrinol. (Copenh.) 106, 199–202.

Li, M. and Boyages, S.C. (1994). Autoimmunity 18, 31–40.

Liesenkotter, K.P., Gopel, W., Bogner, U., Stach, B. and Gruters, A. (1996). Eur. J. Endocrinol. 134, 443–448.

Lindberg, B., Svensson, J., Ericsson, U.B., Nilsson, P., Svenonius, E. and Ivarsson, S.A. (2001). Thyroid 11, 265–269.

Loviselli, A., Velluzzi, F., Mossa, P., Cambosu, M.A., Secci, G., Atzeni, F., Taberlet, A., Balestrieri, A., Martino, E., Grasso, L., Songini, M., Bottazzo, G.F. and Mariotti, S. (2001). Thyroid 11, 849–857.

Marcocci, C. and Chiovato, L. (2000). Thyroid-directed antibodies. In: (eds L.E. Braverman and R.D. Utiger), *The Thyroid*, 8th edn. Lippincott, Philadelphia, PA, pp. 414–431.

Martinez-Weber, C., Wallack, P.F., Lefkowitz, P. and Davies, T.F. (1993). Mt. Sinai J. Med. 60, 156–160.

Mazziotti, G., Premawardhana, L.D., Parkes, A.B., Adams, H., Smyth, P.P., Smith, D.F., Kaluarachi, W.N., Wijeyaratne, C.N., Jayasinghe, A., de Silva, D.G. and Lazarus, J.H. (2003). Eur. J. Endocrinol. 149, 103–110.

McLachlan, S.M. and Rapoport, B. (1992). Endocr. Rev. 13, 192–206.

Mooij, P., Simons, P.J., de Haan-Meulman, M., de Wit, H.J. and Drexhage, H.A. (1994). J. Endocrinol. 140, 503–512.

Nøhr, S.B., Jorgensen, A., Pedersen, K.M. and Laurberg, P. (2000). J. Clin. Endocrinol. Metab. 85, 3191–3198.

Okayasu, I., Hara, Y., Nakamura, K. and Rose, N.R. (1994). Am. J. Clin. Pathol. 101, 698–702.

Okayasu, I., Hatakeyama, S., Tanaka, Y., Sakurai, T., Hoshi, K. and Lewis, P.D. (1991). J. Pathol. 163, 257–264.

Pedersen, I.B., Knudsen, N., Jorgensen, T., Perrild, H., Ovesen, L. and Laurberg, P. (2003). Clin. Endocrinol. (Oxf.) 58, 36–42.

Pisarev, M.A. and Gärtner, R. (2000). Autoregulatory actions of iodine. In: (eds L.E. Braverman and R.D. Utiger), *The Thyroid*, 8th edn. Lippincott, Philidelphia, PA, pp. 85–90.

Premawardhana, L.D., Parkes, A.B., Smyth, P.P., Wijeyaratne, C.N., Jayasinghe, A., de Silva, D.G. and Lazarus, J.H. (2000). Eur. J. Endocrinol. 143, 185–188.

Prentice, L.M., Phillips, D.I., Sarsero, D., Beever, K., McLachlan, S.M. and Smith, B.R. (1990). Acta Endocrinol. (Copenh.) 123, 493–498.

Prummel, M.F., Strieder, T. and Wiersinga, W.M. (2004). Eur. J. Endocrinol. 150, 605–618.

Reinhardt, W., Luster, M., Rudorff, K.H., Heckmann, C., Petrasch, S., Lederbogen, S., Haase, R., Saller, B., Reiners, C., Reinwein, D. and Mann, K. (1998). Eur. J. Endocrinol. 139, 23–28.

Rose, N.R., Rasooly, L., Saboori, A.M. and Burek, C.L. (1999). Environ. Health Perspect. 107, 749–752.

Roth, C., Scortea, M., Stubbe, P., Ruschenburg, M., Zappel, H., Becker, W. and Lakomek, M. (1997). Exp. Clin. Endocrinol. Diabetes 105, 66–69.

Roti, E., Gardini, E., Minelli, R., Bianconi, L. and Braverman, L.E. (1992). Clin. Chem. 38, 88–92.

Ruwhof, C. and Drexhage, H.A. (2001). Thyroid 11, 427–436.

Saboori, A.M., Rose, N.R., Bresler, H.S., Vladut-Talor, M. and Burek, C.L. (1998). Clin. Exp. Immunol. 113, 297–302.

Safran, M., Paul, T.L., Roti, E. and Braverman, L.E. (1987). Endocrinol. Metab Clin. North Am. 16, 327–342.

Salabe, G.B., Salabe-Lotz, H., Ilardi, I., Petracca, C. and Bile, K. (1982). J. Clin. Lab. Immunol. 9, 55–57.

Salvi, M., Fukazawa, H., Bernard, N., Hiromatsu, Y., How, J. and Wall, J.R. (1988). Endocr. Rev. 9, 450–466.

Simescu, M., Varciu, M., Nicolaescu, E., Gnat, D., Podoba, J., Mihaescu, M. and Delange, F. (2002). Horm. Res. 58, 78–82.

Spencer, C.A., Lopresti, J.S., Fatemi, S. and Nicoloff, J.T. (1999). Thyroid 9, 435–441.

Sundick, R.S., Bagchi, N. and Brown, T.R. (1992). Autoimmunity 13, 61–68.

Sundick, R.S., Herdegen, D.M., Brown, T.R. and Bagchi, N. (1987). Endocrinology 120, 2078–2084.

Suzuki, H., Higuchi, T., Sawa, K., Ohtaki, S. and Horiuchi, Y. (1965). Acta Endocrinol. (Copenh.) 50, 161–176.

Szabolcs, I., Bernard, W. and Horster, F.A. (1995). J. Am. Geriatr. Soc. 43, 670–673.

Szabolcs, I., Podoba, J., Feldkamp, J., Dohan, O., Farkas, I., Sajgo, M., Takats, K.I., Goth, M., Kovacs, L., Kressinszky, K., Hnilica, P. and Szilagyi, G. (1997). Clin. Endocrinol. (Oxf.) 47, 87–92.

Teng, W., Shan, Z., Teng, X., Guan, H., Li, Y., Teng, D., Jin, Y., Yu, X., Fan, C., Chong, W., Yang, F., Dai, H., Yu, Y., Li, J., Chen, Y., Zhao, D., Shi, X., Hu, F., Mao, J., Gu, X., Yang, R., Tong, Y., Wang, W., Gao, T. and Li, C. (2006). N. Engl. J. Med. 354, 2783–2793.

Tomer, Y. (1997). Clin. Immunol. Immunopathol. 82, 3–11.

Tomer, Y. and Davies, T. (1993). Endocr. Rev. 14, 107–120.

Tunbridge, W.M., Evered, D.C., Hall, R., Appleton, D., Brewis, M., Clark, F., Evans, J.G., Young, E., Bird, T. and Smith, P.A. (1977). Clin. Endocrinol. (Oxf.) 7, 481–493.

Vanderpump, M.P., Tunbridge, W.M., French, J.M., Appleton, D., Bates, D., Rodgers, H., Evans, J.G., Clark, F., Tunbridge, F. and Young, E.T. (1996). Diabet. Med. 13, 741–747.

Weetman, A.P. and McGregor, A.M. (1994). Endocr. Rev. 15, 788–830.

WHO, UNICEF, and ICCIDD (2001). WHO/NHD/01.1. WHO, Geneva, Switzerland, pp. 1–107.

Yoshida, H., Amino, N., Yagawa, K., Uemura, K., Satoh, M., Miyai, K. and Kumahara, Y. (1978). J. Clin. Endocrinol. Metab. 46, 859–862.

Zimmermann, M.B., Moretti, D., Chaouki, N. and Torresani, T. (2003). Thyroid 13, 199–203.

Zois, C., Stavrou, I., Kalogera, C., Svarna, E., Dimoliatis, I., Seferiadis, K. and Tsatsoulis, A. (2003). Thyroid 13, 485–489.

Zophel, K., Saller, B., Wunderlich, G., Gruning, T., Koch, R., Wilde, J., Mann, K. and Franke, W.G. (2003). Clin. Lab. 49, 591–600.