European Journal of Endocrinology (2006) **155** 219–228 ISSN 0804-4643

CLINICAL STUDY

The Danish investigation on iodine intake and thyroid disease, DanThyr: status and perspectives

Peter Laurberg, Torben Jørgensen[2], Hans Perrild[3], Lars Ovesen[1,4], Nils Knudsen[2,3], Inge Bülow Pedersen, Lone B Rasmussen[1], Allan Carlé and Pernille Vejbjerg[2,3]

Department of Endocrinology and Medicine, Aalborg Hospital, DK-9000 Aalborg, Denmark, [1]Danish Institute for Food and Veterinary Research, Copenhagen, Denmark, [2]Research Centre for Disease Prevention and Health, Glostrup Hospital, Copenhagen, Denmark, [3]Medical Clinic I, Bispebjerg Hospital, Copenhagen, Denmark and [4]The National Heart Foundation, Copenhagen, Denmark

(Correspondence should be addressed to P Laurberg; Email: laurberg@aas.nja.dk)

Abstract

Objective: Denmark was an area of iodine deficiency, and mandatory iodine fortification of table salt and salt in bread (13 p.p.m. iodine) was initiated in 2000/2001. The Danish investigation on iodine intake and thyroid disease (DanThyr) is the monitoring of the iodine fortification program.

Design and methods: DanThyr consists of three main parts: a study of population cohorts initialized before ($n=4649$) and after ($n=3570$) iodization of salt, a prospective identification of incident cases of overt hyper- and hypothyroidism in a population of around 550 000 people since 1997, and compilation of data from the national registers on the use of thyroid medication, thyroid surgery, and radioiodine therapy. Studies were carried-out in parallel in subcohorts living in areas with differences in iodine content of ground water.

Results: The study showed profound effects of even small differences in iodine intake level on the prevalence of goiter, nodules, and thyroid dysfunction. Mild and moderate iodine deficiency was associated with a decrease in serum TSH with age. Other environmental factors were also important for goiter development (increase in risk, smoking and pregnancy; decrease in risk, oral contraception and alcohol consumption), and the individual risk depended on the genetic background. Environmental factors had only a minor influence on the prevalence of thyroid autoantibodies in the population. There were more cases of overt hypothyroidism in mild than in moderate iodine deficiency caused by a 53% higher incidence of spontaneous (presumably autoimmune) hypothyroidism. On the other hand, there were 49% more cases of overt hyperthyroidism in the area with moderate iodine deficiency. The cautious iodine fortification program, aiming at an average increase in iodine intake of 50 μg/day has been associated with a 50% increase in incidence of hyperthyroidism in the area with the most severe iodine deficiency. The incidence is expected to decrease in the future, but there may be more cases of Graves' hyperthyroidism in young people.

Conclusion: A number of environmental factors influence the epidemiology of thyroid disorders, and even relatively small abnormalities and differences in the level of iodine intake of a population have profound effects on the occurrence of thyroid abnormalities. Monitoring and adjustment of iodine intake in the population is an important part of preventive medicine.

European Journal of Endocrinology **155** 219–228

Until recently, the iodine intake of many European populations including the Danish (1) was below the level recommended by the international organizations (2). In Denmark, the low iodine intake was associated with signs of insufficient thyroid hormone production in pregnant women, who showed an increase in serum thyroid-stimulating hormone (TSH) in late pregnancy (3), and a very frequent occurrence of goiter and hyperthyroidism in elderly people caused by autonomous thyroid nodules (4). To rectify this deficiency of iodine, the Danish Food and Veterinary Administration introduced a voluntary program of universal salt iodization. As this turned out to be ineffective, it was subsequently replaced by a mandatory program of iodine fortification of household salt and salt in bread produced in Denmark (5).

It has been part of Danish nutrition policies that fortification of food with vitamins or minerals should be accompanied by a program of monitoring. Moreover, monitoring of iodine intake is specifically recommended by the World Health Organization (2). Iodine interferes with thyroid function and thyroid diseases in many ways. Even if there is consensus on the importance of avoiding iodine deficiency, there are many unanswered questions concerning optimal iodine fortification of food, and optimal iodine nutrition of a population (6).

DOI: 10.1530/eje.1.02210
Online version via www.eje-online.org

In Denmark, it was decided to develop a program of monitoring iodine intake and thyroid diseases that could secure optimal iodine nutrition of the Danish population. The program was designed to improve knowledge on a number of issues such as: how to evaluate iodine status of a population, the epidemiology of thyroid disorders in areas with different levels of iodine intake, and the effects of an increase in iodine intake. An additional aim was to clarify the role of various environmental factors for the development of thyroid disease in the population, and to study how these factors may interact with iodine intake. The steering group organizing the program comprised Peter Laurberg, Torben Jørgensen, Hans Perrild, and Lars Ovesen. Nils Knudsen, Inge Bülow Pedersen, and Lone B Rasmussen were initially involved in different parts of the program as PhD students and are now senior participants, whereas Allan Carlé and Pernille Vejbjerg are young investigators doing their PhDs within the program.

Recently, large programs of iodine fortification of salt have been introduced in many parts of the world in an impressive effort to eradicate developmental brain damage caused by iodine deficiency. We hoped that a systematic study designed in cooperation between experts on thyroidology and iodine, nutrition and food fortification, and epidemiology and disease prevention might lead to knowledge, which could assist in optimizing the iodine fortification programs that now cover billions of people in the world. This article summarizes the present status and some perspectives of this monitoring program, which was entitled the Danish investigation on iodine intake and thyroid disease (DanThyr).

Iodine fortification of salt in Denmark

Figure 1 illustrates the timing of changes in salt iodination in Denmark and the major parts of the accompanying DanThyr program. Before 1998, iodized salt could not be purchased in Denmark. Legislation requested a special permission to sell fortified food in Denmark, and no license to sell iodized salt had been given. Starting from June 1998, a program of voluntary use of iodized salt was launched by the Danish Food and Veterinary Administration in cooperation with salt manufactures and the food industry. The public was informed in several ways about the state of iodine deficiency in Denmark and the beneficial effects of using iodized salt.

The target of the program was to increase the iodine intake of the average Dane by around 50 μg/day, and it was expected that 80% of household salt and salt used by the food industry would be iodized. This corresponds to an average intake of iodized salt around 6–7 g/day, and the amount of iodide added to salt was, therefore, set to 8 p.p.m. After 2 years, it turned out that the program had failed. Only around half of household salt and practically no salt used by the food industry were iodized. The estimated increase in daily iodine intake was well below 10 μg. Therefore, a mandatory program was introduced during the period July 2000–April 2001. Household salt and salt used for commercial production of bread were to be iodized. The intake of iodized salt by this program was estimated to be around 4 g/day, and the iodization level was set to 13 p.p.m. Bread is a staple food in Denmark, and simulation studies performed by the Danish Food and Veterinary Administration based on Danish food surveys had shown that iodized salt in bread would distribute the iodine nearly as evenly in the population as iodization of all salt used by the food industry.

The monitoring program

The DanThyr program consists of three main parts, all of which were started well before the iodization of salt so

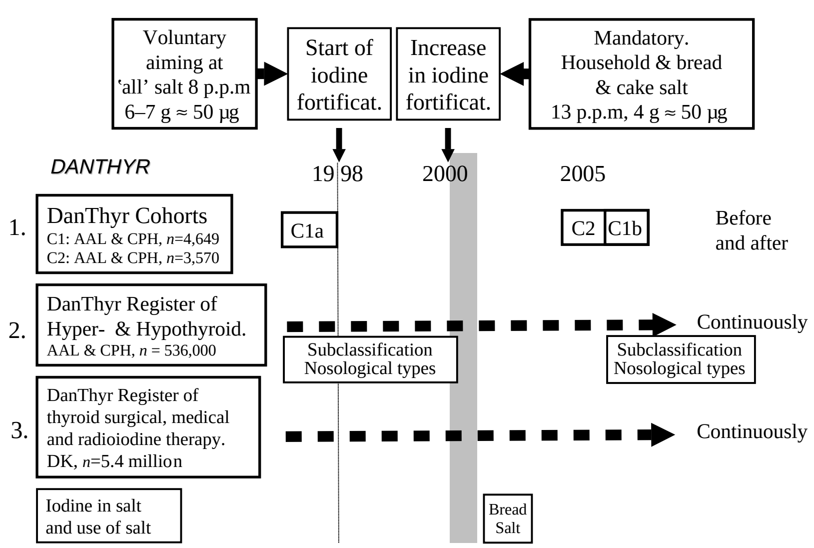

Figure 1 Overview of the DanThyr monitoring program and the relation to the Danish iodine fortification program. Voluntary iodine fortification of salt with 8 μg iodine/g salt was initiated in 1998. As this turned out to be inefficient, a mandatory program was introduced in late 2000 and early 2001. The aim of both programs was to increase average iodine intake by 50 μg/day. DanThyr was started before iodine fortification and consists of a number of cohort studies and registries as described in detail in the text. AAL, Aalborg; CPH, Copenhagen; DK, Denmark.

as to include a control period (Fig. 1). The cohort study (C1, $n=4649$) took place in and around the city of Aalborg in Jutland, and in Copenhagen on Zealand (Fig. 2). The two geographical regions cover the main difference in levels of iodine intake in Denmark caused by different levels of iodine in ground water (7). Details on the random selection of cohorts from the population, participation rates, and analysis of potential bias caused by non-participation have been published (8).

We chose to investigate women in four age intervals (young women, 18–22 years; women in the main reproductive age, 25–30 years; pre-menopausal women, 40–45 years; and post-menopausal women, 60–65 years) and men in the oldest age group. This was done to obtain maximum information from the investment of time and money. Participants filled out an extensive questionnaire on lifestyle, diseases, medication, and food frequency (9), including intake of supplements (10). A clinical investigation for goiter was performed, and weight, height, and blood pressure measured. Subsequently, thyroid ultrasonography was performed (11), and spot urine and blood samples collected. Investigation took place in two centers at Aalborg and Bispebjerg Hospitals. A program of training and control of ultrasonography performance (11), and evaluation of clinical investigation for goiter were used to coordinate centers.

To study the effect of the iodization program at the population level, a new cohort of women and men in the same age groups (C2, $n=3570$) has been investigated in 2005. To evaluate the effect at the level of the individual member of society and to follow-up the thyroid abnormalities detected at the first cohort investigation, permission has been obtained to reinvestigate the C1 cohort in 2006/2007.

The second part of DanThyr is a register of new cases of overt hyper- and hypothyroidism in an open population cohort of more than 550 000 people living in the same two areas (Fig. 2). The details on method of identification and verification of new patients, as well as the various studies performed to evaluate the method, have been published (12). All results of thyroid function tests performed in the areas of the investigation are imported into the study database. The database runs a diagnostic algorithm and excludes the previously diagnosed cases. A list of possible new cases is produced, and the cases are verified by contacting the physician who requested the test. During selected periods of the study, the patients have been asked to come to the centers for a comprehensive investigation including subtyping of the disease. Data on the composition of the population in the study areas are obtained each year from the Danish Statistical Bureau.

The third part of DanThyr is a central register for surgical, medical, and radioiodine treatment of thyroid disease in Denmark. Data are extracted from several national databases on surgical activity and prescription of medication. This part of the program gives information on therapeutic activity, and allows estimation of the costs associated with therapy of thyroid disorders in Denmark (13).

Independently, the Danish Institute for Food and Veterinary Research includes measurements of iodine in food in their program of monitoring nutrition contents of food in Denmark.

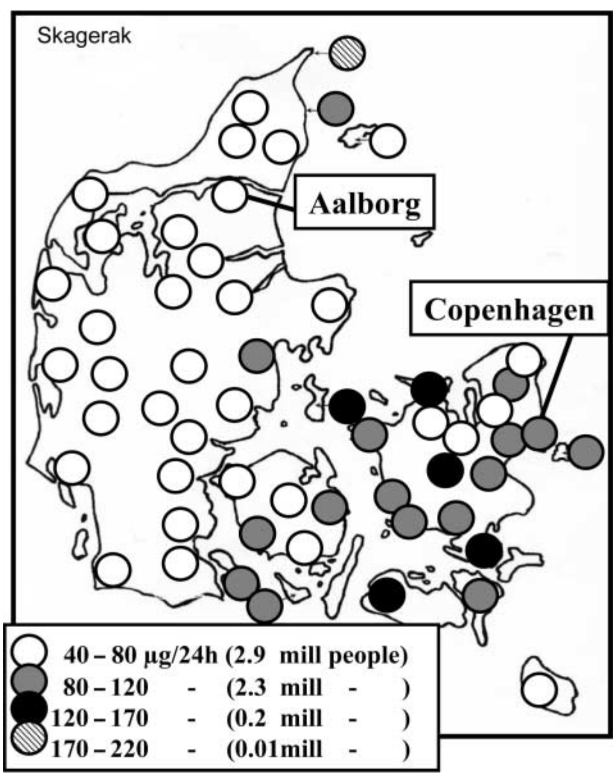

Figure 2 Median urinary iodine in various Danish cities before iodine fortification of salt. Values were compiled from different studies of urinary iodine excretion, or estimated from measurements of ground water iodine contents. The table indicates the estimated number of people living in areas with different levels of urinary iodine excretion.

Iodine nutrition in Denmark before and after salt iodization

The participants ($n=4649$) of the C1 cohort had iodine measured in a spot urine sample. Median urinary iodine concentration was 53 µg/l in participants from Aalborg, and 68 µg/l in Copenhagen (14) corresponding to the difference in ground water iodine content (5 µg/l in Aalborg, 20 µg/l in Copenhagen (7)). Some of the participants took iodine-containing vitamin and mineral supplements (14). Participants who did not take iodine supplements had median urinary iodine concentrations of 45 (Aalborg) and 61 µg/l (Copenhagen). Thus, it was confirmed that participants from Copenhagen had mild iodine deficiency, and those from

Aalborg moderate iodine deficiency (2). From the food frequency registration, it could be shown that even the subgroup with the highest intake of fish and dairy products (rich in iodine) had iodine deficiency if they lived in Aalborg (14). Milk and milk products alone contributed about 44% of iodine intake, and iodine deficiency was more severe in the group with little intake of milk products. This group is expected to be covered by the present use of iodized salt.

Complete details on the effect of the present iodization of salt are awaiting the completion of measurements of iodine and analyses of data from the recently investigated C2 cohort. The Danish Institute for Food and Veterinary Research investigated the iodine content of bread and salt sold in Danish stores in 2002. This showed that the present legislation is followed; 97% of rye bread and 90% of other breads sold in Denmark were iodized. Using databases on food consumption in Denmark, it was calculated that iodine intake had increased to 62 μg/day (15).

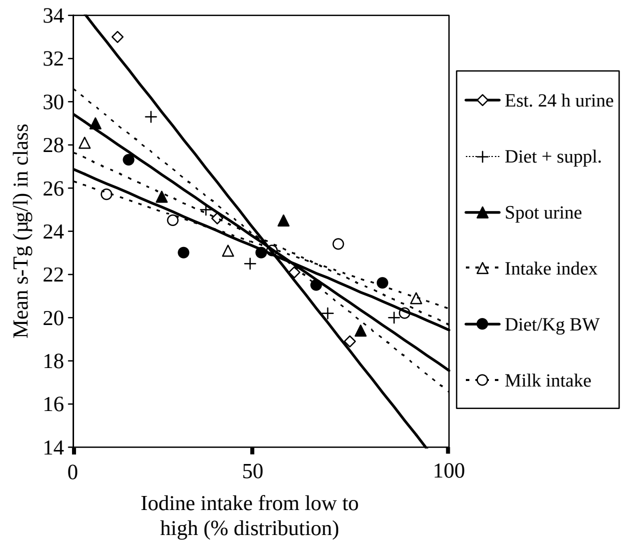

Figure 3 The association between serum thyroglobulin and various measures of iodine intake in groups of participants from the population. The measures of iodine intake were: (1) 24-h urinary iodine excretion estimated from urinary concentrations of iodine and creatinine; (2) daily iodine intake calculated from a food frequency questionnaire including iodine from supplements; (3) Iodine concentration in a spot urine sample; (4) an index of intake of iodine rich food; (5) daily iodine intake per kilogram body weight (BW) calculated from a food frequency questionnaire; and (6) daily intake of milk. Data from Rasmussen *et al.* (16).

Markers of iodine deficiency in the population

Studies of the C1 cohort have revealed a series of abnormalities that correlate to the degree of iodine deficiency. The most sensitive marker is an elevation of the concentration of thyroglobulin in serum (16). However, many types of thyroid abnormalities may stimulate the thyroid release of thyroglobulin, and a high serum thyroglobulin cannot be used as a marker of iodine deficiency in an individual (17). Figure 3 shows how iodine intake evaluated by various methods correlates to serum thyroglobulin in the population. Estimated 24 h urinary iodine excretion (18) was a good marker of low vs high iodine intake. A somewhat similar pattern was observed when serum thyroglobulin concentration was replaced by thyroid volume in these calculations (16).

The results suggest that the estimated 24 h urinary iodine excretion as described in Fig. 3 is a convenient measure of the iodine status. In the entire DanThyr C1 cohort (including participants who took vitamin/mineral supplements), the median 24-h iodine excretion estimated from the concentration of iodine and creatinine in a non-fasting urine sample and the known average 24-h creatinine excretion in people of similar age and sex (18) were 111 μg/day in Copenhagen (iodine concentration in urine 68 μg/l) and 74 μg/day in Aalborg (iodine concentration in urine 53 μg/24 h) (14). This illustrates that a rough measure of the median 24-h urinary iodine excretion (μg/24 h) in a group can be obtained by multiplying the median non-fasting day time spot urine iodine concentration (μg/l) by 1.5 (19).

Thyroid enlargement, clinical goiter, and thyroid nodules

The study of the C1 cohort illustrated the high frequency of goiter in areas with mild to moderate iodine deficiency (Fig. 4) (20). Goiter was more common in Aalborg than in Copenhagen. In the women, thyroid size and the prevalence of goiter increased with age up to the 40–45 year-old group, but not much thereafter. On the other hand, the prevalence of multiple thyroid nodules >1 cm increased with age from 15% in 40–45 year old women to 25–30% in women age 60–65 years. Around 10% of the elderly women had a solitary >1 cm thyroid nodule by ultrasonography.

Median thyroid volume was larger in the elderly men than in the women, but all thyroid abnormalities were considerably more common in the women. The problem of goiter when iodine intake is moderately low can be illustrated by the findings in the 60–65-year-old women in Aalborg (Fig. 4) as follows: 13% had previous goiter diagnosis, 33% had enlarged thyroid by ultrasonography (>18 ml), 24% had palpable goiter, and 6% had undergone goiter surgery (data on surgery not shown).

Environment and goiter

The C1 cohort study enabled identification and characterization of a number of environmental factors

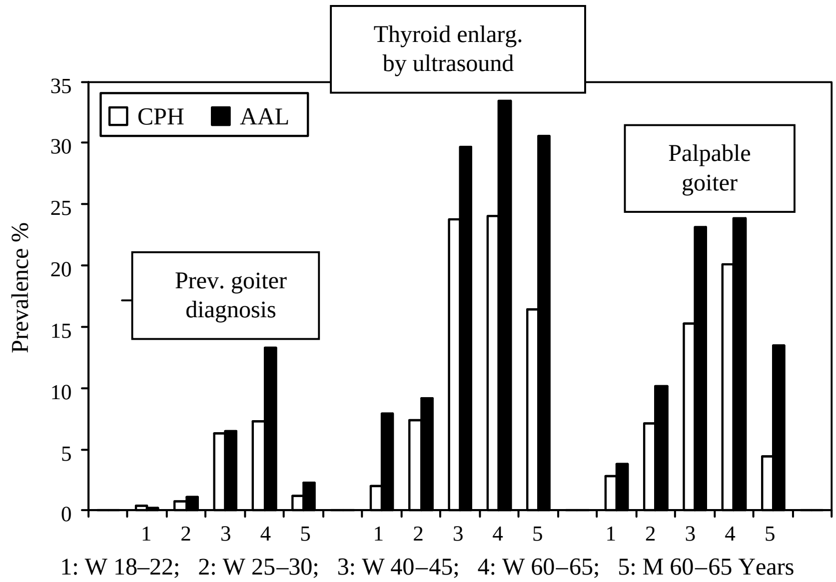

Figure 4 Prevalence rate of goiter in the DanThyr C1 cohort. Columns indicate rates in Copenhagen (CPH) with mild iodine deficiency and Aalborg (AAL) with moderate iodine deficiency. Numbers 1–5 on the abscissa indicate the various groups of people participating in the study (four groups of women (W) aged 18–22, 25–30, 40–45, and 60–65 years, and one group of men (M) aged 60–65 years). The groups of columns indicate from left to right: how frequently participants knew they had goiter, the prevalence of enlarged thyroid by ultrasonography (women >18 ml, men >25 ml), and the prevalence of palpable goiter by clinical examination.

of importance for the development of goiter. As discussed and illustrated (Fig. 4), the iodine intake level was very important. However, even after adjustment for differences in iodine intake, environment had major effects (Table 1). Goiter was more common in smokers (21) and women with previous childbirths (22). On the other hand, regular alcohol consumption (23) and current use of oral contraceptives (24) were associated with less prevalence.

In the C1 cohort, 115 women had a combined low-risk profile (no smoking, no childbirths, regular alcohol consumption, and use of oral contraceptives), whereas 671 women had a combined high-risk profile. None of the 115 low-risk women had visible goiter in the study, and 4.3% had thyroid enlargement by ultrasonography. In the 671 high-risk women, 7.2% had visible goiter, and 32.9% enlarged thyroid by ultrasonography. The estimated odds raio for finding visible goiter in the high-risk group was 12 (Table 1).

The effects of smoking (21) and previous childbirths (22) were more pronounced in Aalborg with the lowest iodine intake, suggesting interaction between these factors and low iodine intake. Smoking may worsen iodine deficiency by thiocyanate inhibition of the sodium-iodide-symporter (25), and pregnancy increases iodide demands for thyroid hormone production (26). Alcohol intake and use of oral contraceptives affected goiter prevalence without interaction with iodine intake. Participants with a high alcohol intake had an odds ratio below 0.5, relative to alcohol abstainers for having thyroid enlargement or nodules No difference in effect was observed between consumption of beer and wine. This seemingly protective effect of alcohol is unexplained (23).

The effect of oral contraceptives was considerable with a 50% reduction in the risk of goiter. We hypothesized that oral contraceptives may suppress endogenous synthesis of goitrogenic by-products of female sex hormone production (24).

Goiter and thyroid nodules were more prevalent in participants with lower levels of education. This association was diminished markedly by adjustments for differences in smoking, alcohol consumption, and iodine intake (27).

The studies illustrate the profound influence of environment on thyroid size and development of goiter. However, familial occurrence of goiter was associated with the presence of goiter in the DanThyr cohort (odds ratio 2.5; 95% confidence interval (CI), 1.6–3.9, relative to no familial occurrence of goiter) (27). Even if underestimation of familial clustering of risk factors cannot be excluded, the genetic background seems to be important for the effect of a certain environment. The importance of genes is in accordance with Danish studies of twins (28). The conclusion of the twin study was that genetic background is the dominant cause for

Table 1 Estimated risk for visible goiter in pre-menopausal women with low- and high-risk profiles in the DanThyr cohort.

	Low-risk profile	High-risk profile
Number with profile[a]	115	671
Current smoker[b]	No	Yes
No. of childbirths[c]	0	≥ 1
Current use of oral contraceptives[d]	Yes	No
Alcohol intake, drinks per week[e]	≥ 7	<7
Odds ratio	1.0 (reference)	12.0

Data were adjusted for differences in age and region of inhabitance. [a]Total number of pre-menopausal women investigated: 2821. [b]Data for calculation from Knudsen *et al.* (21). [c]Data for calculation from Knudsen *et al.* (22). [d]Data for calculation from Knudsen *et al.* (24). [e]Data for calculation from Knudsen *et al.* (23).

goiter in Denmark, which is not in accordance with results of our studies. Underestimation of the importance of environmental factors for development of common diseases has been a general phenomenon in studies of twins (29). The discussion on genetic modification of environmental effects is complex. Genetic factors are difficult to change, but some of the important environmental factors identified in our studies are certainly modifiable.

Iodine intake and thyroid function in the population

In an iodine-replete population, like that of the US, serum TSH tends to increase with age (30). A major factor responsible for this phenomenon seems to be a gradual thyroid failure in many people caused by autoimmunity. Accordingly, the prevalence rate of treated and untreated overt or subclinical hypothyroidism is high in such populations (30). In the DanThyr C1 cohort, the pattern was different; serum TSH decreased with age. This decrease was more pronounced in Aalborg with the lowest level of iodine intake (Fig. 5). The mechanism behind this fall seems to be responsible for the high frequency of the development of autonomous thyroid nodules with age, in people living in a low iodine intake area. When participants with any type of thyroid abnormality by ultrasonography were excluded from analysis, serum TSH did not decrease with age (8).

In the C1 cohort study, 95% of participants (percentiles 2.5–97.5) with no known thyroid disease, no thyroid peroxidase antibodies in serum, and normal thyroid size and structure by ultrasonography had serum TSH between 0.4 and 3.6 mU/l (8). Many participants with abnormal thyroid gland or antibodies in serum had serum TSH outside this range and the prevalence increased with age. Among the participants never treated for thyroid disease, but otherwise not selected ($n=4360$), the prevalence of low TSH among 60–65 year-old women and men in Aalborg (moderate iodine deficiency) was 11.1/7.2%, and in Copenhagen (mild iodine deficiency), it was 8.3/4.0%. On the other hand, the prevalence of elevated TSH as a sign of some thyroid failure was: Aalborg 8.2/2.1% and Copenhagen 9.1/2.7%. Accordingly, 15–20% of the post-menopausal women and 5–10% of the men had abnormal serum TSH. In Aalborg, more people had low rather than high serum TSH, but both abnormalities were common. The majority of participants with abnormal TSH had serum-free thyroxine (T_4) and free tri-iodothyronine (T_3) within the reference ranges (8).

Thyroid function and overweight in the population

It is much debated whether small abnormalities in thyroid function are of clinical importance and should be treated. In contrast, there is consensus that overweight is in general not caused by some metabolic abnormality, but by excess energy intake relative to energy expenditure. Resting energy expenditure is rather sensitive to small alterations in thyroid function (31). Resting energy expenditure is the dominant type of energy expenditure constituting approximately 60% (40–80%) of total energy expenditure, depending on the activity level of the individual.

In the DanThyr C1 cohort, even small differences in serum TSH within the reference range were associated with differences in body mass index (BMI) (Fig. 6) (32). The difference in average body weight between the group with slightly elevated TSH (median 4.5 mU/l) and slightly low TSH (median 0.28 mU/l) (all had normal free T_4 and free T_3) was 5.5 kg, and the group with elevated TSH was on average 4.0 kg heavier than the group with a TSH of 1–1.99 mU/l. Serum-free T_4 showed a negative association with BMI, which demonstrates that the elevated TSH is caused by thyroid failure and not by a central effect such as leptin stimulation of hypothalamic thyrotropin-releasing hormone secretion. The findings, which have been confirmed in a Norwegian population study (33)

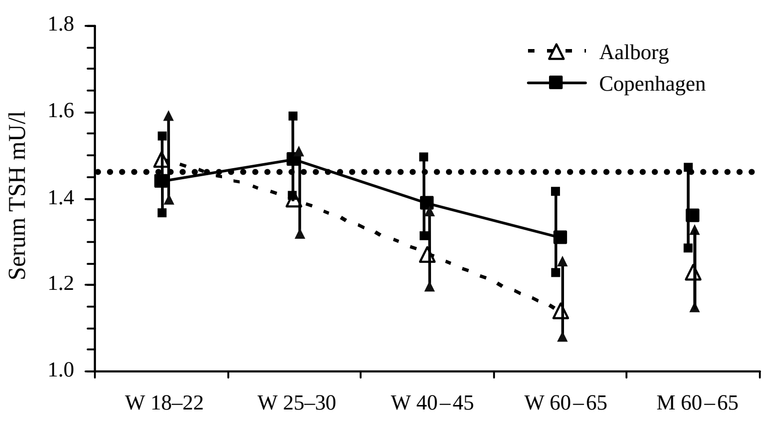

Figure 5 Median serum TSH in the groups of participants in the DanThyr C1 cohort study. The dotted line indicates average values in young healthy participants from both cities (Four groups of women (W) aged 18–22, 25–30, 40–45 and 60–65 years, and one group of men (M) aged 60–65 years). Vertical lines are 95% confidence of median. The figure illustrates the age-associated decrease in serum TSH in the population, and that this was more pronounced in Aalborg with the lowest iodine intake. Data from Knudsen *et al.* (8).

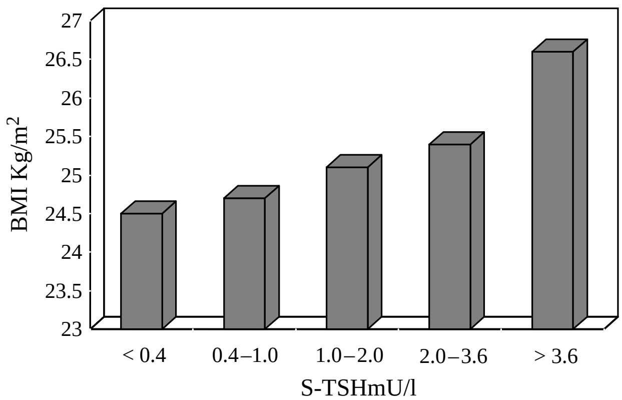

Figure 6 Association between serum TSH (s-TSH) and body mass index (BMI) in the DanThyr C1 cohort. Participants previously treated for thyroid dysfunction or with overt dysfunction when studied were excluded. Data from Knudsen *et al.* (32).

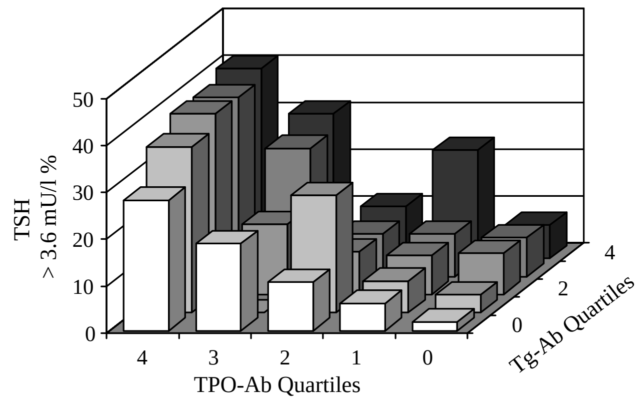

Figure 7 Frequency of finding elevated serum TSH (>3.6 mU/l) in people with thyroid peroxidase antibodies (TPO-Ab) and thyroglobulin antibodies (Tg-Ab) in serum. The group marked '0' had no antibodies. People with antibodies were split into quartiles according to the level of the antibody. Both TPO-Ab and Tg-Ab were associated with an increase in the risk of having elevated serum TSH, but the presence of TPO-Ab was a much stronger predictor than the presence of Tg-Ab. Data from Bülow Pedersen *et al.* (36).

illustrate that even small differences in thyroid function in a population may have clearly detectable effects on other variables in the population. Recently, an association has been detected between small differences in thyroid function and blood pressure in the C1 cohort (34).

Thyroid antibodies and thyroid function in the population

Thyroid autoimmunity is common in all populations with some differences in prevalence associated with genetic background (30). In the DanThyr C1 cohort, thyroid peroxidase autoantibodies (TPO-Ab) and thyroglobulin autoantibodies (Tg-Ab) were measured with sensitive and specific methods (35). The overall prevalence of one or both antibodies was 18.8%. The prevalence rates of the two antibodies were similar (TPO-Ab/Tg-Ab: 13.1/13.0%). Antibodies were more common in women than in men, and in the women, the prevalence increased with age (35). The prevalence rates of thyroid antibodies measured by other assays in National Health and Nutrition Examination Survey III were TPO-Ab, 13.0% and Tg-Ab, 11.5% (30).

The distribution of the two antibodies was similar in many ways (35). This may suggest that the generation of one or the other antibody in an individual is more or less by chance. However, the functional consequence of harboring one or the other antibody is very different (Fig. 7). The frequency of impaired thyroid function with an elevated serum TSH increased dramatically with the amount of TPO-Ab in serum (36). There was an additional association between an elevated TSH and the level of Tg-Ab, but this was much less (Fig. 7). In the cohort, there was an association between elevated serum TSH, the presence of TPO-Ab, and large thyroid volume (36). In the clinic, this is the picture of a patient with Hashimoto's goitrous thyroiditis and subclinical hypothyroidism.

We found no association between thyroid autoantibodies and parity, which argues against microchimerism as a trigger of thyroid autoimmunity (37). Interestingly, use of hormone-replacement therapy by post-menopausal women was associated with a low prevalence of Tg-Ab (37).

In the cohort, there was a moderate association between hypoechogenicity by thyroid ultrasonography, impaired thyroid function and the presence of TPO-Ab (38). The forthcoming follow-up study of the C1 cohort will show the importance of the different markers for future development of overt thyroid disease.

Iodine intake and the incidence of overt hyper- and hypothyroidism

The DanThyr register of overt hypo- and hyperthyroidism revealed considerable differences in incidences of hyper- and hypothyroidism between the two study areas (39). Hyperthyroidism was more common in Aalborg and hypothyroidism in Copenhagen. Hyperthyroidism was in general more common than hypothyroidism. This pattern was observed in both men and women, and in different age groups (Fig. 8). The pattern in Aalborg is similar to previous findings in another part of Jutland (40), and the differences between Aalborg and Copenhagen are qualitatively similar to the previously observed differences between Jutland with a low iodine intake and Iceland with a much higher iodine intake (41).

Probably, the high incidence of hyperthyroidism is caused by a high incidence of multi-nodular toxic goiter (41), but subtype analyses of data have not yet been finalized. Recently, the data on incidences of subtypes of

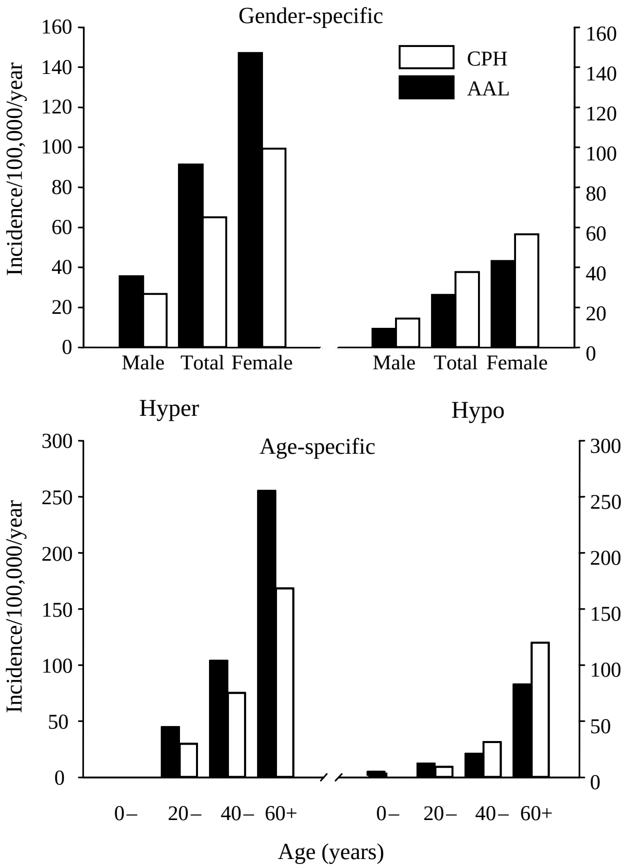

Figure 8 Incidences of overt hyper- and hypothyroidism in Copenhagen (CPH) with mild iodine deficiency, and in Aalborg (AAL) with moderate iodine deficiency. The upper panel shows values for women and men, and the lower panel for different age groups. The incidence of overt hyperthyroidism (hyper) was significantly higher in Aalborg, whereas the incidence of overt hypothyroidism (hypo) was significantly higher in Copenhagen. Both disorders were more common in women than in men, and the incidences increased with age. Data from Bülow Pedersen *et al.* (39).

hypothyroidism before mandatory salt iodization were published (Table 2) (42). The difference in the incidence of hypothyroidism between areas is entirely caused by a difference in incidence of spontaneous hypothyroidism, which is presumably caused by autoimmune destruction of the thyroid gland. The findings suggest that an increase in iodine intake of a population may, over time, lead to fewer cases of hyperthyroidism, whereas there may be more cases of autoimmune hypothyroidism – even when iodine intake is changed from deficient to adequate.

The mechanism behind an iodine-induced increase in the incidence of hypothyroidism is unknown, but several mechanisms could be involved. Iodine has been associated with thyroid autoimmunity (43), and it has a number of autoregulatory inhibitory effects on thyroid hormone production and secretion (44). Moreover, excess iodine may lead to apoptosis of thyroid follicular cells as studied in *in vitro* systems (45).

Table 2 Standardized incidence rates (SIR) of subtypes of overt hypothyroidism in Aalborg with moderate and Copenhagen with mild iodine deficiency.

	SIR[a] Copenhagen	SIR[a] Aalborg	SIRR[b] (CI[c])
Hypothyroidism	38.9	29.2	1.33 (1.15–1.55)*
Spontaneous	35.0	23.1	1.53 (1.29–1.80)*
Non-spontaneous	3.9	6.1	0.64 (0.43–0.96)*
PPTD[d]	1.4	1.8	0.77 (0.40–1.50)
Amiodarone	0.8	1.6	0.60 (0.26–1.37)
SAT[e]	0.3	0.9	0.30 (0.06–1.35)
Radiation/surgery	0.3	0.9	0.26 (0.06–1.20)
Lithium	0.4	0.6	0.69 (0.18–2.66)
Congenital	0.7	0.4	1.72 (0.50–5.95)

*Statistically significant ($P<0.05$). Reproduced from Carlé *et al.* (42). [a]Incidence rates (per 100 000 persons/year) age adjusted to the Danish population January 1, 1999. [b]Standardized incidence rate ratio (SIRR). A SIRR value above 1.0 indicates a higher frequency of hypothyroidism in Copenhagen compared with Aalborg. The difference is statistically significant, if 1.0 is not included in the confidence interval of SIRR. [c]95% Confidence intervals of standardized incidence rate ratios. [d]*Post partum* thyroid dysfunction presenting with hypothyroidism. [e]Subacute thyroiditis presenting with hypothyroidism.

Central register for surgical and medical treatment

Data from a number of central registers have been used to compare the use of antithyroid drugs, levothyroxine, number of patients treated with radioiodine, and number of thyroid operations in areas of Denmark with different iodine intake levels. Before iodine fortification of salt, the use of antithyroid drugs was 60% higher in the west than in the east part of Denmark, 20% more people were treated with radioiodide, and thyroid surgery was 25% more common (13). This corresponds to the higher incidence of hyperthyroidism and high prevalence of goiter in Jutland with the lowest iodine intake.

From 1999 to 2003, the quantity of antithyroid drugs sold increased 42% in North Jutland and the number of patients treated increased 32% (from 6.5 to 8.6/1000). Little change occurred in Copenhagen; the quantity of drugs sold was unaltered, and the number of patients treated increased from 2.9 to 3.0/1000 per year (46). This difference corresponds to the more severe increase in incidence of hyperthyroidism in Aalborg than in Copenhagen after iodide fortification of salt (47).

Effect of the salt iodization program on iodine intake and thyroid diseases in Denmark

As described above, much of the data analyses so far have been devoted to the description of the conditions during mild and moderate iodine deficiency in the two study areas. Collection and analyses of data after iodization of salt have just begun. Studies on food indicate that legislation works as planned (15), and urinary iodine data from the cohort investigated in 2005 (C2) will become available in 2006. In addition, C2 data on thyroid size and structure as well as thyroglobulin in serum will be available for comparison with the C1 cohort investigated before iodization of salt. Data on serum TSH, T_4, and T_3 will show if the iodization program has changed thyroid function in the population, and data on thyroid antibodies in serum if iodization has affected thyroid autoimmunity in the population.

Incidences of overt hyperthyroidism were expected to increase transiently after the increase in iodine intake (48). Such an increase should presumably be caused by hyperthyroidism in elderly subjects harboring autonomous thyroid nodules in a goiter. We have observed a 50% increase in the incidence of hyperthyroidism in Aalborg and a somewhat smaller increase in Copenhagen (47). Unexpectedly, this increase has predominantly occurred in young subjects, where Graves' disease is the dominant cause of hyperthyroidism. It may suggest a change in disease pattern from multi-nodular toxic goiter in the elderly towards Graves' disease in the young. Such a pattern is found in Iceland with a relatively high iodine intake (41).

Large differences have been observed between the occurrence of hypothyroidism in different countries (49). It is at present unclear if this is caused by genetic or environmental differences. Hypothyroidism is relatively rare in Denmark compared with some other countries (49) and it is our hope that the present cautious and monitored program of salt iodization will not alter this state. So far, no major changes have been observed.

Conclusion

The DanThyr, which monitors the Danish iodine fortification program, has given considerable information on the importance of small systematic differences in iodine intake for the occurrence of thyroid diseases in a population. It has shown that not only iodine intake, but also a number of other environmental factors alter the risk of thyroid disease. Some factors seem to act via interaction with iodine intake, whereas others are independent of iodine. Information on risk factors is useful for the guidance of individual patients, and it gives a basis for studying pathogenic mechanisms. DanThyr has verified the high occurrence of multi-nodular thyroid disease and hyperthyroidism in low intake areas. The program will allow a comprehensive description of the changes occurring in thyroid diseases in a population when the iodine intake is increased to a recommended level from being insufficient. Such knowledge may be of value for optimizing the iodine fortification program in Denmark and elsewhere. Moreover, it may show whether more control is needed with the use of iodine-containing substances in farming, food industry, and elsewhere.

References

1 Vitti P, Delange F, Pinchera A, Zimmermann M & Dunn JT. Europe is iodine deficient. *Lancet* 2003 **361** 1226.

2 International Council for the Control of Iodine Deficiency Disorders, The United Nations Children's Fund, World Health Organization. Assessment of iodine deficiency disorders and monitoring their elimination 2nd ed. Geneva: *World Health Organization*, 2001.

3 Pedersen KM, Laurberg P, Iversen E, Knudsen PR, Gregersen HE, Rasmussen OS, Larsen KR, Eriksen GM & Johannesen PL. Amelioration of some pregnancy-associated variations in thyroid function by iodine supplementation. *Journal of Clinical Endocrinology and Metabolism* 1993 **77** 1078–1083.

4 Rasmussen LB, Andersson G, Haraldsdottir J, Kristiansen E, Molsted K, Laurberg P, Overvad K, Perrild H & Ovesen L. Iodine. Do we need an enrichment program in Denmark? *International Journal of Food Sciences and Nutrition* 1996 **47** 377–381.

5 Bekendtgørelse om tilsætning af jod til husholdningssalt og salt i brød og almindeligt bagværk m.v. *Fødevareministeriets bekendtgørelse nr. 627* af 29. juni, 2000.

6 Laurberg P. Iodine intake – what are we aiming at? *Journal of Clinical Endocrinology and Metabolism* 1994 **79** 17–19.

7 Pedersen KM, Laurberg P, Nohr S, Jorgensen A & Andersen S. Iodine in drinking water varies by more than 100-fold in Denmark. Importance for iodine content of infant formulas. *European Journal of Endocrinology* 1999 **140** 400–403.

8 Knudsen N, Bülow I, Jorgensen T, Laurberg P, Ovesen L & Perrild H. Comparative study of thyroid function and types of thyroid dysfunction in two areas in Denmark with slightly different iodine status. *European Journal of Endocrinology* 2000 **143** 485–491.

9 Rasmussen LB, Ovesen L, Bülow I, Jorgensen T, Knudsen N, Laurberg P & Perrild H. Evaluation of a semi-quantitative food frequency questionnaire to estimate iodine intake. *European Journal of Clinical Nutrition* 2001 **55** 287–292.

10 Knudsen VK, Rasmussen LB, Haraldsdottir J, Ovesen L, Bulow I, Knudsen N, Jorgensen T, Laurberg P & Perrild H. Use of dietary supplements in Denmark is associated with health and former smoking. *Public Health Nutrition* 2002 **5** 463–468.

11 Knudsen N, Bols B, Bülow I, Jorgensen T, Perrild H, Ovesen L & Laurberg P. Validation of ultrasonography of the thyroid gland for epidemiological purposes. *Thyroid* 1999 **9** 1069–1074.

12 Pedersen IB, Laurberg P, Arnfred T, Knudsen N, Jorgensen T, Perrild H & Ovesen L. Surveillance of disease frequency in a population by linkage to diagnostic laboratory databases. A system for monitoring the incidences of hyper- and hypothyroidism as part of the Danish iodine supplementation program. *Computer Methods and Programs in Biomedicine* 2002 **67** 209–216.

13 Jorgensen T, Laurberg P, Perrild H & Ovesen L. Direct costs of handling patients with thyroid diseases in areas with mild and moderate iodine deficiency. *European Journal of Public Health* 2002 **12** 66 (abstract).

14 Rasmussen LB, Ovesen L, Bülow I, Jorgensen T, Knudsen N, Laurberg P & Perrild H. Dietary iodine intake and urinary iodine excretion in a Danish population: effect of geography, supplements and food choice. *British Journal of Nutrition* 2002 **87** 61–69.

15 Rasmussen LB, Bülow I, Knudsen N, Perrild H, Jorgensen T, Ovesen L & Laurberg P. Monitoring of the Danish iodine fortification programme. Eighteenth International Nutrition Congress, Durban, South Africa, 2005. Abstract 6.2.7:157. Available at http://www.puk.ac.za/iuns/(accessed March 06 2005).

16 Rasmussen LB, Ovesen L, Bulow I, Jorgensen T, Knudsen N, Laurberg P & Perrild H. Relations between various measures of iodine intake and thyroid volume, thyroid nodularity, and serum thyroglobulin. *American Journal of Clinical Nutrition* 2002 **76** 1069–1076.

17 Knudsen N, Bülow I, Jorgensen T, Perrild H, Ovesen L & Laurberg P. Serum Tg – a sensitive marker of thyroid abnormalities and iodine deficiency in epidemiological studies. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 3599–3603.

18 Knudsen N, Christiansen E, Brandt-Christensen M, Nygaard B & Perrild H. Age- and sex-adjusted iodine/creatinine ratio. A new standard in epidemiological surveys? Evaluation of three different estimates of iodine excretion based on casual urine samples and comparison to 24 h values *European Journal of Clinical Nutrition* 2000 **54** 361–363.
19 Laurberg P, Andersen S, Bjarnadóttir RI, Carlé A, Hreidarsson AB, Knudsen N, Ovesen L, Pedersen IB & Rasmussen LB. Evaluation of iodine deficiency in pregnant women and young infants. Complex physiology with risk of misinterpretation. *Public Health Nutrition* (In Press).
20 Knudsen N, Bülow I, Jorgensen T, Laurberg P, Ovesen L & Perrild H. Goitre prevalence and thyroid abnormalities at ultrasonography: a comparative epidemiological study in two regions with slightly different iodine status. *Clinical Endocrinology* 2000 **53** 479–485.
21 Knudsen N, Bülow I, Laurberg P, Ovesen L, Perrild H & Jorgensen T. Association of tobacco smoking with goiter in a low-iodine-intake area. *Archives of Internal Medicine* 2002 **162** 439–443.
22 Knudsen N, Bülow I, Laurberg P, Ovesen L, Perrild H & Jorgensen T. Parity is associated with increased thyroid volume solely among smokers in an area with moderate to mild iodine deficiency. *European Journal of Endocrinology* 2002 **146** 39–43.
23 Knudsen N, Bülow I, Laurberg P, Perrild H, Ovesen L & Jorgensen T. Alcohol consumption is associated with reduced prevalence of goitre and solitary thyroid nodules. *Clinical Endocrinology* 2001 **55** 41–46.
24 Knudsen N, Bülow I, Laurberg P, Perrild H, Ovesen L & Jorgensen T. Low goitre prevalence among users of oral contraceptives in a population sample of 3712 women. *Clinical Endocrinology* 2002 **57** 71–76.
25 Laurberg P, Nohr SB, Pedersen KM & Fuglsang E. Iodine nutrition in breast-fed infants is impaired by maternal smoking. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 181–187.
26 Glinoer D. The regulation of thyroid function during normal pregnancy: importance of the iodine nutrition status. *Best Practice and Research. Clinical Endocrinology and Metabolism* 2004 **18** 133–152.
27 Knudsen N, Bülow I, Laurberg P, Ovesen L, Perrild H & Jorgensen T. Low socio-economic status and familial occurrence of goitre are associated with a high prevalence of goitre. *European Journal of Epidemiology* 2003 **18** 175–181.
28 Brix TH, Kyvik KO & Hegedus L. Major role of genes in the etiology of simple goiter in females: a population-based twin study. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 3071–3075.
29 Hopper JL. Why common environmental effects are so uncommon in the literature. In *Advances in Twin and Sib-Pair Analysis*, Eds. TD Spector, H Sneider & AJ MacGregor, London: Greenwich Medical Media Ltd., 2000.
30 Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA & Braverman LE. Serum TSH, T4, and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 489–499.
31 al-Adsani H, Hoffer LJ & Silva JE. Resting energy expenditure is sensitive to small dose changes in patients on chronic thyroid hormone replacement. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 1118–1125.
32 Knudsen N, Laurberg P, Rasmussen LB, Bülow I, Perrild H, Ovesen L & Jorgensen T. Small differences in thyroid function may be important for body mass index and the occurrence of obesity in the population. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 4019–4024.
33 Nyrnes A, Jorde R & Sundsfjord J. Serum TSH is positively associated with BMI. *International Journal of Obesity* 2006 **30** 100–105.
34 Knudsen N, Laurberg P, Perrild H, Ovesen L, Bülow I, Jorgensen T & Rasmussen L. Elevated blood pressure is associated with small differences in serum TSH in a general population. *Thyroid* 2005 **15** S-10 (abstract).
35 Pedersen IB, Knudsen N, Jorgensen T, Perrild H, Ovesen L & Laurberg P. Thyroid peroxidase and thyroglobulin autoantibodies in a large survey of populations with mild and moderate iodine deficiency. *Clinical Endocrinology* 2003 **58** 36–42.
36 Bülow Pedersen I, Laurberg P, Knudsen N, Jorgensen T, Perrild H, Ovesen L & Rasmussen LB. A population study of the association between thyroid autoantibodies in serum and abnormalities in thyroid function and structure. *Clinical Endocrinology* 2005 **62** 713–720.
37 Bülow Pedersen I, Laurberg P, Knudsen N, Jorgensen T, Perrild H, Ovesen L & Rasmussen LB. Lack of association between thyroid autoantibodies and parity in a population study argues against microchimerism as a trigger of thyroid autoimmunity. *European Journal of Endocrinology* 2006 **154** 39–45.
38 Vejbjerg P, Knudsen N, Perrild H, Laurberg P, Pedersen I, Ovesen L, Rasmussen L & Jorgensen T. The association between hypoechogenicity at thyroid ultrasonography and thyroid function in the general population. *Thyroid* 2005 **15** S-194 (abstract).
39 Bülow Pedersen I, Knudsen N, Jorgensen T, Perrild H, Ovesen L & Laurberg P. Large differences in incidences of overt hyper- and hypothyroidism associated with a small difference in iodine intake: a prospective comparative register-based population survey. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 4462–4469.
40 Laurberg P, Bülow Pedersen I, Pedersen KM & Vestergaard H. Low incidence rate of overt hypothyroidism compared with hyperthyroidism in an area with moderately low iodine intake. *Thyroid* 1999 **9** 33–38.
41 Laurberg P, Pedersen KM, Vestergaard H & Sigurdsson G. High incidence of multinodular toxic goitre in the elderly population in a low iodine intake area vs. high incidence of Graves' disease in the young in a high iodine intake area: comparative surveys of thyrotoxicosis epidemiology in East-Jutland Denmark and Iceland. *Journal of Internal Medicine* 1991 **229** 415–420.
42 Carlé A, Laurberg P, Pedersen IB, Knudsen N, Perrild H, Ovesen L, Rasmussen LB & Jorgensen T. Epidemiology of subtypes of hypothyroidism in Denmark. *European Journal of Endocrinology* 2006 **154** 21–28.
43 Braverman LE. Iodine and the thyroid: 33 years of study. *Thyroid* 1994 **4** 351–356.
44 Markou K, Georgopoulos N, Kyriazopoulou V & Vagenakis AG. Iodine-induced hypothyroidism. *Thyroid* 2001 **11** 501–510.
45 Langer R, Burzler C, Bechtner G & Gartner R. Influence of iodide and iodolactones on thyroid apoptosis. Evidence that apoptosis induced by iodide is mediated by iodolactones in intact porcine thyroid follicles. *Experimental and Clinical Endocrinology and Diabetes* 2003 **111** 325–329.
46 Perrild H, Jorgensen T, Knudsen N, Vejbjerg P, Laurberg P, Bülow I & Ovesen L. An increase in antithyroid drugs sold as well as number treated predominantly in a moderately iodine deficient area compared to an area with mild iodine deficiency after national iodination program. *Thyroid* 2005 **15** S-180 (abstract).
47 Bülow Pedersen I, Laurberg P, Knudsen N, Jorgensen T, Perrild H, Ovensen L & Rasmussen L. Predominant increase in incidence of hyperthyroidism in young people after iodine fortification of salt in Denmark. *Thyroid* 2005 **15** S-175 (abstract).
48 Stanbury JB, Ermans AE, Bourdoux P, Todd C, Oken E, Tonglet R, Vidor G, Braverman LE & Medeiros-Neto G. Iodine-induced hyperthyroidism: occurrence and epidemiology. *Thyroid* 1998 **8** 83–100.
49 Laurberg P. Global or Gaelic epidemic of hypothyroidism? *Lancet* 2005 **365** 738–740.

Received 28 March 2006
Accepted 15 May 2006