Risk factors for benign thyroid disease

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INTRODUCTION

The prevalence of goitre and thyroid dysfunction in the population may be influenced by a number of factors, and the aetiology of goitre is still not known in detail. One factor has been offered much attention in the past, namely iodine intake; and iodine intake undoubtedly takes a dominating position in the determination of the occurrence of thyroid diseases, particularly goitre. This dominating role of iodine intake in the determination of goitre prevalence may have discouraged scientists from searching for further risk factors. Thus, further research is needed for an understanding of the aetiology of goitre with possible therapeutic advances to follow. However, also potential prophylactic initiatives may not have been engaged.

Iodine deficiency as a risk factor for goitre has been known at least since 1820 (14). The association between severe iodine deficiency and endemic goitre is well established (15), but the impact of lesser degrees of iodine deficiency and iodine excess is still under discussion. Consequently, the optimal level of iodine intake has not been established (16), though recommendations of iodine intake have been published and give some clue to the optimal levels of iodine intake (15).

One reason why the optimal iodine intake has not been established is that few studies were actually designed with this purpose. Such studies should use precise and evaluated methods and proper epidemiological designs intending to compare levels of iodine intake in different populations with the occurrence of goitre and other manifestations of iodine deficiency. Different ways of measuring iodine intake and the lack of accuracy of these estimates of iodine intake have given rise to misclassification. Further, no consensus exists about the measurement of indicators of iodine deficiency. Thus, even if thyroid volume determined with ultrasonography has been the preferred indicator in the past decades, a lack of standardisation has been an obstacle for precise comparisons. Schoolchildren have often been the target group for epidemiological surveys (15). It is very likely, however, that mild degrees of iodine deficiency do not have manifestations in childhood but only after several decades of exposure to iodine deficiency – e.g. multinodular goitre among the elderly part of the population (V, 17). The time required for full impact of changes in iodine intake of a population on thyroid volume is not known. It is probably dependent on the group investigated and the degree of iodine deficiency before the increase in iodine intake took place and on the magnitude of the increase. This cohort effect may confound comparisons between different areas if iodine intake has not been stable for generations.

Only few studies have used methods from analytical epidemiology in the investigation of thyroid disease. Thus, only few studies have searched for associations between life-style and thyroid disease, and the proper analytical tools to identify the individual risk factors have not been applied. Goitrogens in water and food have been investigated (18), and the impact of smoking on the occurrence of goitre and especially Graves disease with ophthalmopathy have been described in a number of studies (19, 20). The importance for the occurrence of thyroid disease of life-style compared to the impact of iodine status of the population in general and to the impact genetic factors has still not been established, even if some estimates have been suggested. In twin studies, a higher concordance rate has been found for monozygotic than for dizogotic twins for goitre as well as autoimmune thyroid disease indicating a role of genetic background in the pathogenesis (21-23). The relative importance of environment and genes in the pathogenesis of different thyroid diseases has not been settled, and the probably very complex interactions between genes and environment also remain to be elucidated (23).

More than 1.5 billion people live in iodine-deficient areas (24), and now $^2/_3$ of the population exposed to iodine deficiency have access to iodized salt (25). Still, goitre has a high prevalence in many countries also in Europe, illustrated by our report of previous goitre operation for non-toxic goitre in 4-6% of 60-65 years old Danish women (V). In Germany with mild to moderate iodine deficiency as in Denmark, the annual costs of iodine-deficiency goitre treatment was recently estimated to be 2.1 billion D-Mark (1 billion Euro) (26). This could suggest annual costs of iodine-deficiency goitre treatment of approximately 600 million DKK (80 million Euro) in Denmark. Thus, the potential benefit from goitre prevention is considerable for public health as well as health care costs.

AIM

The main aim was to describe the prevalence of thyroid disease in Denmark. Special attention was paid to the regional differences in order to evaluate the impact of differences in iodine intake in areas with mild to moderate iodine deficiency on the prevalences of thyroid diseases, as the DanThyr cohort was also meant to be the first part of the monitoring of an iodization program in Denmark. Variation in the prevalence of thyroid disease with age and sex and the interplay with iodine status were to be part of the analyses.

Other aims were to analyse the impact of modifiable, life-style-related factors such as smoking, alcohol consumption, use of oral contraceptives and previous pregnancies on the occurrence of thyroid disease. This was intended both to investigate the risk associated with exposure to these potential risk factors and to ensure that observed regional differences in the occurrence of thyroid disease were not explained by different exposure to these risk factors. Again, interplay between different factors and in particular the interplay with iodine status was to be considered. Likewise, influence of non-modifiable risk factors such as family history and social status was investigated.

Going through the literature, a need for validation of methods used in thyroid epidemiology was identified, as validation of the most used principles for monitoring of iodine status in a population was scarce. Thus, a further aim was validation of these methods. Measurement of iodine excretion and of thyroid volume was evaluated through precise descriptions of the methods and modifications to ensure reproducibility and comparability from different studies. This was not only for use in the present studies but also for the applicability in thyroid epidemiology in general. Testing of serum thyroglobulin as a useful marker of iodine deficiency in a population was part of these validations.

METHODS

COHORTS

The data for the analyses in this thesis were obtained from four different cohorts: two population-based cross-sectional cohorts for the investigation of determinants of thyroid disease, and two smaller cohorts for the evaluation of methodology used for estimating iodine excretion in urine and estimating thyroid volume using ultrasonography.

DanThyr

The Danish Investigation of Iodine Intake and Thyroid Diseases (DanThyr) was initiated to assess the occurrence of thyroid disease in Denmark before an intended iodization programme and subsequently to monitor the consequences of the iodization programme. It was established in co-operation between the departments of endocrinology at Bispebjerg Hospital and Aalborg Hospital, The Danish Food Administration, and Centre for Preventive Medicine at Glostrup Hospital. The first cohort in the DanThyr investigation was generated with the specific purpose of obtaining information on the prevalence of thyroid diseases in two parts of Denmark. It was designed as a cross-sectional, comparative study in order to focus on regional differences in the occurrence of thyroid diseases in Denmark in two regions with a difference in iodine intake (27). Due to the difficulties with individual estimation of iodine intake and the yearlong exposure with possible changes in iodine intake over time, this design with characterisation of iodine status in large groups is the preferred design when investigating associations between iodine intake and thyroid disease.

Two regions were chosen for the investigation. Representing the eastern part of Denmark with a lesser degree of iodine deficiency, the northern part of the municipality of Copenhagen was chosen. Representing the more severe iodine deficiency in the western part of Denmark, the central part of Aalborg was chosen. Thus, in both regions urban areas were investigated. With a relatively uniform iodine excretion in the western part of Denmark, Aalborg is probably representative for this part of Denmark (28). In the eastern part of Denmark, larger variations in iodine excretion has been found, but still within the limits of mild iodine deficiency (28). Thus, the Copenhagen part of the cohort is representative for the major part of eastern Denmark, but small variations in iodine status should be considered.

Five groups were chosen for the investigation; women aged 18-22, 25-30, 40-45 and 60-65 years and men aged 60-65 years. The age groups were chosen to represent women before childbearing age, in childbearing age, after childbearing age, and postmenopausal women. An over-representation of women was chosen to increase the statistical power of the study owing to the higher prevalence of thyroid diseases in women. One group of men aged 60-65 years was included for comparisons between the genders.

The sample was drawn from the Civil Registration System that generated complete lists of subjects within the age groups in the two areas. The samples were restricted to subjects born in Denmark. This was done to avoid participants who had grown up under different and possibly unknown iodine exposure. Further it reduced the number of participants not able to participate for linguistic reasons. The subjects in these lists were assigned random numbers within each group by a computer programme and they were invited to participate in the order of the random numbers.

The number of participants required to detect a regional difference in thyroid volume of 1 ml between each group was 416; the SD was estimated to 4 ml and α and β were both set to 5%. This number would also allow the detection of a 4% difference in the prevalence of palpable goitre overall if the prevalence was 10% in the "control" area. Thus, as these were the primary endpoints, the aim was to reach 500 participants in each group. The numbers invited in each group were adjusted throughout the study to ensure that the number of participants was the same in all groups irrespective of different rates of participation.

The investigation was conducted in 1997 and 1998. The overall participation rate turned out to be 50.1%, as 4, 649 participated out of 9, 274 invited (**Table 1**). With this participation rate it was essential to ensure the external validity of the study. A short questionnaire was sent to the non-participants, and another 27% of the original cohort completed this questionnaire. From this short questionnaire, a higher occurrence of previously diagnosed thyroid disease was reported among participants than among respondents to the short questionnaire (odds ratio 1.9; confidence interval 1.5-2.5), suggesting an over-estimation of the prevalence of known goitre in the population from the participants. The degree of bias was similar in the two regions, and thus comparisons between the regions were unbiased. No selection bias was identified regarding previous thyroid dysfunction.

Table 1. Participation rates in the two regions in Denmark in the first DanThyr cohort.

	Copenhagen	Aalborg	Total
Invited	5209	4065	9274
Full participation	2429 (46,6%)	2220 (54,6%)	4649 (50,1%)
Short questionnaire	1406 (27,0%)	1079 (26,5%)	2485 (26,8%)
No response	1372 (26,3%)	768 (18,9%)	2139 (23,1%)

The participation rate differs significantly between the two regions ($p<0.001$; Pearson chi-square).

Table 2. Odds ratios for previous goitre operation, goitre diagnosis or thyroid dysfunction registered in the Danish National Patient Register for subjects from the DanThyr study. Participants in the full examination are reference group. Odds ratios are given with 95% confidence interval.

	Participants	Respondents to short questionnaire	Non-respondents
Goitre operation	1 (reference)	0.30 (0.15-0.59)	0.35 (0.18-0.67)
Goitre diagnosis	1 (reference)	0.37 (0.18-0.76)	0.53 (0.27-1.03)
Thyroid dysfunction diagnosis	1 (reference)	0.41 (0.22-0.78)	0.67 (0.39-1.17)

Records of previous goitre operations and goitre diagnoses were obtained from the National Patient Register. From these records, a higher incidence of goitre operations was reported amongst participants than amongst non-participants, whereas the group with no response to the short questionnaire exhibited an intermediate incidence between the participants and the respondents to the short questionnaire (**Table 2**). Again, no difference was found between the regions.

In conclusion, the occurrence of previously diagnosed goitre could be overestimated when based on the reports of the participants only. Comparisons between the regions should not be biased by selection. The selection of subjects is an important factor to consider when making correlation studies, in particular if subjects with awareness of thyroid disease are included. On the other hand, prevalences of goitre, and especially enlargement of the thyroid as indicator of goitre, were probably not biased to any major extent, as very few subjects were aware of these conditions beforehand. Nonresponder data have previously been presented in greater detail (29).

It should be noted that the study was designed as a comparative study, and the intention was not to allow correlation studies. This would be questionable due to the distribution on age and sex, which is not representative for the population. We would probably underestimate the prevalence from our cohort compared to the general population as we have relatively few participants from the elderly part of the population. Before comparing data with other studies, these reservations should be considered. From our parallel register-based study, we have precise data on the incidence of thyroid dysfunction – but not goitre – in the general population (30).

The MONICA10 cohort

The MONICA10 investigation represents follow-up on a cohort (MONICA-1) that was generated in 1982 with the aim of monitoring risk factors for cardiovascular events. At the 10-year follow-up in 1993 and 1994, all subjects from the initial investigation were invited including non-participants, and at this time, ultrasonography of the thyroid, biochemical markers of thyroid function, and questions regarding previous thyroid disease were included in the Glostrup part of MONICA.

The participants represented both sexes and four age groups: 41, 51, 61 and 71 years, and 2656 subjects participated (65% of the in-

vited) (I, III). As thyroid disease was not the major topic of the investigation, the specific selection regarding thyroid disease was expected to be small. In telephone questionnaires with non-participants, answered by 11% of the cohort, the prevalence of self-reported thyroid disease was similar to prevalences from the participants (1). The prevalence of smoking was higher among non-participants that among participants; the exact influence of this selection on prevalences of thyroid disease was not evaluated further.

The urinary iodine excretion evaluation study

Urine samples were collected by 31 subjects, who collected a total of 123 24-hour urine samples and corresponding spot urine samples. The study group consisted of hospital staff and relatives; 24 men and 7 women aged 27 to 71 years. The cohort was originally initiated to investigate variations in iodine excretion over time and with different levels of iodine supplementation, but data collection was expanded with the purpose of validating different measures of iodine excretion.

The ultrasonography evaluation study

The ultrasonography evaluation study consisted of two parts. For the inter-observer variation study, 25 consecutive participants from the "DanThyr" study were included for blinded duplicate evaluations. For the precision study, 35 consecutive deceased patients referred to the department of pathology at Bispebjerg Hospital for autopsy were evaluated for the study. Seven of these patients were excluded, two owing to extreme obesity, two owing to recent surgery or radiation to the neck region, and three owing to poor echo conditions on the neck.

QUESTIONNAIRES

The questions in the questionnaires for the two population-based studies were generally adapted from the questionnaires used in the "Glostrup Population Studies" and the MONICA studies.

Four new questions were constructed concerning thyroid disease: "Has a doctor ever told you that you had hyperthyroidism/hypothyroidism/goitre/thyroid nodule(s)?" These questions were evaluated in "DanThyr", where the results from the questionnaires were compared to results from a subsequent interview with an M.D., and in some cases also tracing of medical records (V, VI). The prevalence of previous thyroid dysfunction was overestimated by self-reporting, whereas regarding goitre prevalence, misclassification was common but the estimate was unbiased.

Questions regarding life-style and socio-economic factors were standard questions in MONICA10, and they were adapted to "DanThyr" with minor changes, for instance regarding physical activity, where illogical categorisation was corrected. Smoking questions have previously been discussed in detail (29), and they have been validated by the developers in the MONICA group (31, 32). Besides questions concerning previous thyroid disease, also medications and dietary supplements were recorded at a personal interview. Further, the reported information on the life-style factors were confirmed and in most cases explored further at the interview.

STATISTICS

All data processing was performed by the author with SPSS statistical software version 7.5, 8.0, 8.0.2 or 10.0.

Method comparison studies

Two parts of the study involved comparison of measurement by different observers (II) or of different principles of measurement (IV). For these comparisons, data were evaluated in difference-versus-mean plots (not shown) as suggested by Bland and Altman (33). No obvious influence of the mean of the measurements was found on the differences between the measurements. Thus, the agreement of the methods or between observers was described with a correlation coefficient and with either median of the two methods or a regression coefficient to evaluate and quantify a possible bias between the two observers or methods.

Descriptives

The estimates of prevalence were given in tables as crude values without adjustment for possible confounding or explanatory factors to allow comparisons with other studies. It was also tested for the DanThyr data, however, in multivariate models that regional differences were not due to confounding caused by different prevalences of life-style-related risk factors in the two regions. Statistical evaluation was done with non-parametric tests for values of serum TSH, TPO antibodies, urinary iodine concentrations and thyroid volume, as they were not normally distributed, whereas thyroid hormone levels were evaluated by t-tests and ANOVA.

Risk factor analyses

Multivariate statistics

There is little tradition in thyroid research for the use of multivariate statistics. However, when analysing data from epidemiological studies, this is the standard method today. As the risk factors may be unevenly distributed within the study population on for instance age and gender, and as also the occurrence of thyroid disease has a clear association with age and gender, possible confounding has to be considered.

Confounding is an interplay between the endpoint (dependent variable) as for instance thyroid volume and two potential risk factors (independent variables) as for instance gender and tobacco smoking. A precondition for confounding is that the confounder is associated with both the endpoint and the other risk factor. This could be true in an example where thyroid volume could be higher in men than in women and smoking more prevalent among men than among women. Thus, high thyroid volumes among smokers could in fact be because smokers are more often men and not an effect of smoking per se. This is adjusted for in multivariate analyses.

In multivariate analyses, adjustment is made from the impact of all other independent variables in the statistical model. In the example above, an adjustment would be made in a linear model with – in principal – adding the sex difference in thyroid volume to the thyroid volume of all women for the analysis of the impact of smoking on thyroid volume. Thus, gender would no longer confound the analysis of thyroid volume and smoking. Such adjustment can be made for a large number of other risk factors if the number of cases is large (>10-20 times the number of factors in the model).

The same principle applies for endpoints that are dichotomous variables. Then logistic regression analysis is used where the natural logarithm of odds ratios between for instance exposed and non-exposed individuals is computed from a mathematically more complicated procedure through maximal likelihood approximation. The principle with adjustment for possible confounding from other risk factors is similar to linear models, however.

Before adjusting for confounding, a test for statistical interaction (effect modification) should be done. This is done by including the product of two factors in a multivariate model along with the main effect of the two factors. Interaction is present if the risk attributable to one risk factor is different dependent on the exposure to another factor in the model. In the example from before this could be that smoking increases thyroid volume among women but not among men. This can be handled by making new variables with all combinations of the two factors or by inclusion of the product of the two factors in the model, but simple adjustment for confounding would not make sense in that case.

End-points

Different end-points were considered in the risk factor analyses. Serum thyroglobulin was included as a sensitive but unspecific marker of thyroid abnormalities (VII). Thyroid volume was considered as a continuous variable and as a dichotomous variable – thyroid en-

largement or not. Thyroid volume was evaluated as a continuous variable to detect variation also within the normal range and to increase the statistical power of the analyses. The dichotomous classification was based on reference values suggested by Gutekunst et al. (34) as mean + 3SD in iodine sufficient populations; thyroid enlargement was accordingly defined as a thyroid volume exceeding 18 ml for women and 25 ml for men.

Goitre at the clinical evaluation was categorised according to WHO criteria (15). For most purposes, only goitre grade 1b was included as end-point owing to the low number of participants with goitre grade 2 or 3 and owing to the uncertainty of the precision and relevance of palpable, but invisible goitres (grade 1a).

Thyroid structure was classified as normal, uninodular or multinodular on the basis of the ultrasonographies. Though nodules were registered at diameters ≥5 mm for follow-up studies, only nodules at diameters ≥10mm were included in the present analyses in order to reduce misclassification and to obtain a higher clinical relevance of the registered thyroid abnormalities. Thus, a single nodule ≥10 mm in diameter as well as multiple (>1) nodules with at least one nodule ≥10 mm were used as end-points. This separation of single and multiple nodules was done, as single and multiple nodules could represent different disease entities as discussed later.

Serum TSH as a continuous variable was evaluated for all risk factors, whereas thyroid dysfunction was only evaluated for iodine status and smoking, where important impacts could be anticipated. For other risk factors they were not considered owing to the limited number of participants with current, overt thyroid dysfunction. Further, we had the possibility of evaluating these associations in a parallel case-control study involving a large number of newly diagnosed cases of thyroid dysfunction (not yet published). The level of TPO antibodies was included as end-point in the analyses from the MONICA10-cohort, where it was also documented that the true cut-off value between "normal" and elevated levels of TPO antibodies was 200 kU/l with this generation of TPO antibody assays (I), as only levels of TPO antibodies above 200 kU/l were associated with other indications of thyroid disease.

Statistical modelling

Data were analysed in linear models and logistic regression analyses. In the linear models, the dependent variables were transformed by natural logarithms, as all these variables were skewed towards higher values but were normally distributed after transformation.

As age, sex and region of inhabitancy (reflecting iodine status) were potential confounders of all independent variables, these variables were included in all models. The most appropriate description of each risk factor was considered in these simple models, and variables with a significant association to the end-point or with an obvious trend were further evaluated.

The options considered in the description of risk factors were possible categorisation, number of categories and cut-points between categories. Data gathered as a continuous variable were evaluated in the statistical models as a continuous variable with a check for linearity but also as categorised variables. Further, the most appropriate categorisation was evaluated. This was also the case for data sampled as categorical variables, where combination of categories was evaluated. This was evaluated not only from mathematical considerations, but also from clinical practise and relevance. Thus, smoking for instance showed linearity with thyroid volume as a continuous variable, but was used in four categories for a comprehensive view and due to traditions in the field of smoking related diseases.

The potential risk factors were evaluated in multivariate models, and insignificant factors were removed manually from the models as described below. The categorisation was not always the same with a factor as the explanatory variable as when the factor was included as a potential confounder. Smoking was used as a variable with four parameters when smoking was investigated as the explanatory variable, but was used uncategorized in the same four groups (as a continuous variable with four possible values) when included as confounder to reduce the number of parameters in the model. Likewise, alcohol consumption was used with four parameters as explanatory variable and with two parameters as confounder. The appropriateness of this simplification was tested through the increase in deviance, which was minor in both cases.

Interactions

Statistical interaction (effect modification) was generally considered between the explanatory variable and all other independent variables in the final multivariate models. The identified significant interactions were then quantified either by doing the analysis without inclusion of the main effect of one of the variables in the interaction term, or by generating new variables with all combinations of the interacting variables. In the papers, results were in some cases presented without the interaction terms, stating that the estimates represent average of effects from different groups. This was done in order not to extend the data material and only where effects were not counteracting, but showing the same tendency.

Only first-order interactions were analysed, as it was out of the scope of these papers in general endocrinological papers to interpret more advanced interaction models, and as the relevance of such second order interactions for the interpretation of data is doubtful.

Control for confounding in the DanThyr study

The overall strategy was identification of possible confounders for the different risk factors before publication of any data from the DanThyr studies. A presentation of data with identified associations that would later have to be corrected should obviously be avoided. Thus, before doing risk factor analyses in detail including analyses of regional differences, potential risk factors were analysed in broad outline in order to establish a provisional, comprehensive, multivariate model.

From this initial model, a number of risk factors could be excluded as confounders generally or in specific contexts. Excluded at this early stage was marital status, strain of problems and time in menstrual cycle, as these factors had no association with the endpoints, though time in menstrual cycle have previously been found associated with thyroid volume (35). Other factors showed associations with one or more endpoints in simple statistical models, but not in multivariate models and were also excluded as confounders. These included occupational status, housing conditions, self-assessed health and physical condition. Another group of factors showed significant associations to some endpoints in multivariate models, but could be removed without any influence on other parameter estimates. These were season, time of the day (except TSH analyses), family history and physical activity levels. Also height, weight and Body Surface Area showed significant associations with thyroid volume, but could be removed without influence on the estimates for the other factors in the model.

The second stage was to analyse the risk factors for which the hypothesis was a possible association with thyroid volume or thyroid function. Test for effect modification was performed, and the specific confounder control for each factor was estimated and the best categorization of the factor of interest evaluated. At this stage the final model for the factor was constructed and data published. An overview of the confounder control in each paper is given in **Table 3**.

The control for these factors was not reported consequently in all papers. The factors that turned out to be confounders and were included in the final models for adjustment were reported in all papers. Also factors with obvious potential confounding such as tobacco smoking as confounder for the thyroid – alcohol association were reported. However, mentioning the remaining factors in the analyses tended to make reviewers demand full presentation of all factors. Such data were often not analysed in sufficient detail for

Table 3. Confounder control in the six papers from the DanThyr studies investigating other risk factors than iodine deficiency. Only factors that were considered potential confounders in the first, provisional model are included.

		Confounder								
Paper	**Risk factor**	**Age, sex**	**Region**	**Alcohol**	**Smoking**	**Oral Contrac.**	**Parity**	**Education**	**Iodine suppl.**	**Iodine excretion**
VIII	Alcohol	Conf.	Conf.	x	Conf.	N.C.	N.C.	N.C.*	N.C.*	N.C.
IX	Smoking	Conf.	E.M.	N.C.	x	N.C.*	N.C.*	N.C.*	E.M.	N.C.
X	OC	Conf.	Conf.	N.C.	N.C.	x	N.C.	N.C.	N.C.	N.C.*
XI	Parity	E.M.	E.M.	N.C.	N.C.	Conf.	x	N.C.*	N.C.	N.C.*
XII	Smoking	Conf.	E.M.?	N.C.	x	N.C.*	N.C.*	N.C.*	N.C.*	N.C.
XIII	Education	E.M.	Conf.	Inter	Inter	N.C.	N.C.*	x	Inter	Inter

Conf.: Confounding was found and adjustment was made; E.M.: Effect modification; Inter: Possible intermediate factor; N.C.: No confounding found at test; *: Not reported in original paper.

presentation at this stage, and further, the proper space for description and discussion of data were not at disposal. Thus, a brief presentation of these factors as confounders would hamper later presentation in detail, and correct presentation and discussion of data might not be possible. In certain cases, some confounders were reported at the specific request of the reviewers. In the initial paper from the cohort (V), descriptive statistics were used in the presentation, as observed values were supposed to be easier to interpret than results of multivariate analyses. It was tested in multivariate models before presentation, however, that the regional differences were not confounded in any larger extent by the other risk factors.

Finally, the complete models could be constructed after all the detailed analyses as presented in a later chapter.

Causality

In most cases it is essential to establish whether an identified association is causal. The concept of causality can be discussed from a philosophical angle, but for the practical work with data, the question is often: is the exposure to a risk factor increasing the risk of disease, not vice versa or merely as co-incidental occurrence of the disease and risk factor or due to insufficient control for confounding.

Generally, randomisation to exposure to a risk factor (or removal of an exposure) would be considered a gold standard, but this is often not practicable for logistic or ethical reasons. In stead the causality must be evaluated on the basis of the design and results available. For an estimation of causality in epidemiological studies, the Hill criteria (originally: guidelines) (36) have been well accepted by most epidemiologists. However, the applicability of these criteria has been discussed the recent years, and the use of some of the criteria has been toned down (37, 38).

Consistency from different studies is often regarded an important criteria of causality. This is almost always discussed in papers, and from the DanThyr analyses, this is discussed in detail for each reported risk factor. Also the strength of the association is regarded as a marker of causality. This is in itself not logical as weak associations can be causal, but strong associations are less likely to be explained by yet undiscovered confounding factors. The association between goitre and use of oral contraceptives from the DanThyr analyses is being discussed with this criterion among others.

Ideally, it should be documented that exposure precedes disease, but temporality is often not possible to estimate in a cross-sectional study. This is also the case for our analyses, but temporality may be elucidated in a planned follow-up of the cohorts.

A dose-response relationship (biological gradient) is also regarded as an indicator of causality. This was found in our analyses for tobacco, alcohol and education, but not for parity. For dichotomous factors this could not be evaluated. Dose-response has also been shown for different measures of iodine intake in the association with thyroid disease in DanThyr (39). A special issue not normally included in the criteria is reversibility. It could be argued that reversibility as found in our analyses for tobacco smoking and use of OC gives some indication of temporality and causality.

Finally the criteria concerning the biological plausibility and experimental support for the association should be discussed. An association discovered by chance when exploring data and not as result of a hypothesis generated before collecting data should be considered with particular reservation. This was to some extent true for the thyroid-alcohol association in DanThyr, though a mechanism has previously been suggested (40). Regarding the association between goitre and use of OC, the association was directed oppositely from the original hypothesis. This was however, reviewing the experimental evidence, not as meaningless as initially thought as discussed in the paper and in a later section of this thesis. For tobacco smoking and parity, the identified interactions together with experimental studies strongly supported a causal relationship. It should be noted that apparently plausible explanations for an association is always easily provided, and consequently the evidence should be reviewed carefully.

Other coherences may support causality in a specific context. In the DanThyr analyses, the effect modification between smoking or parity and iodine status together with the supposed pathogenesis and previous epidemiological findings was a further support for causality, though this was in fact a specific lack of consistency. In the alcohol analysis, the independent association with thyroid enlargement and nodules for different types of alcohol consumed was also a further support of causality as lifestyle was a less likely confounder.

Thus, these criteria may be useful in discussion of possible causality in order to reduce "wishful thinking", but objectivity is hardly reached, and discussions must also consider the specific context.

Epidemiological concepts

The population attributable fraction (PAF) describes the proportion of cases that could have been prevented if the population had not been exposed to a certain risk factor. This is dependent on the relative risk (RR) for the exposed individuals compared to the non-exposed individuals and on and the frequency of the exposure in the population. As a causal association between the disease and the risk factor is a pre-requisite for a meaningful estimation of the population attributable fraction, a discussion of the likelihood of such causality should preceed the calculations.

The PAF was computed according to the following formula: PAF = [p (RR-1)]/[p (RR-1) + 1], where p is the fraction of exposed individuals in the cohort (41). We used odds ratios (OR) instead of RR in order to have estimates from multivariate models adjusted for potential confounding from other risk factors. This is an approximation, but with the odds ratios relatively close to one or a low prevalence of the disease, the difference between RR and OR is minor.

The preventive fraction (PF) describes the fraction of cases prevented compared to a hypothetical population where no individuals were exposed to the preventive factor. This was computed as PF= p (1-RR), where p represents the fraction exposed to the preventive factor, and RR again was replaced by OR. This formula was based on theoretical considerations by Miettinen (42).

METHODS IN THYROID EPIDEMIOLOGY

IODINE STATUS

Iodine deficiency is the most important risk factor for goitre, and the characterisation of iodine status of a population has a central

role in thyroid epidemiology. The preferred way of estimating iodine status in epidemiological surveys is still under debate, and no gold standard exists. Different principles may be applicable dependent on the type of study and aim of the analyses.

Generally iodine status is characterised from iodine excretion in urine. This is plausible, as approximately 90% of ingested iodine is excreted in the urine, whereas the remaining 10% is excreted with faeces, however with large inter-individual differences (43).

Iodine intake

A straightforward approach to investigate iodine intake would be dietary records with calculation of iodine intake. This is hampered by variation in – and insufficient information about – iodine contents in the elements in the food. Further, due to large iodine contents in some foods not eaten regularly as for instance fish, long periods of registration is warranted for precise estimates at an individual level. The latter objection is overcome through food frequency questionnaires, asking for an average consumption of selected foodstuff over a longer period (44). Still, knowledge of the precise iodine content of the foodstuff is a prerequisite and recall bias may be introduced. Another approach is through duplicate portions, where participants store a duplicate of all ingested food for later analysis. This is, however, troublesome for participants and analytically if used over several days, and the procedure may affect the choice of food. And as with most of the principles of registration of food intake, it only provides an up-to-the-minute account. Registration of iodine intake is used rarely in thyroid epidemiology.

Iodine excretion

Measurement of urinary iodine excretion is the preferred method for estimation of iodine status. The most precise estimate of iodine excretion is obtained from collection of 24-hour urine samples. Owing to the large day-to-day variation in iodine excretion (45), a series of iodine collections is needed for a precise estimate of iodine status, and since even collection of a single 24-hour urine sample is troublesome in epidemiological surveys, other procedures may be justified even if precision is lower. With low compliance and the risk of incomplete sampling, the results from 24-hour samples may not be valid anyhow.

Iodine concentration in spot urine samples has been used as an estimate of iodine excretion at the population level, and WHO classification of possible iodine deficiency is based on urinary iodine concentration values (15). The large variations in 24-hour urine volume and thereby dilution of the urine prohibits any individual estimate of iodine excretion from iodine concentration in spot urine samples, but in large groups, a median iodine concentration provides an estimate of iodine status (46). As mean 24-hour urine volume in a population is generally larger than 1000 ml (IV, 45, 47), iodine concentrations should not be compared to 24-hour iodine excretions. Differences in the dilution of the urine samples may also confound comparisons of median iodine concentrations, as 24-hour urine volumes may differ between populations or subgroups in a population (48, 49).

As creatinine is excreted with a fairly constant rate through the day independent of urinary volume (45), and also day-to-day variation is small compared to variations in iodine excretion (50-53), creatinine concentration has been used to adjust the iodine concentrations in spot urine samples: the iodine/creatinine ratio. With this estimate, misclassification caused by differences in the dilution of the urine is diminished, and the iodine/creatinine ratio is closer correlated to 24-hour iodine excretion than iodine concentration (4). Values are still not comparable to 24-hour iodine excretion (IV), as creatinine excretion is in average more than 1 g/day (43, 50, 51, 53-55), and 1 g creatinine is used for the denominator of the expression. An objection to the iodine/creatinine ratio has been variations in creatinine excretion between populations of different ethnicity (56), but in industrialised societies creatinine excretion does not vary much (43, 50, 51, 53-55). However, creatinine excretion is not constant between different age- and sex groups (53), and bias may be introduced if the age- and sex distribution of the cohorts differ, and no stratification or individualisation is feasible.

Improvement of the iodine/creatinine ratio has been suggested by taking into account the sex difference in creatinine excretion (43, 55, 57). We elaborated on this principle by introducing correction factors adjusting also for the age-related decline in creatinine excretion. An age- and sex adjusted iodine/creatinine ratio was computed as: Iodine (μg/l)/[creatinine (g/l)/expected creatinine excretion (g/day)]. The expected creatinine excretion was adapted from a large Belgian population study (53), **Table 4**.

In our comparison to 24-hour urine samples of the three estimates of iodine excretion based on spot urine samples, the adjusted iodine/creatinine ratio was superior, but it did still not provide estimates identical to 24-hour samples (IV) (**Table 5**). The correlation coefficients should be interpreted with reservation, as the samples were not independent but collected from a limited number of participants in periods with different iodine intake. This could explain the relatively high correlation between 24-hour iodine excretion and the unadjusted iodine/creatinine ratio. The high correlation between the adjusted iodine/creatinine ratio and 24-hour iodine excretion but tendency to underestimation of iodine excretion was also found in two other studies (44, 45). The tendency towards underestimation was probably conditioned by diurnal variations, as the ratio was lowest in the morning (45), where spot urine samples are most often collected. On the other hand, other studies have shown a minor diurnal variation in iodine excretion (45, 47, 58, 59). The substantial differences between 24-hour iodine excretion and the unadjusted iodine/creatinine ratio or iodine concentration underline that these estimates apart from possible differences in the distribution in the population also have considerable bias. This was expected but is often overlooked in epidemiological surveys, thus confusing iodine intake with these estimates.

In a survey from the DanThyr study it was shown that the adjusted urinary iodine/creatinine ratio had a close association with various estimates of thyroid diseases with an expected association with iodine deficiency (39). On the other hand, only a weak association was found between urinary iodine concentration and thyroid disease.

Conclusion

Iodine status is generally evaluated from casual urine samples in epi-

Table 4. Expected 24-hour creatinine excretion values in different age and sex groups.

	Creatinine (g/day)	
Age (years)	**Males**	**Females**
25-49	1,74	1,23
50-59	1,63	1,15
60-69	1,47	1,07
70-	1,39	1,00

Table from (IV), original data from (53).

Table 5. Four ways of expressing iodine excretion in epidemiological surveys. Percentiles and comparison between 24-hour urine values and three estimates based on corresponding casual urine samples (n = 112).

	5th	25th	median	75th	95th	r
24-hour iodine excretion (μg/day)	75	112	143	185	297	
Adjusted iodine/Cr ratio (μg/day)	58	92	126	161	319	0.62
Iodine / gram creatinine (μg/g)	41	55	77	105	198	0.61
Iodine concentration (μg/l)	39	68	87	133	290	0.37

r = regression coefficient compared to 24-hour iodine excretion. Data from (IV).

demiological surveys. Iodine concentration has the advantage of being simple and widely used in the past, and WHO recommendations are based on this estimate. An age- and sex adjusted iodine/creatinine ratio provides a better estimate of 24-hour iodine excretion, and it allows for stratification and comparisons at the individual level for instance in regression models. Still, large day-to-day variations are not accounted for.

THYROID ULTRASONOGRAPHY

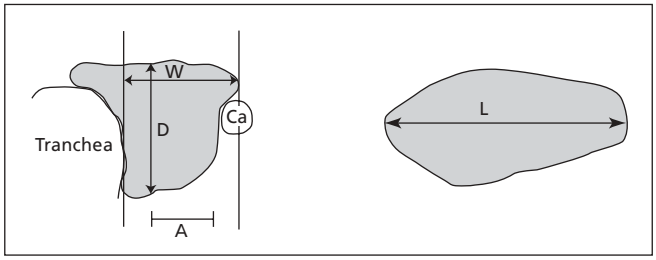
The most obvious consequence of iodine deficiency is increased volume of the thyroid gland eventually leading to a visible and enlarged thyroid gland – goitre. The primary endpoint in most epidemiological surveys of iodine deficiency disorders has therefore been the median thyroid volume or the prevalence of goitre. The principles of estimating a possible increase in thyroid volume have not been settled, though thyroid volume measurement by ultrasonography has gained widespread use. Procedures for the most common method for volume measurement with thyroid ultrasonography have only been described briefly (60) until our recent paper (II).

Some authors still advocate clinical examination of the thyroid, as moderate to good agreement between trained observers has been found and the investigation is easy to perform (61-63). But the accuracy of palpation has been found low when compared to ultrasonography (64-67), the sensitivity of palpation is modest (63, 68), and nodules are often overlooked (64, 66, 69). Consequently, palpation may be sufficient in areas with severe endemic goitre, but may not be able to discriminate the changes found in the thyroid gland in less pronounced degrees of iodine deficiency, unless very large cohorts are investigated.

Thyroid volume

Thyroid volume measurement by ultrasonography has been used since the 1960'ies as summarised by Brown (70). Two quite different methods have been applied; a method based on planimetri of consecutive transverse scans, and a method based on the length of the three axes of each lobe. The planimetric method was thoroughly described in a paper by Rasmussen and Hjort (71) and was later further evaluated (72-74). It has only been used to limited extend as specialised equipment is demanded. The principle based on the three axes of each lobe was described in a paper by Brunn (60), and has been used in many surveys, although sometimes with modifications. Three-dimensional ultrasonography has been evaluated and was found superior to the three-axes method (75), but their results with three-dimensional ultrasonography was apparently not better than what had been obtained in other studies with the three-axes method (II). Also planimetri in three perpendicular planes has been described (76), but the results have not been reproduced by other groups and the method is complicated to perform (II).

The description of the three-axes method was published before high-resolution grey-scale images were available, and few details on the measuring procedure were included. The need for precise measuring procedures has increased with modern high resolution grey-scale images, where presentation of thyroid anatomy and demarcation from adjacent tissue has been improved. Our experience from use of high-resolution ultrasonography was that interpretation of width and depth of the thyroid gland could vary substantially between observers, not only in irregularly shaped glands but also in quite normal glands. In correlation studies, this raises the question if observed differences are due to actual differences in thyroid volume or methodological differences. When large differences in thyroid volume are found between populations with similar iodine status (III, 67, 77), methodological differences in thyroid volume measurement could be one reason, goitrogens another (18). Such methodological differences have recently been documented for thyroid ultrasonography of children (78). We have therefore defined precise guidelines to make the ultrasonic thyroid volume measuring procedure reproducible between observers (II) (**Figure 1**).



W = Width (from most lateral part to medial part in cross-sectional picture, no further medial than the lateral wall of trachea)
D = Depth (maximal depth within "A" in cross-sectional picture)
A = area for measurement of thyroid depth (middle ½ of thyroid width)
Ca = Carotid artery
L = Length (maximal length in longitudinal picture)

Figure 1. Measuring procedure for ultrasonographic thyroid volume determination. Two perpendicular planes of the thyroid: left, cross-sectional picture; right, longitudinal picture. Figure from (II).

Blinded duplicate evaluations showed that the inter-observer variation was low. The coefficient of variation was 9.6%, which is in the lower end compared to the few previous methodological studies (II, 79-81). Even compared to the cross-sectional planimetric method, this inter-observer variation was acceptable (71, 82). No significant bias between the observers was found (**Table 6**). The trend, however, was a slightly higher thyroid volume measured by the observer from the area with less pronounced iodine deficiency; the mean difference for each thyroid lobe was 0.4 ml (95% confidence interval –0.3 to 1.2 ml). The regional difference in thyroid volume between the two regions in the DanThyr studies thus tend to be underestimated and was not explainable by bias between the observers.

The validity of the thyroid volume measurements was evaluated by comparisons to the results of autopsies. The agreement between ultrasonography and autopsy volumes was smaller than the inter-observer variation, which was possibly due to the less optimal scanning conditions and changes appearing during the autopsy (II). Still, a satisfactory correlation was found with a correlation coefficient of 0.93 and a slight bias (regression coefficient 0.90). In this particular case, bias was less important than correlation, since the two methods are not required to be interchangeable, as only ultrasonography is used in epidemiology. Higher as well as lower correlation to surgical specimens has been reported for the planimetric method (71, 72), but as the variation in thyroid volume was much larger in those studies, correlation coefficients were expected to be higher.

Table 6. Agreement between results of ultrasonography of 49 thyroid lobes by two sonographers.

	Sonographer A	Sonographer B	
Volume three-axes method (ml)	8.8 (3.5-44.6)	8.2 (3.4-52.6)	p=0.34
Volume planimetric method (ml)	9.2 (3.7-43.1)	9.8 (3.8-50.8)	p=0.03
Lobes with nodules	16	14	κ=0.72*
Number of nodules	25	24	p=0.80
Size of nodules (mm)	13.7 (5.2-54.5)	13.4 (5.3-44.7)	p=0.82
Hypoechogenecity	10/49	4/49	κ=0.19***
Micronodularity	19/49	28/49	κ=0.42**

For continuous variables medians (and ranges) are given; * Good agreement; ** Moderate agreement; *** Poor agreement; Table from (II).

Thyroid structure

Besides thyroid volume, thyroid structure is also of interest in an epidemiological context, as iodine status as well as other goitrogens might affect the prevalence of nodules and thyroid echogenecity (VIII, XII, 67). Registration of these variables is probably even more subjective than thyroid volume measurement, and in a clinical setting, large inter-observer variation in detection of nodules has been reported (83).

We found good agreement between the two observers in blinded duplicate observations of distinct thyroid nodules, reflected by a κ-value of 0.72, and first and foremost, the estimates were unbiased. On the other hand registration of hypoechogenecity and micronodularity (irregular echo pattern) showed little agreement and considerable bias (II) (Table 6). Thus, these variables seemed not to be suitable for evaluation in epidemiology, at least not when evaluated by different observers. Only small differences in echogenecity were observed in the 25 participants in the study, and it is possible that more pronounced differences in echogenecity could be estimated with higher agreement. Consequently, it is possible that echogenecity is a useful variable in clinical contexts, where more severe degrees of hypoechogenecity may be observed. Hypoechogenecity has been suggested as a good marker of e.g. autoimmunity (84, 85), atrophic thyroiditis (86) and subacute thyroiditis (87).

Conclusion

Ultrasonography is the preferred method for evaluation of thyroid volume and thyroid enlargement, though palpation may be sufficient in field studies in areas with high goitre prevalence. The widespread method based on measurement of the three axes of each thyroid lobe is reproducible and sufficiently valid at least when the measuring procedure is well defined. For comparison studies, possible bias in thyroid volume measurements should be considered, and comparative studies are preferable. Prevalence of distinct nodules is an applicable variable, but echogenecity and micronodularity seemed unfit for comparison between observers.

THYROGLOBULIN

The use of serum thyroglobulin (Tg) measurement in daily clinical practise is almost entirely restricted to follow-up of patients with differentiated thyroid cancer for the detection of recurrence (88). Other applications have been suggested and some may be useful in particular situations. Thyrotoxicosis due to subacute thyroiditis can be differentiated from Graves' disease by an earlier and more distinct rise in serum Tg (89), and in thyrotoxicosis factitia, serum Tg is low contrary to all other types of hyperthyroidism (90, 91). Serum Tg measurement could have some value in follow-up of patients after surgery for non-toxic goitre, as early detection of recurrence is possible (92). As marker of outcome of thyroid hormone treatment of non-toxic goitre the value was limited (93, 94).

Analytical interference with Tg Antibodies and lack of standardisation have been obstacles for the use of serum Tg measurements. However, with newer assays, interference is reduced, and a standard to which all assays should be calibrated (CRM 457) has been introduced (88).

Determinants of serum thyroglobulin level

The diagnostic use of serum Tg measurement is hampered by the low specificity, as not only cancer but also almost all types of pathological changes in the thyroid lead to increased serum Tg levels (88). Increased serum Tg is found in patients with goitre (95-99) and in patients with thyroid nodules (96, 97, 100-104). Correlation between goitre size and Tg levels has been found by some authors (100, 103) but not by others (98), and the association with thyroid volumes measured at ultrasonography has been controversial (82, 105, 106). Also thyroid hyperfunction is associated with increased serum thyroglobulin, whether caused by TSH receptor antibodies in Graves disease (107, 108) or by autonomous thyroid nodules (95-97, 101, 109).

The individual associations with serum Tg levels of thyroid volume, structure and activity have previously been difficult to estimate as precise registration of thyroid volume and thyroid function was only available in few studies, and as multivariate statistics have not been applied. The relative importance of these factors has consequently not been established. We found in multivariate models that thyroid volume was the factor with the closest association with serum Tg, but that also thyroid nodularity, iodine excretion and thyroid hyperfunction had independent associations with serum Tg in that order of importance (VII). So, each of these factors may contribute to increased serum Tg levels, and serum Tg will only be an unspecific marker of thyroid pathology.

An age- and gender dependency of serum Tg levels has been a matter of discussion, as conflicting results have been found as reviewed by Feldt-Rasmussen (107, 110, 111). An age- and gender dependency has primarily been found in iodine-deficient areas as ours (VII), where also the occurrence of goitre and hyperthyroidism shows age- and gender dependency (I, III, V, VI). Thus, the association of serum Tg levels with age and gender may be secondary to an association with thyroid volume and hyperthyroidism.

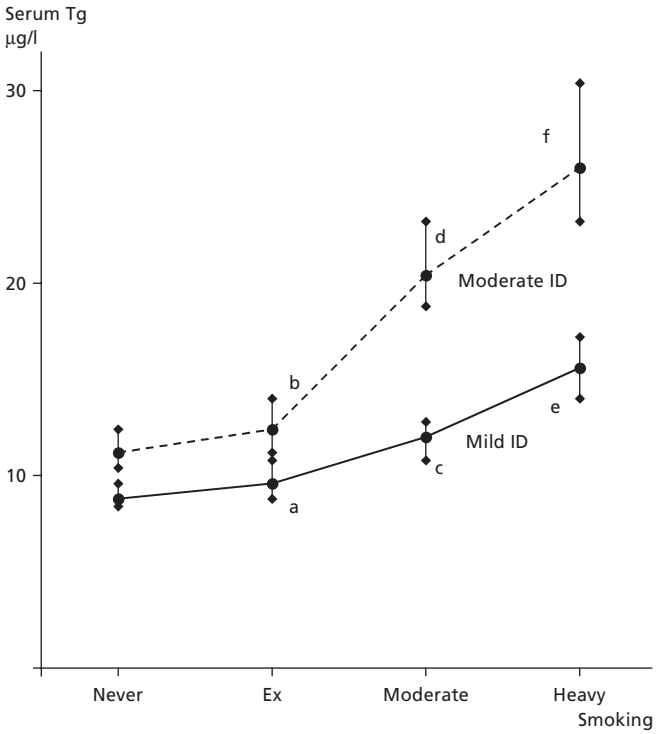
TSH has an acute stimulatory effect on Tg secretion by the thyroid (112, 113), possibly mediated by a general increase in thyroid activity. Increased serum TSH in severe iodine deficiency has been found associated with increased serum Tg indicating a direct stimulatory effect of TSH on Tg secretion (114-116), but the increased thyroid volumes in severe iodine deficiency might as well explain the increased serum Tg levels. And in other situations where serum TSH is not closely associated with increased thyroid volumes, the association between TSH and Tg has been questioned (VII, 96, 105, 110).

A direct genetic determination of serum Tg level has been suggested from twin studies (117), but this determination could simply be a reflection of the genetic predisposition for goitre, which has a similar pattern of inheritance (21).

Iodine intake and thyroglobulin

Serum Tg level is apparently determined by a number of factors (thyroid mass, thyroid structure, thyroid function), which are in turn determined by among other things iodine status. It is also possible, however, that iodine has a direct effect on Tg secretion due to lower iodization of the Tg molecule in the follicular cells (98), and that thyroid mass is not an intermediate factor between iodine deficiency and Tg levels, but simply another consequence of the iodine deficiency. This view is supported by the lack of association between thyroid volume and serum Tg levels in an iodine-replete area (82). Further, apart from the distinct association with iodine status, serum Tg level is closely associated with tobacco smoking (IX), and synergistic effects of iodine deficiency and smoking on serum Tg levels were found (**Figure 2**). This is in line with the impact of tobacco smoking being primarily mediated through inhibition of iodine uptake (118). Conversely, thyroid volume and serum Tg showed different associations with alcohol consumption (VIII) and use of oral contraceptives (X); and these factors have a non-iodine-dependent association with thyroid volume.

Serum Tg level has previously been suggested as a marker of iodine status (115, 116, 119, 120), and in comparative studies, a distinct difference has been found in serum Tg levels between areas with iodine deficiency and iodine-replete areas (67, 121, 122). We confirmed the usefulness of serum Tg level as marker of iodine status in a population with the finding of a large difference in serum Tg level between two regions with only a moderate difference in iodine excretion (Figure 2). Furthermore, intake of iodine-containing vitamin tablets, iodine-rich foodstuff, and individual iodine excretion values were associated with serum Tg level (VII, 39). Thus, expressions of iodine intake at the group level, individual here-and-now intake, and habitual intake were all associated with serum Tg level, even when included in the model together. This implies that serum Tg level could be a better marker of iodine status than iodine excre-



a: Compared to never smokers, p = 0.24
b: Compared to never smokers, p = 0.20
c: Compared to ex smokers, p = 0.004; compared to never smokers, p < 0.001
d: Compared to ex smokers, p < 0.001; compared to never smokers, p = 0.001
e: Compared to all other groups, p < 0.001
f: Compared to moderate smokers, p = 0.008; compared to other groups, p < 0.001

Figure 2. Serum thyroglobulin in 3764 participants from two Danish regions with different iodine status and in groups with different tobacco smoking habits. Results from multivariate statistics after adjustment for age and sex. Significant statistical interactions between iodine status and smoking were found. Figure from (IX).

tion, even when the most accurate estimates of iodine excretion are provided.

With the possibility of measuring serum Tg from dry blood spot samples (116) it is also a convenient method in many situations, as serum samples can be stored and transported from remote areas. In other situations, serum samples from population studies may be available, and in that case it is a less time consuming procedure than ultrasonography.

Conclusion

The current use of serum Tg measurements is limited to follow-up of patients with differentiated thyroid cancer, though a use as marker of iodine status has been suggested. Serum Tg level has close, independent associations with thyroid volume, thyroid nodularity and thyroid hyperfunction; all being factors also associated with iodine deficiency. But serum Tg level is further associated with iodine intake, and may have a direct dependency from iodine status apart from effects mediated from the factors above. Tg is a sensitive marker of iodine status; perhaps the most sensitive marker available, and it may be useful in many situations, especially with the newer assays with less analytical problems.

IODINE STATUS AND PREVALENCE OF BENIGN THYROID DISEASE

Iodine status is probably the major determinant of the prevalence of benign thyroid disease in a population. As iodine status is usually only determined at the population level, an estimate for the increased risk associated with iodine deficiency is not easily provided at an individual level, and much of our knowledge is derived from correlation studies. Structural abnormalities as well as thyroid dysfunction has been determined with many different methods and different standards, differences that complicate these correlation studies. Still, some patterns seem to apply regarding occurrence of thyroid disease in areas with different iodine status. Iodine status may determine not only the prevalence of thyroid disease but also the prevailing type of thyroid disease and the distribution within populations, as the diseases may occur in different age groups dependent on iodine status (123).

GOITRE

The most obvious consequence of iodine deficiency is an increase in thyroid volume eventually leading to a clinically detectable goitre. The occurrence of this phenomenon has been measured either as the prevalence of palpable and visible goitre, as median thyroid volume at ultrasonography, or as the prevalence of increased thyroid volume at ultrasonography.

Palpable goitre

The association between iodine status and goitre prevalence is well established as reviewed by Delange (15). The association is only clear in severe and moderate iodine deficiency; no increase in goitre prevalence was seen in mildly iodine-deficient areas. However, in the DanThyr study we found a significant difference in goitre prevalence between the two regions (V). In the two regions with only a modest difference in iodine excretion, palpable goitre was found in 10% of the participants in the mildly iodine-deficient region and in 15% of the participants from the moderately iodine-deficient region. The palpations were not done by the same observers, but the difference was parallel to the difference in thyroid enlargement (15 vs. 22%).

Not only iodine deficiency can lead to goitre; in areas with a very high iodine intake, a high goitre prevalence has been observed too (124). In a study comparing towns with different iodine intake, an increased prevalence of goitre was seen among children in towns with median urinary iodine concentrations exceeding 800 µg/l (125), and in two towns with iodine concentrations in drinking water of 463 and 54 µg/l, goitre prevalences of 65 and 15%, respectively, were found (126).

Thyroid volume

More precise comparisons of thyroid volume may be obtainable from ultrasonographic studies. However, as previously mentioned, bias may still be present. A summary of studies using ultrasonography for determination of thyroid volume is shown in **Figure 3**. The

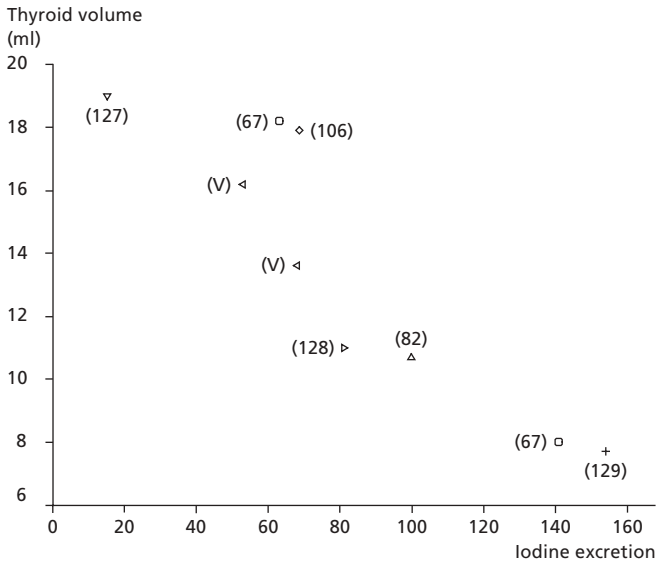


Figure 3. Correlation between urinary iodine excretion and thyroid volume at ultrasonography. Studies selected to include studies of Caucasians and studies with determination of thyroid volume at ultrasonography and iodine excretion in both genders.

trend is decreasing thyroid volume with increasing iodine excretion (V, 67, 82, 106, 127-129). Other studies from areas with presumed mild iodine deficiency confirmed the tendency (III, 72, 77, 130-132). A recent, African study from a moderately iodine-deficient area showed high thyroid volumes (68), and a comparative study of nursing home residents showed higher thyroid volumes in an iodine-deficient area than in an iodine-sufficient area, but no further decrease in thyroid volume in an area with abundant iodine intake (122). The association in Figure 3 seems strongest in the range of iodine excretions corresponding to mild iodine deficiency, but some variation is present. This may be due to methodological differences, as iodine excretion was given as either an iodine concentration or as an iodine/creatinine ratio, estimates that are not directly comparable (IV). Further, thyroid volumes are computed with different formulas producing a 10% difference in thyroid volume, and measuring procedures may be different (II, 78). Another explanation for the variation could be impact from other goitrogens in food, differences in status regarding life-style associated risk factors, or genetic differences. The methodological differences were diminished in the two comparative studies, however, and they showed a marked association between iodine excretion and thyroid volume (V, 67).

Cohort effects are also possible, as only few populations have had a stable iodine intake in their entire lifespan. The time required for a change in thyroid volume following an increase in iodine intake is not known. In DanThyr, participants who had grown up in the moderately iodine-deficient area and later moved to the mildly iodine-deficient area had thyroid volumes similar to the thyroid volumes of permanent residents in the mildly iodine-deficient area. Participants who had moved from mild to moderate iodine deficiency also had thyroid volumes similar to permanent residents in mild iodine deficiency (V). Thus, life-long exposure to moderate iodine deficiency was a prerequisite for the increased thyroid volumes observed in that area. The exact time span for these changes in thyroid volume could not be evaluated.

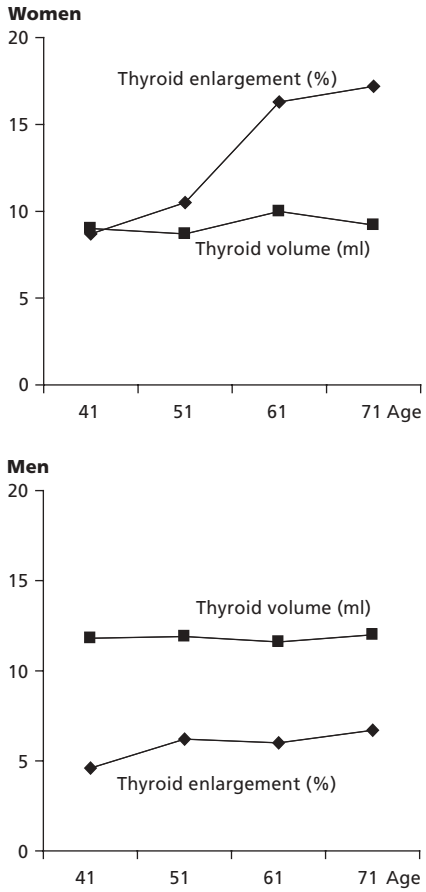


Figure 4. Median thyroid volume and prevalence of thyroid enlargement at ultrasonography in different age- and sex groups from a Danish population study including 2656 subjects. Thyroid enlargement was defined as a thyroid volume exceeding 18 ml for women and 25 ml for men (34).

Age and gender

Thyroid volume has generally been found higher in men than in women except in one study (106), probably because thyroid volume is correlated with lean body mass (129, 133). Thyroid enlargement, defined as a thyroid volume exceeding mean + 3SD in iodine-sufficient populations (34), was on the other hand more prevalent among women in some studies (III, V, 106), though similar prevalences in the two genders have been reported (77, 134). The prevalence of palpable goitre was in all studies higher among women than among men (V, 135-137). This indicates that women do not have larger thyroids in general, and that it is not a continuum of larger thyroid volumes that leads to increased goitre prevalence among women; rather, a larger proportion of women develop growth of the thyroid when exposed to for instance iodine deficiency.

The association between age and goitre prevalence or thyroid volume seems to be dependent on iodine status. Increasing thyroid volumes are found up to an age around 40 years, but after that age, no further increase is seen (III, V, 72, 130, 131, 138). In areas with mild or no iodine deficiency, the tendency was even a decline in thyroid volume after the age of 40 (129), possibly associated with a decline in lean body mass (128). This was confirmed in a Danish study of centenarians, where low thyroid volumes were found, but in an extreme age group, a "healthy survivor bias" should be considered (139).

The pattern is different for thyroid enlargement or goitre. In severe iodine deficiency, the prevalence of goitre has a maximum for subjects in the teenage years (15). In areas with mild iodine deficiency, a more heterogeneous pattern has been found; in one of our studies we found a steady increase in the prevalence of thyroid enlargement from age 41 to age 71 years in spite of unchanged median thyroid volume (III). The data, which are shown in **Figure 4**, support that a subset of women are responsible for the increase in goitre prevalence. In other studies, a more constant goitre prevalence (V, 134) or even decreasing goitre prevalence with age have been found (131). Decreasing goitre prevalences after the age of 40 years have also been found in iodine sufficient areas (135, 136), but one major study found increasing goitre prevalences with age (140). In the last study, a cohort effect was likely, however, due to marked increase in iodine intake of the population in the lifetime of the oldest part of the cohort. In studies including old age, goitre prevalence decreases after the age of 70 years (134, 135).

Bias should be considered in these comparisons, as they were all cross-sectional studies, and cohort effect are possible, especially as the iodine supply in most areas has not been stable for the life-time of the investigated cohorts. Further, the selection may be different in the different age groups, and often patients with previous diagnosis of goitre were not included in the studies, which could have contributed substantially to the estimates.

Conclusion

Goitre and increased thyroid volume are closely associated with iodine status with a U-shaped association, since low as well as high iodine intake may lead to increased goitre prevalence. Although thyroid volume is larger in men than in women, goitre is 2 to 10 folds more prevalent in women than in men. Thyroid volume generally increases up to an age around 40 years with a subsequent decline in thyroid volume in iodine-sufficient areas. Goitre prevalence has a peak early in life in severely iodine-deficient areas, whereas the peak appears around middle age or in the elderly in mildly iodine-deficient areas.

THYROID NODULES

The association between iodine intake and prevalence of thyroid nodules is less clear than the association between iodine intake and goitre. The natural history of goitre development could be a development of diffuse goitre, transition to nodular goitre, and eventu-

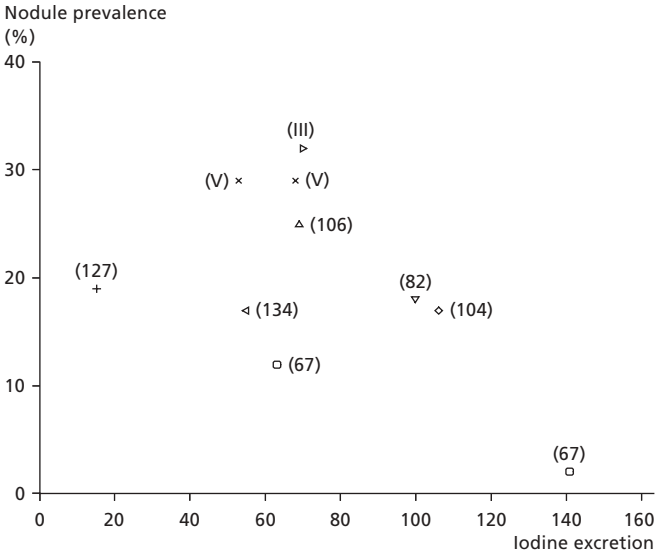


Figure 5. Correlation between urinary iodine excretion and prevalence of thyroid nodules at ultrasonography. Studies selected to include studies of Caucasians and studies with determination of nodule prevalence and iodine excretion in both genders.

ally appearance of autonomous thyroid tissue with hyperthyroidism. Accordingly a high prevalence of non-toxic goitre among the young and of multinodular toxic goitre among the elderly in iodine deficiency has been found (121).

Iodine intake

A summary of studies giving results on the prevalence of thyroid nodules at ultrasonography and iodine status is shown in **Figure 5** (III, V, 67, 82, 104, 106, 127, 134). The association is not convincing, but it should be noted that in the two comparative studies, a clear association between nodule prevalence and iodine intake was found in one (67), whereas we found no association in our study with only a modest difference in iodine excretion between the regions (V). Another comparative study of nursing home residents showed significantly higher nodule prevalence in iodine deficiency and lower nodule prevalence in abundant iodine intake when compared to an area with sufficient iodine intake (122). The low correlation between iodine excretion and nodule prevalence in Figure 5 is supported by other studies without precise indication of iodine status or with only men or women included. In areas with presumed mild iodine deficiency, nodule prevalences of 14 to 26% were reported (77, 131, 132, 138, 141), compared to prevalences of 14 to 56% in areas with presumed high iodine intake (104, 142-145).

The lack of association in the ecological comparison does not rule out a true association, however, as a number of factors may confound or bias the results. The ultrasonographic equipment was different, and higher resolution of the images leads to detection of more nodules. A considerable observer variation is likely; the definition of a nodule is not unambiguous. A cohort effect was possible in most cases, as iodine intake had varied within the lifetime of the participants. The composition of the cohorts was different with respect to age and gender, and the selection procedure was different. Further, though the prevalence of nodules did not seem convincingly related to iodine status, the type of nodules may differ as suggested from the different characteristics of the nodules in the two regions in our comparative study (V).

Another approach to elucidate the association between thyroid nodules and iodine status is through registration of food intake. With specially designed questionnaires, the intake of iodine was evaluated in the DanThyr cohort (44). A pronounced association was found between iodine intake and thyroid multinodularity, whereas the association was less clear regarding solitary thyroid nodules (39). Particularly concerning thyroid multinodularity, a closer association with food questionnaires than with iodine excretion might have been anticipated, as such questionnaires indicate iodine intake over a longer period of time. Thyroid nodularity correspondingly is evolved over a longer time span than simple thyroid enlargement.

The less distinct association between iodine intake and solitary thyroid nodules could be because some of these nodules represent less pronounced nodularity but cases that will later develop into multinodularity. As discussed later, some of these nodules will, however, represent benign neoplasms with a pathogenesis that may not be dependent on iodine intake. Such misclassification is inevitable in cross-sectional studies, but in this case, an attempt to discriminate between multiple and solitary seems relevant as the association with iodine intake as well as some of the other risk factors were different.

In many studies, thyroid nodularity is described irrespectively of the number of nodules; this may blur differences between cohorts. In the studies where distinctions were made, solitary nodules were predominant in the areas with the less iodine deficiency (104, 141), whereas multiple nodules were predominant in areas with more pronounced iodine deficiency (III, V, 77).

Age and gender

In all studies, nodules were found more prevalently in women than in men, but the ratio between the genders was smaller than what was observed for clinically detectable goitre (146). Likewise, increasing nodule prevalence with increasing age was a constant observation, at least up to the age of 70 years (77, 134, 141). In the few studies with enough observations to allow stratification, a relatively constant prevalence of solitary nodules was found in different age groups, whereas the prevalence of multinodular glands increased with age (III, V, 77). These studies were all from mildly iodine-deficient areas.

Increasing prevalence of palpable nodules with increasing age and a higher prevalence among women is also a general tendency in studies of palpable nodules as reviewed by Mazzaferri et al (146). The distinct increase in clinically detectable goitre with age may be caused by the group of women developing large, nodular goitres with age in areas with iodine deficiency, whereas the slightly enlarged, diffuse glands in younger age are often not detectable.

"Thyroid incidentalomas"

The clinical significance of the high number of nodules detected in most studies has been questioned, and the very varying prevalences of nodules observed in different studies support that most of the nodules have no clinical relevance. Very few nodules in these studies gave rise to symptoms, and most of the nodules were not detectable at a clinical examination. These nodules detected by chance have been named thyroid incidentalomas.

Few studies have investigated the risk of malignancy in incidentalomas. By cytological investigation at the time of detection of the nodule, low or immeasurable malignancy risks have been reported (143, 147). In follow-up studies, no signs of malignancies were found after two (III) or five years (148). The size of the nodule in itself does not seem to be a useful marker of possible malignancy (149). The general recommendation is a conservative approach to incidentalomas with a simple clinical follow-up (104, 141, 143, 144, 148, 150), but one study has found a malignancy rate of 4% and advocates fine-needle aspiration of incidentalomas (151). However, there is a discrepancy between detection of thyroid nodules in $^1/_3$ to $^1/_4$ of the population, scintigraphically cold nodules in 2-5% of the population (III, 152) and an incidence of thyroid cancer of only approximately 2/100,000/year (153).

Conclusion

The association between iodine intake and the occurrence of thyroid nodules is not obvious, possibly in part owing to methodological differences. Nodules may represent different pathogenic mechan-

Table 7. Prevalence of thyroid dysfunction in 10 epidemiological studies focusing on the occurrence of thyroid dysfunction in the general adult population.

Author	Iodine status	Age	Hyperthyroid	Mild hypothyroid	Unknown hypothyroid	Mild hypothyroid
Seck et al. (1997) Germany	Moderate ID	50-80	2.2%	6.6%	0.9 %	0%
Knudsen et al. (1999) Denmark	Mild ID	41-71	2.0%	1.4%	1.4 %	0.6%
Tunbridge et al. (1977) UK	Sufficient	min. 18	1.6%		1.1 %	5.0%
Ericsson et al. (1990) Sweden	Sufficient	41 + 55	0.7%		0.2 %	1.1%
Eggertsen et al. (1988) Sweden	Sufficient	min. 18	0.2%	1.0%	4.3 %	2.9%
Sawin et al. (1985) USA	High	60-69*			1.6 %	4.1%
Hollowell et al. (2002) USA	High	Min. 12	0.5%	0.7%	0.3 %	4.3%
Okamura et al. (1987) Japan	High	min. 40	0.9%		0.3 %	4.2%
Konno et al. (1993) Japan	High	adults	0.4%	0.3%	0.6 %	1.3%

*: The figures represent a stratum from the investigation in order to have comparable ages. Adapted from (I).

isms dependent on iodine intake; in iodine repletion, some nodules may be due to autoimmunity or focal autoimmune processes may appear as nodules at ultrasonography, whereas in iodine deficiency, the development of multinodular, colloid goitres may be facilitated. Thyroid nodules occur in large numbers in old age when investigated by ultrasonography, and the risk of malignancy is low in incidentalomas.

THYROID FUNCTION

Iodine deficiency could be associated with an increased risk of hypothyroidism due to the lack of substrate for thyroid hormone synthesis as observed in areas with severe iodine deficiency (154). It has now become clear that milder degrees of iodine deficiency are not associated with a high occurrence of hypothyroidism. Correlation studies should be interpreted with care, as comparisons may be biased by the use of different assays and of different definitions of hypo- and hyperthyroidism, by the composition of cohorts with respect to age and gender, by selection of the cohorts, by different diagnostic activity and thereby different levels of known thyroid dysfunction, and by recent changes in iodine intake of the population among other things. Further, genetic differences may determine some of the differences between populations. An influence of genetic background on serum TSH levels and the occurrence of thyroid dysfunction has been demonstrated (22, 155, 156). Also an increased susceptibility to other risk factors may be dependent on genetic background (gene-environment interactions). Thus, no evidence of causality is provided from correlation studies. A summary of thyroid dysfunction in selected studies, investigating samples of both genders from the general population is given in **Table 7** (I, 55, 135, 137, 157-161).

With all reservations mentioned above, a pattern is seen with increasing prevalences of hypothyroidism and decreasing prevalences of hyperthyroidism with increasing iodine intake. However, more evidence is obtained from comparative studies where methodological bias has been diminished. Comparing Iceland with a stable high iodine intake and Jutland in western Denmark with a stable low iodine intake, elevated serum TSH was found with a high prevalence in Iceland and a low prevalence in Jutland (121). Conversely, suppressed TSH levels were often found in Jutland, but were not identified in Iceland. The same pattern was found in a study of nursing home residents in different areas of Hungary: a high prevalence of elevated TSH levels in iodine repletion and a high prevalence of suppressed TSH levels in iodine deficiency (122). In our study, no statistically significant regional differences in thyroid dysfunction were found with the cut-off value used in daily clinical practise, but with a wider definition of mild thyroid dysfunction, which is relevant for epidemiological purposes, a tendency towards higher prevalence of elevated TSH values was found in mild iodine deficiency and a higher prevalence of low TSH values was found in moderate iodine deficiency (VI). The term "mild thyroid dysfunction" is preferred to the previously used "subclinical thyroid dysfunction" in this thesis for cases with serum TSH outside the reference range and levels of thyroid hormones within the defined range. With a wider definition of mild thyroid dysfunction (narrowing the reference interval for serum TSH), more cases are identified, but obviously, the price could be an increased misclassification, if a part of the additional cases in reality have no thyroid dysfunction. However, the recent years the trend is towards narrowing the reference interval for serum TSH particularly in the high end as increased risk of later clinical overt disease is seen already with TSH levels in the high end of the normal interval (162-164). It is now advocated that subjects with positive TPO Antibodies should be excluded when normal levels of serum TSH is defined, which was the procedure that we followed in our new definition of mild thyroid dysfunction.

Looking just at median TSH values in the cohorts, the tendency is similar. Higher serum TSH levels are found in areas with a higher iodine intake (VI, 67) even among children (126).

Age and gender

Both hypothyroidism and hyperthyroidism are more common among women than among men; this is a constant finding from all studies. Investigating only mild thyroid dysfunction, the gender difference is less pronounced especially for hyperthyroidism (I, VI, 55, 159, 161) but also for hypothyroidism (165). No explanations have been proposed for this paradox, but part of the explanation could be a larger non-differentiated misclassification with wider definitions of thyroid dysfunction.

Hypothyroidism is found with increasing prevalence with age, as it is in most cases a chronic disease; but also the incidence increases with age (30, 162, 166). This age relation is more pronounced in iodine-replete areas (VI, 30).

The occurrence of hyperthyroidism has different relations to age in iodine-replete and in iodine-deficient areas. No association was found between age and the prevalence of previously undiagnosed hyperthyroidism in Norway with iodine sufficiency (165), contrary to the increase with age observed in our mildly and especially moderately iodine-deficient area (I, VI). This association with age is, however, demonstrated even more clearly in studies of the incidence of thyrotoxicosis from areas with different iodine intake (30, 167), where the height of the incidence of hyperthyroidism is seen in the age around 30-40 years in iodine-sufficient areas, but in the very old in iodine-deficient areas. Corresponding to the changes in the prevalences of thyroid dysfunction, a steeper decrease in serum TSH levels with age was found in the region with the most pronounced iodine deficiency in our study (VI) (**Figure 6**); and increasing TSH values with age have been found in iodine sufficiency (168) compared to decreasing values in an iodine-deficient region (134).

Pathogenesis of thyroid dysfunction

In order to understand the difference in the prevalence of hypo- and hyperthyroidism and the different distribution in age groups between areas with different iodine intake, one has to look at the different distribution of the subtypes of thyroid dysfunction.

Hyperthyroidism

In iodine-replete areas, the dominating type of hyperthyroidism

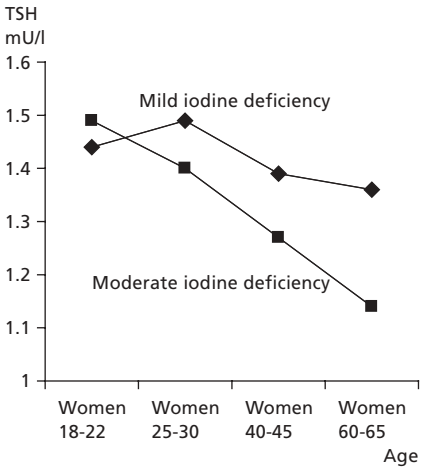


Figure 6. Mean serum TSH in 3,505 women from two areas in Denmark with different iodine intake. Women previously treated for thyroid disease were excluded.

is Graves disease caused by autoimmune stimulation of TSH receptors, whereas in iodine-deficient areas, most cases of hyperthyroidism is caused by autonomous functioning thyroid nodules in multinodular goitres (123, 167). Accordingly we found thyroid nodules in almost all participants with low TSH values in the region with the more severe iodine deficiency (VI). In the region with only mild iodine deficiency, the prevalence of nodules in participants with low TSH was lower than in the area with moderate iodine deficiency. This appearance of thyroid autonomy could also explain the low TSH values in goitrous subjects in areas with mild or moderate iodine deficiency (134, 169) and the general decline in TSH levels with age in these areas (VI) (Figure 6). The occurrence of solitary toxic thyroid nodules does not seem to be related to iodine status in the same way as multinodular toxic goitre is; almost similar incidences of solitary toxic nodules were reported from iodine repletion and iodine deficiency (170). This emphasises that single and multiple thyroid nodules may represent different disease entities.

As Graves' disease has a maximal incidence in young adults and multinodular toxic goitre is almost entirely found among the elderly and old part of the population, this explains the different age distribution of hyperthyroidism in areas with different iodine intake.

The role of iodine in the aetiology of Graves' disease and multinodular toxic goitre is not known. The lack of substrate for thyroid hormone synthesis may inhibit the outbreak of thyrotoxicosis in the presence of TSH receptor stimulating antibodies in iodine-deficient areas, but the tendency is also that the maximal incidence of Graves' is seen in older age groups in iodine deficient areas (167).

The evolution of toxic nodules in most or probably all cases involves a TSH receptor mutation or a mutation in the intracellular pathway of the TSH signal; various mutations have been identified in the TSH receptor and the α-subunit of the guanine nucleotide binding protein (Gsα) in toxic nodules or hyper functioning areas in multinodular glands (171-174). Somatic activating mutations have been reported initially in solitary "hot" nodules, but later the same types of mutations have also been identified in multinodular glands (175). For multinodular glands, such mutations are thought to be clinically apparent only when combined with growth stimulators (176), growth stimulators that could be iodine deficiency in areas with a low iodine intake (172). The assumed high occurrence of somatic thyroid mutations in iodine deficiency could be mediated by the H_2O_2 generating system which is stimulated by iodine deficiency among other things (177). It has also been suggested that activation of mesenchymal, normally inactivated, thyroid cells may be part of the explanation for thyroid nodularity in iodine deficiency (178). The molecular mechanisms leading to growth and in some cases increased function and the influence of genetic background has not been fully elucidated, and further research is desirable.

Hypothyroidism

The dominating aetiology of spontaneous hypothyroidism is thyroiditis, either in the form of Hashimoto's thyroiditis or atrophic thyroiditis (I, 162). In a review, high iodine intake seemed associated with higher prevalences of thyroid antibodies (thyroid peroxidase (TPO) antibodies or thyroglobulin (Tg) antibodies) (179). In recent comparative studies, this tendency was not retrieved (121, 180). Thyroid antibodies may not be specific for thyroiditis, however, as other thyroid diseases could be associated with antigen presentation and antibody production. We found a high prevalence of TPO Ab in participants with multinodular goitre (I), and thyroid multinodularity was associated with TPO Ab in an area with mild (borderline) iodine deficiency (III). This might represent antibody production due to the multinodular changes (17) or that thyroiditis could lead to the formation of distinct thyroid nodules.

Thus, one possibility is that high iodine intake is associated with higher prevalences of thyroiditis and thereby hypothyroidism. Another possibility is that the difference in the prevalence of thyroiditis is minor, whereas the well-known inhibitory effect of iodine on thyroid function acts synergistically with the thyroiditis and thus is responsible for the high prevalence of hypothyroidism in areas with abundant iodine intake (166, 181). We found no differences in the occurrence of TPO Ab between our two regions in DanThyr (180). Approximately half the participants with mild hypothyroidism had high TPO Ab titres. Our findings, the apparent reversibility of some cases of hypothyroidism at iodine restriction (124, 182) and the high TSH levels observed in children from areas with abundant iodine intake (126) are in favour of a direct inhibitory effect of iodine on thyroid function. The interaction between iodine intake and thyroid antibody levels in serum may play a role but needs further clarification.

Conclusion

The prevalence of thyroid hyperfunction is high in areas with moderate to mild iodine deficiency, particularly among the elderly part of the population. This is probably due to autonomous thyroid nodules in multinodular goitres. On the other hand, the prevalence of thyroid hypofunction is increasing with increasing iodine intake already in areas with an iodine intake below the recommended 150 µg/day. Whether this is due to a high prevalence of thyroiditis, a direct inhibitory effect of iodine on the thyroid, or a combination of the two has not been settled.

OTHER RISK FACTORS FOR GOITRE

TOBACCO SMOKING

Goitre and thyroid volume

Different trends have been found concerning the association between tobacco smoking and goitre. Some authors found no association between smoking habits and goitre prevalence (168, 183, 184) or thyroid volume (82, 127, 129). Others have found a positive association between smoking habits and goitre prevalence (185-188) or thyroid volume (128, 131, 185, 189). It has been suggested that the discrepancies could be caused by differences in iodine status, as the association seems to be stronger in iodine-deficient areas (19, 20). This is not an unequivocal trend, however, as no association was found in a German study from an area with moderate to severe iodine deficiency (127), whereas an association was found in three studies from iodine-repleted areas (186, 187, 190) and a in French study with just mild iodine deficiency (128). Thus, methodological differences or interaction with other environmental or genetic factors seem to play a role.

In our comparative set-up, a difference between the two geographical regions in the impact of smoking habits on thyroid volume (**Figure 7**) and particularly serum Tg was found (Figure 2). The associations were stronger in the region with the most severe iodine deficiency, but were still significant in the region with the mildest degree of iodine deficiency (IX). Serum Tg levels have pre-

viously been found positively associated with smoking habits (185, 187). Further, thyroid enlargement and goitre were more common among smokers than non-smokers with odds ratios for current smokers compared to never-smokers of 2.5 (2.0-3.0) and 3.5 (2.0-5.8), respectively. For these variables no significant interaction with iodine status was found (IX).

Partly reversibility of the increased goitre prevalence among smokers has been reported. Ex-smokers have been found to have intermediate thyroid volumes between smokers and non-smokers (IX, 128). An intermediate goitre prevalence was also reported among the ex-smokers in a Swedish population study (186), but among the younger in that population and from another study, a goitre prevalence similar to or even lower than never-smokers was reported for ex-smokers (186, 187). Thus, reversibility of the smoking-induced increase in thyroid volume was suggested particularly among the young.

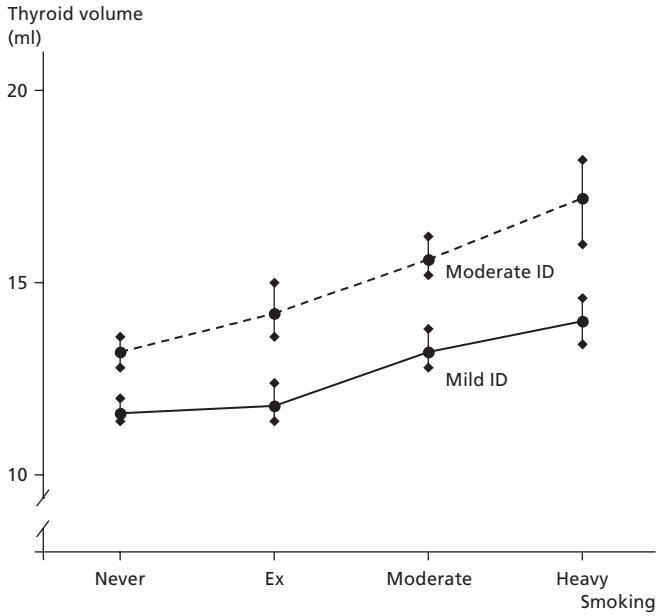


Figure 7. The association between tobacco smoking and thyroid volume in two areas with different iodine intake. Data from a Danish population study including 4412 subjects after exclusion of subjects with known thyroid disease. Significant associations with tobacco smoking were found in both areas. Figure from (IX).

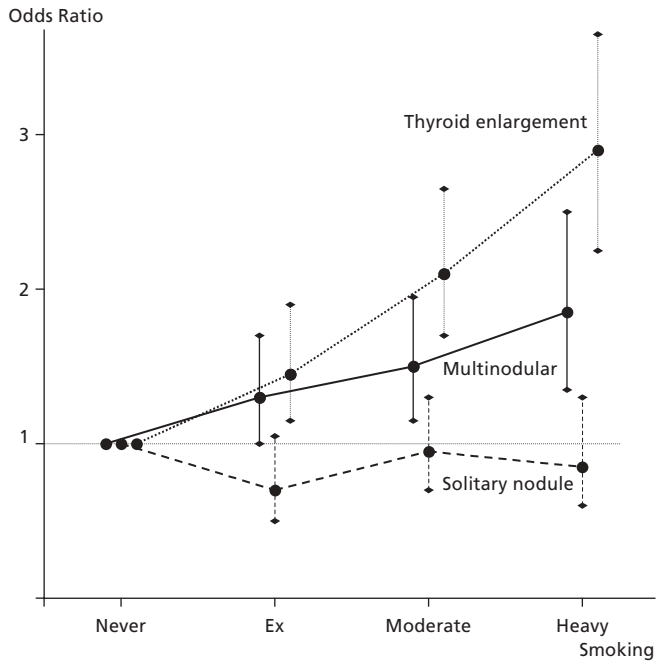


Figure 8. The association between smoking and thyroid enlargement, multiple nodules, and a solitary nodule at an ultrasonographic investigation of 4412 unselected Danes with no previous thyroid disease. Vertical bars represent 95% confidence interval. Figure adapted from (XII).

Thyroid nodules

Less attention has been paid to the association between thyroid nodules and smoking habits. In one study with an association between smoking and goitre, nearly all goitres were multinodular (187). Similarly, nodules were more common in goitres among smokers than among non-smokers (191), and nodular goitre has been found associated with smoking (190) or an insignificant trend towards an association was found (186). We separated participants into groups with multiple nodules or a solitary nodule. For the multinodular group, a positive association with smoking was found, whereas no association was found for the solitary nodule group (XII) (**Figure 8**).

It could be speculated that the group of participants with solitary thyroid nodules was not homogenous, as some nodules in this group will represent the early stage of colloid, multinodular goitres, whereas others will be true – mostly benign – solitary thyroid neoplasms. As the first group has a positive association with smoking, the latter group with true adenomas may have a negative association. This could be in line with a reduced risk of thyroid malignancies among smokers (192, 193).

Thyroid function

Decreased serum TSH levels in smokers are found in most studies (168, 185, 186, 194-196), although in some, the difference did not reach statistical significance (187, 197) possibly due to limited statistical power of the studies. One study reported a tendency towards higher TSH levels among smokers (198).

No specific pattern was found for the association between smoking and thyroid hormone levels; most of the studies above reported an absent association. Two minor studies found a positive association between smoking and serum T3, however, possibly leading to the decreased serum TSH levels (187, 197). These findings have previously been reviewed (19).

We found lower serum TSH levels among smokers, and in addition higher serum free T4 levels (XII). The difference in serum free T4 between smokers and non-smokers was significant, but small. We further demonstrated a lower prevalence of mild hypothyroidism among smokers. There are some controversies in the literature regarding the association between smoking and hypothyroidism. Some studies have found no association (183, 184, 186), whereas one recent study found a significantly higher occurrence of hypothyroidism among smokers (199). The latter study only included hospitalised cases and thus represents highly selected cases with complicated hypothyroidism and probably other medical diseases in many cases. This may explain the discrepancy. Another possibility might be that smoking is associated with a decreased risk of mild hypothyroidism; the induction of clinically overt hypothyroidism may on the other side involve other mechanisms as well and thus show a different association with smoking.

Mild hyperthyroidism showed no significant association with tobacco smoking in our study (XII). That is surprising as the association with Graves' disease is well established as reviewed previously (20, 200). Also a higher occurrence of autonomously functioning nodules could have been anticipated leading to an increased prevalence of hyperthyroidism, though the association between tobacco smoking and toxic nodular goitre has not been settled (200).

Mechanisms of action

Several pathogenetic mechanisms for the impact of smoking on thyroid morphology and function have been suggested (20). Thiocyanate is a degradation product of cyanide in tobacco smoke and it is found in increased concentrations in blood and urine among smokers (197). Thiocyanate inhibits uptake and organification of iodine

competitively, and experimentally it has been shown to be the compound in tobacco smoke with the major impact on the thyroid (118). Part of the effects of tobacco smoke on the thyroid could be explained by the generation of thiocyanate from cyanide. An inhibition of uptake and organification of iodine would mimic a lower iodine intake, and a consequence of lower iodine intake is a higher prevalence of goitre and multiple thyroid nodules, but not solitary thyroid nodules. The distinct association between tobacco smoking and serum Tg levels and the interaction with iodine deficiency (IX) also favour that the inhibition of iodine uptake is involved. The association between iodine status and serum Tg levels is close, possibly through a direct effect of higher or lower iodization of the intrathyroidal Tg in the determination of serum Tg levels (VII, 98).

Thyroid cancer, in particular papillary cancer, is less prevalent both among smokers (192, 193) and in iodine deficiency (201). We found no association between solitary thyroid nodules and smoking (IX). This might be explained by the inhomogeneous group of solitary nodules, as some nodules probably represent the early stage of colloid goitres with an increased risk among smokers, whereas other represent benign neoplasms, possibly with negative association with smoking.

This apparent accordance between observed associations of smoking and expected effects of thiocyanate does not rule out other mechanisms. Nicotine from tobacco smoke has been suggested to cause humeral abnormalities, but nicotine has been shown experimentally not to affect the thyroid in rats (202). Sympathetic nerve stimulation by smoking might explain decreased serum TSH levels through a direct stimulatory effect on thyroid hormone production (203), but it has not been settled whether sympathetic stimulation of the thyroid could also lead to proliferation of thyroid cells. Stimulation of thyroid hormone turnover by tobacco smoke is possible (204), but would not lead to the observed effects. A direct effect on the pituitary has been speculated (20), but this lacks experimental evidence, and it should be associated with low levels of thyroid hormones. Finally inhibition of peripheral deiodinase activity by pyridine compounds in tobacco smoke has been suggested, but the clinical relevance was questioned (198).

The association between tobacco smoking and Graves' disease and especially ophthalmopathy is probably not dependent on thiocyanate generation but involves other pathogenetic mechanisms. This association seems not to be iodine dependent and involves impairment of immunosuppression by components in tobacco smoke (20) and possibly stimulation of cytokines with a specific proliferative effect on fibroblast in eye muscles or unspecific stimulation of fibroblasts by hypoxia (205).

Lower iodine intake – and thereby possibly also cigarette smoking due to increased thiocyanate levels – is associated with lower serum TSH levels either through a repeal of the direct inhibitory iodine effect on the thyroid or through a higher prevalence of thyroid autonomy in iodine deficiency (121). Overt hypothyroidism may, however, also involve other mechanisms modulating autoimmune responses, possibly in synergy with an impact of iodine status.

Conclusion

Smoking is associated with increased thyroid volumes and an increased prevalence of goitre. The association is stronger in areas with concomitant iodine deficiency. Serum Tg is also positively associated with smoking habits, and multiple nodules but not solitary nodules are found more often in smokers than in non-smokers. Lower levels of serum TSH and a lower prevalence of mild hypothyroidism are found among smokers, whereas overt hypothyroidism may be more prevalent among smokers. These associations are most likely mediated by thiocyanate acting as a competitive inhibitor of iodine uptake and organification. Tobacco smoking is a well-established life-style factor that increases the risk of goitre at least when combined with iodine deficiency. A causal association is supported especially for the morphological changes in the thyroid by fairly consistent findings in different studies, a strong association, less pronounced changes among ex-smokers, and reasonable pathogenetic mechanisms to explain the differences.

ALCOHOL

Alcohol and goitre

The association between alcohol consumption and mortality and morbidity from cardiovascular diseases has received much attention, but only few studies have reported data concerning the association between alcohol consumption and thyroid disease. Low thyroid volumes and a low goitre prevalence were found in hospitalised alcoholics (206). This association was independent from alcoholic liver disease (40). The association observed among alcoholics raises the question of a general effect of alcohol on the thyroid. Two minor studies have reported an absent association between alcohol consumption and thyroid volume at ultrasonography (82, 129), but the size of the studies limits the value of these negative findings.

In the DanThyr cohort, we found low thyroid volumes and a low prevalence of goitre in the groups with a moderate or high alcohol consumption compared with the groups with no or low alcohol consumption (VIII). Likewise, a significantly lower occurrence of solitary thyroid nodules was found in alcohol consumers (**Figure 9**). A somewhat similar but statistically insignificant association was found with multinodular thyroid structure (VIII). The low levels of solitary nodules, most likely representing mainly benign neoplasms, could be parallel to the low level of malignant neoplasms found in alcohol consumers (192). Higher serum TSH levels accompanied the lower thyroid volumes among alcohol consumers (VIII).

Causality?

As previously described, a cross-sectional study like ours does not provide evidence of causality. Certain indicators of a causal association were found, however. A dose-response relation was found with a threshold around 7 drinks/week for the onset of the association. We reached a stable statistical model with little influence by inclu-

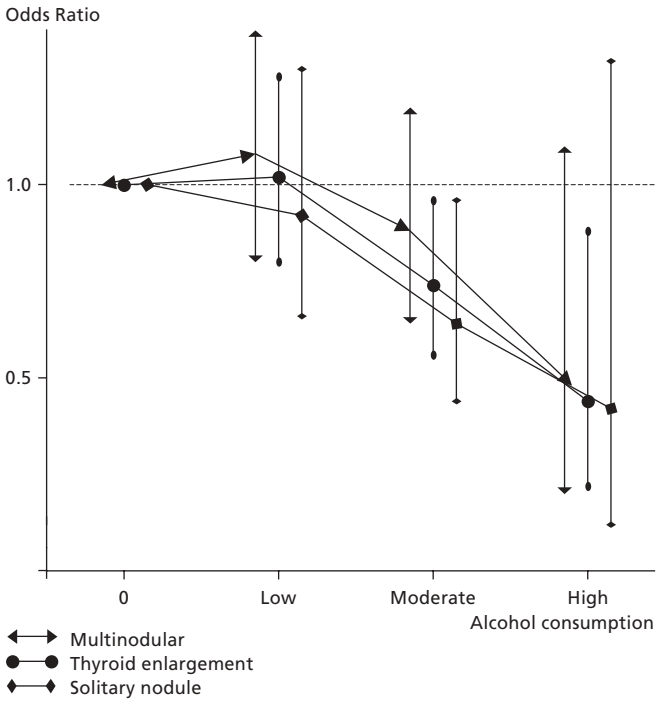


Figure 9. The association of alcohol consumption with the presence of multiple nodules, thyroid enlargement or a solitary nodule at ultrasonography. Data from 4408 subjects in a Danish population study. Low alcohol consumption = 1-7 drinks/week; moderate = 8-28/week (women), 8-42/week (men); high = above 28/42 drinks/week. Values are odds ratios compared to abstainers; vertical bars represent 95% confidence intervals. Figures were adjusted for age, sex, region and smoking habits of the subjects. Differences are significant at the 95% level if CI does not include 1 (dotted line). Figure from (VIII).

sion of other potential confounders in the analysis. The association was found for beer drinkers as well as wine drinkers, which would not have been the case if other life-style related risk factors were confounding the analyses, as beer- and wine drinkers have different lifestyles (207-209). Further, lower thyroid volume has previously been found in alcoholics (206), whose lifestyle in many ways differs from that of ordinary alcohol consumers.

An obvious possibility is that alcohol consumers have a higher iodine intake due to the slightly higher iodine content in beer and wine than in soft drinks and water (210). But inclusion of an estimate of 24-hour iodine excretion in the regression model did not influence the alcohol-thyroid association, and the association was similar in the two regions with different iodine intake contrary to the effect of smoking (IX) and intake of iodine containing vitamin tablets (VII). Further, the association with serum Tg does not show the pattern that would have been expected if the association were mediated by iodine. However, residual confounding from iodine can still not be completely ruled out, as a single estimate of iodine excretion does not take the large variations in iodine excretion into account.

Mechanisms

There is little experimental research to provide an explanation for the association. Among the possibilities could be inhibition of thyroid hormone metabolism, but this should lead to lower TSH levels contrary to our observations. A direct inhibitory effect of alcohol on thyroid cell proliferation is possible, which would also be compatible with the slightly higher serum TSH levels among alcohol consumers. Increased sensitivity of thyrocytes to TSH in presence of high concentrations of alcohol has been reported (211), but the relevance for physiological conditions is unclear, and increased sensitivity to TSH should lead to higher or unchanged thyroid volumes and lower TSH levels. Some fibrosis of the thyroid was found in the study of alcoholics with liver cirrhosis, suggesting a toxic effect on the thyroid (40). But again, extrapolation to normal levels of alcohol consumption is hazardous, and thyroid function was normal in these patients as well as in our groups of alcohol consumers. Lower levels of serum Tg – contrary to the high levels observed in cirrhotic patients (212) – and lower prevalence of thyroid nodules in alcohol consumers in our study do not support a toxic effect either.

Conclusion

Alcohol consumption seems to be associated with a lower prevalence of goitre and solitary thyroid nodules. In our study, some indications of a causal relation were found, but possible confounding from iodine intake should still be considered. The mechanism behind this apparent association is at present unclear. Confirmatory studies for this association are needed as well as experimental studies of the pathogenesis of the association.

ORAL CONTRACEPTIVES

The preponderance of women among patients with goitre and other thyroid diseases has been dealt with in previous sections. It is not known whether this is caused by direct genetic effects on the susceptibility to thyroid disease or more indirectly by e.g. differences in the levels of sex hormones. Environmental factors may be unevenly distributed between the genders, and a complex combination of the mentioned possibilities may exist.

Estrogens and the thyroid

The possible impact of estrogens on thyroid growth and function was studied extensively around the middle of the last century as reviewed by Noach (213). Conflicting results were reported from these studies, but two major effects were suggested: Inhibition of TSH release by the pituitary and increase of thyroid sensibility to TSH under the influence of estrogens. The net effect was increased thyroid weight. Two more recent studies of cultured thyroid follicle cells also showed increased proliferation under the influence of estrogens (214, 215). It could be speculated that the use of oral contraceptives (OC) could lead to goitre owing to the high estrogen levels. This is a clinically important issue: should women with goitre or disposition for goitre be advised to seek other contraceptive methods? Thyroid function seems to be affected as well; most studies have reported increased serum TSH levels under administration of estrogens (216-220).

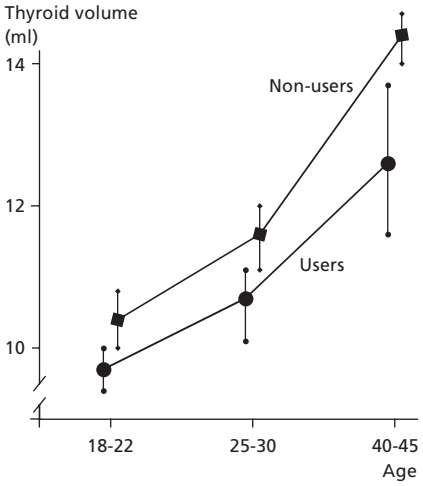


Figure 10. Thyroid volume at ultrasonography among current users and non-users of oral contraceptives. Data from 2510 participants in a population study, analysed in a linear model adjusting for age and region of inhabitancy. Values are means and vertical bars represent 95% confidence interval for means. The difference between users and non-users of OC was significant in all age groups ($p < 0.005$). Figure from (X).

Epidemiology

In a prospective study, a tendency towards a decreased risk of goitre was observed among users of OC (Relative risk 0.69, non-significant) (221), and a recent cross-sectional study found lower thyroid volumes ($p = 0.05$) at ultrasonography in users of OC (128). Further, use of OC was evaluated as a risk factor for benign thyroid nodules in patients exposed to radiation on the neck in childhood; no significant association was found (222). After the publication of our study, also a markedly reduced risk of hyperthyroidism has been reported associated with the use of OC (223).

We found significantly lower thyroid volumes (**Figure 10**) and lower goitre prevalence (OR = 0.25) among users of OC in multivariate models (X). The strength of the association was illustrated by the preventive fraction: 29% of goitre cases in a hypothetical population without use of OC was prevented by use of OC in this cohort assuming a causal association. The strong association, concordance with the few previous studies, support from experimental data, and a similar trend for postmenopausal estrogen substitution supported such a causal association.

There were no significant differences in nodule prevalence, though the tendency was a reduced prevalence of nodules as well. Similarly to previous studies, serum TSH was slightly higher in users of OC. Differences were also found for serum free T3 and free T4 with higher levels of T3 and lower levels of T4, but these differences should be interpreted with reservations, as assays may not be able to produce comparable values between the groups due to the large variations in serum thyroid hormone binding globulin (TBG) (224, 225). Serum Tg was slightly higher among users of OC.

Similar differences were found in postmenopausal women between users of estrogen hormone replacement therapy and non-users (X). These differences were not statistically significant, as they were slightly less pronounced, the number of participants was smaller, and variation in thyroid volume was larger among the elderly.

Pathogenesis

An overview of the pathogenesis of the association between goitre and OC is not easily provided, as it may be a combination of several interplays between estrogens and thyroid function, and each of these interplays have not been described in detail.

Some of the described effects of estrogens on the thyroid would rather be associated with an increased goitre prevalence: Increased TBG concentration leading to higher levels of total T3 and T4 in order to obtain unchanged levels of free hormones, and increased deiodinase activity leading to increased turnover of thyroid hormones (226). The latter mechanism might explain the increased T3/T4 ratio. Androgens could play a role, as androgens have been shown to inhibit proliferation of thyroid follicular cells (227); the levels of androgen are lower under estrogen therapy, however (228). Another effect in play may be inhibition by estrogens of the expression of the Na/I symporter (214), and iodine uptake (229). This could explain the elevated serum Tg levels in users of OC, as Tg is a very sensitive marker of iodine status as discussed in a previous chapter. Furthermore, increased Tg RNA levels were found after addition of estradiol to cultured thyroid follicles (215). The same study suggested increased thyroid growth with estrogens, though unchanged levels of the c-myc proto-oncogene were found, even if this is normally associated with thyroid growth. Increased sensitivity of the pituitary to TRH during use of OC might explain the slightly higher serum TSH levels (230, 231), but it does not explain the lower thyroid volumes. The higher serum TSH levels could also be secondary to increased demands for T4 because of the increased TBG levels. This could be parallel to increased demands for T4 substitution with estrogen therapy (232). Impaired binding of TSH to thyrocytes has been reported with high-dose estrogen administration to rats (233), but the relevance for pharmacological doses of estrogens in women is unknown.

Other mechanisms are most likely responsible for the decreased thyroid volumes in users of OC. A possibility is the suppression of estrogen metabolites with toxic effect on the thyroid, metabolites that are not generated in the metabolism of the ethinylestradiol in OC. Such a toxic effect was reported for 2-methoxyestradiol, a metabolite of the naturally occurring 17β-estradiol, with changes in thyroid morphology and increased apoptosis (234).

We also suggested another possibility, based on the observation that LH and FSH binds to and stimulates the TSH receptor (235-237). Such stimulation was not unexpected, as large structural similarities between the gonadotropins and TSH are present with identical α-chains. hCG is known to stimulate the TSH receptor during pregnancy, sometimes leading to completely suppressed TSH levels (238). With suppression of LH and FSH during therapy with OC and with postmenopausal estrogen therapy (228), less stimulation of thyroid growth could be a consequence. We observed an increased thyroid volume among subjects previously under work-up for infertility independent from present parity status (unpublished data). This could be a consequence of increased LH levels in infertility either as part of the pathogenesis of infertility (239) or as consequence of the treatment. Also levels of estrogens in serum may be different under these conditions, however.

Conclusion

Use of oral contraceptives is associated with a decreased risk of goitre. Indications of causality were found with a strong association and no confounding from other life-style-associated factors. However, even if such a causal association was confirmed, OC have no obvious place in the prevention of thyroid disease due to other effects and side effects of OC use. Experimental data suggest possible mechanisms for an association, but the pathogenesis of this association remains to be settled; it is probably a combination of several direct and indirect effects of sex steroids on the thyroid.

PARITY

Pregnancies have been claimed to explain the gender difference in goitre prevalence. At least, the increased goitre prevalence observed during pregnancies could contribute to the higher occurrence of goitre among women (238).

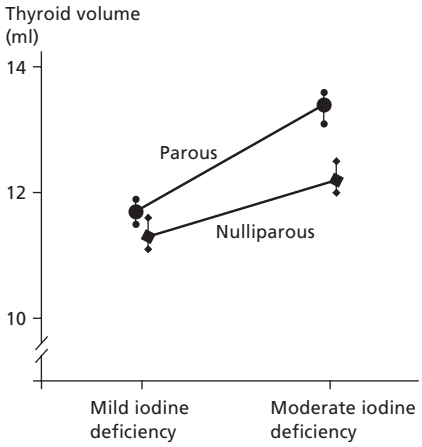


Figure 11. The association between thyroid volume and parity in two Danish regions with mild and moderate iodine deficiency, respectively. Data from 3,361 unselected women with no previous thyroid disease and without present or recent pregnancy. Figures are mean values from a linear model after adjustment for age and use of oral contraceptives; thyroid volume was analysed after logarithmic transformation. Vertical bars represent Standard Error. Comparison of parous to nulliparous in moderate iodine deficiency, p<0.001; in mild iodine deficiency, p=0.24. Figure from (XI).

Epidemiology

There are two major arguments against pregnancy as a major explanatory factor for the gender difference in goitre prevalence. Firstly, the gender difference is present already in adolescents (15, 240). Secondly, whereas the gender difference is a constant phenomenon from all investigations, pregnancy only seemed associated with goitre prevalence or thyroid volume in iodine-deficient areas (241-247). In iodine-sufficient areas, no association has been found (248-251). A comparative study of palpable goitre revealed no increase in goitre prevalence during pregnancy in an iodine-replete area, but a goitre prevalence twice as high in pregnancy as in controls in an iodine-deficient area (252). The long-term consequences of pregnancies for goitre prevalence have only been evaluated in few studies, as the follow-up in the studies above was no more than one year. In a small study from an iodine-sufficient area, no association between parity and thyroid volume was found (129), but in a study from an area with moderate iodine deficiency, a pronounced, positive association between parity and thyroid volume was found (253). In our comparative study, the association between parity and thyroid volume was strongest in the area with the most pronounced iodine deficiency (XI) (**Figure 11**) also after adjustment for amongst other things various life-style factors, use of OC, and Body Surface Area. Thus, even the long-term effects of pregnancy on thyroid volume depend on iodine status. The association between parity and serum Tg was quite parallel with the association between parity and thyroid volume in our study (unpublished data).

One previous study found an association between parity and thyroid nodules, apparently in a univariate analysis (254). We also found this association in a univariate analysis, but after adjustment for age, no association was found (XI). Apart from the difference in statistical methods, also the older age of the women in the German study may explain their positive association. Further, a difference in iodine status could attribute to the discrepancy, but iodine excretion was supposedly similar in the two studies. An increase in thyroid nodule volume during pregnancy was observed in an area with borderline iodine deficiency (255), but with no blinding and no control group the validity of the small differences remains unsettled.

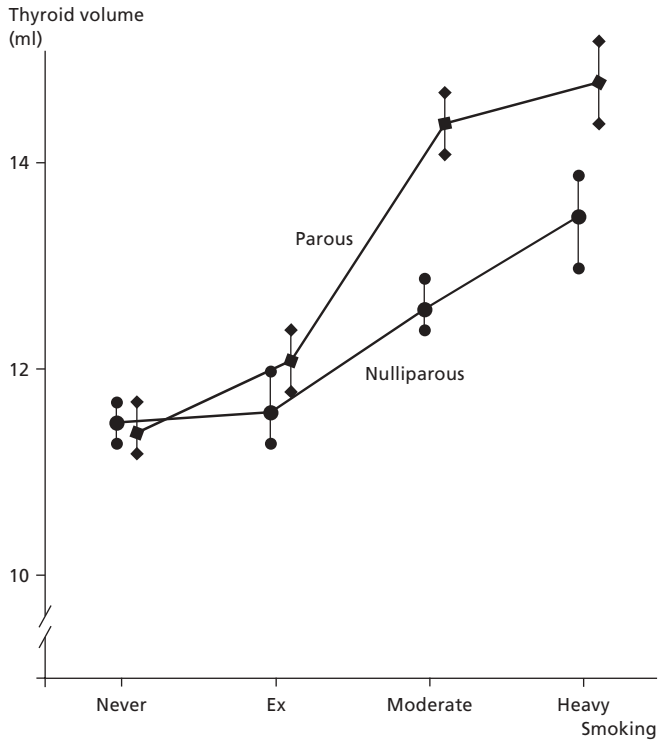


Figure 12. The dependency to smoking of the association between parity and thyroid volume. Data from 3,361 unselected women with no previous thyroid disease and without present or recent pregnancy. Figures are means in a linear model after adjustment for region of inhabitancy, age and use of oral contraceptives; logarithmic transformation of thyroid volumes was used in the analyses. Vertical bars represent Standard Error. Comparison of parous to nulliparous women within each group according to smoking habits: never smokers, p=0.82; ex-smokers, p=0.27: moderate and heavy smokers, p<0.001. Figure from (XI).

Mechanism

The iodine-dependency of the association between pregnancy and goitre seems clear and is supported by successful prevention by iodine supplementation (244, 246, 256, 257). Increased iodine demands throughout gestation may lead to aggravation of existing iodine-deficiency disorders when these increased demands are not met (238), and increased renal loss of iodine through gestation may aggravate the iodine deficiency (258).

We observed synergistic effects of parity and smoking on thyroid volume (**Figure 12**), and also of parity and degree of iodine deficiency (XI). Both synergies are in line with the iodine-dependency of the association, as also smoking seems to exert a goitrogenic effect through aggravation of iodine deficiency (IX). The association between thyroid volume and parity was weaker in the oldest age group (XI), indicating that pregnancies accelerate the iodine-deficiency induced changes in the thyroid, changes that may have appeared under all circumstances, but at a later time. Another possibility is that other, stronger risk factors dominate in old age. Smoking had a similar, age-dependent association (IX) further supporting that parity and smoking influence goitre prevalence by similar mechanisms: worsening of iodine deficiency.

Conclusion

Pregnancy is associated with increased thyroid volume and increased goitre prevalence in iodine-sufficient areas but not in iodine-replete areas. A similar pattern applies to the association with previous pregnancies, but the association was weaker in elderly women; nullipara may have a later "catch-up" growth of the thyroid due to the low iodine intake. Apart from the interaction with iodine status, a synergistic effect with smoking was identified. Thus, the impact of parity is most likely mediated by aggravation of existing iodine deficiency.

SOCIAL IMBALANCES

Epidemiology

Few studies have investigated the association between social status and the occurrence of thyroid disease. A higher prevalence of goitre was found among children from low socio-economic levels than among children from higher socio-economic levels (259-263), but this was not confirmed in the three other available studies (264-266). Most of these studies were performed in developing countries, and they all only used palpation of the thyroid as marker of thyroid enlargement.

We used educational level as a marker of socio-economic status and found significant associations between educational level and serum thyroglobulin, thyroid volume, goitre prevalence, and prevalence of thyroid multinodularity with lower occurrence of goitre and thyroid nodules among participants with a high educational level (XIII). This association was still significant after adjustment for other known risk factors in the statistical models but diminished considerably. Especially inclusion of tobacco smoking and iodine excretion affected the associations. An association with thyroid volume or serum thyroglobulin was found for most other investigated variables indicative of living conditions such as housing (higher serum Tg among tenants than house-owners) and work (higher serum Tg among blue-collar workers than among white-collar workers). These associations were found in simple statistical models adjusting only for age, gender and region of inhabitancy but became insignificant after adjustment for smoking and other life-style associated factors.

Interpretation

It seems reasonable to assume that the association between educational level and thyroid disease is not mediated by education per se. Rather, education is a marker of living conditions and life-style, and these variables are associated with thyroid disease. We demonstrated that the association was mediated to some extent by tobacco smoking and iodine intake, which is in accordance with a lower frequency of smoking and a more varied food intake in higher socio-economic groups. Other factors are possibly involved. Deficiency of other micronutrients could be associated with an increased risk of thyroid disease as it has been shown for iron deficiency (267, 268). Still, residual confounding is another possibility leaving space for an even further part explained by smoking and the iodine contents of ingested food.

COMBINATION OF RISKS

From the analyses in the first "DanThyr" cohort it is possible to identify certain groups with an increased risk of goitre and other thyroid disease. It is statistically correct to combine risk estimates such as odds ratios by multiplication if they are obtained in the same analysis, given that the basal conditions for the statistical model are fulfilled and statistical interactions are taken into account. The appropriateness can be estimated with for instance a goodness-of-fit test (Hosmer-Lemeshow).

In our case, the identified statistical interactions hamper combination of different risks, as the most correct model combining all risk factors would include too many subgroups for a reliable analysis. Consequently the examples below do not combine all risk factors or ignore some interactions.

It can be estimated for the premenopausal female population in our cohort without interactions in the model that a high-risk group of present smokers, with alcohol consumption less than seven drinks per week, without use of oral contraceptives, and with at least one childbirth has an odds ratio for thyroid enlargement at ultrasonography of 8.3 compared with a low-risk group without smoking, drinking 8 drinks or more per week, taking oral contraceptives, and without previous childbirths. For palpable and visible goitre at the clinical examination the odds ratio was 12.0 comparing the groups with high and low risk. If region of inhabitancy was included

adding moderate iodine deficiency to the high-risk group and only mild iodine deficiency to the low-risk group, the odds ratio was 13.7 for thyroid enlargement and 20.9 for clinical goitre. An adjustment for age was made in all models, and the goodness-of-fit showed p values around 0.8 indicating good agreement between the estimates and the observed data.

Comparing these odds ratios with observed prevalences of goitre in the groups, the impact from childbirths was ignored, as age adjustment was not possible for the observed data and particularly parity was very age dependent. The high-risk group with tobacco smoking, low alcohol consumption and no use of oral contraceptives included 671 women, and the prevalence of thyroid enlargement was 32.9% in this high-risk group compared to 4.3% among the 115 women in the low-risk group. The prevalence of clinical goitre was 7.2% in the high-risk group, and no case of goitre was observed in the low risk group.

In a model taking all interactions into consideration the picture was similar. The analysis was, because of the interactions, restricted to women aged 40 to 45 years living in Aalborg. The high-risk group with smoking, low alcohol consumption, previous childbirth and no use of oral contraceptives had an odds ratio for thyroid enlargement of 13.9 compared with the corresponding low-risk group.

As mentioned, these figures should be interpreted with some reservation as the confidence intervals are wide and not directly calculable, and the handling of interactions was difficult. The apparent correspondence between the statistical models and the observed values does not serve as a validation of the statistical model. These calculations illustrate, however, the remarkable impact of life-style on the risk for goitre with an approximately tenfold increased risk of goitre for a group with an inexpedient combination of these life-style related variables even when iodine status was not included.

The risk estimates are for mild degrees of thyroid disease and may not be transmittable to clinical cases. As the risk factors were more important for goitre than the cases only identified by ultrasonography, it could be speculated by extrapolation that these risk factors would also have greater impact on the occurrence of more severe disease. A great number of clinical cases would be required to test this hypothesis, and the selection bias would make data difficult to interpret.

PREVENTION

The major preventive initiative against goitre has been eradication of iodine deficiency. Today iodization programmes affect more than $^2/_3$ of the population of the 130 countries exposed to iodine deficiency (25), and implementation and regulation of iodization programmes, primarily through iodization of salt, should still have high priority. In many regions – also in Denmark as demonstrated from "DanThyr" and in other parts of Europe – the iodine supply is still not sufficient.

During the past decade, the specific aim of iodization programmes has been discussed with the growing awareness of possible side effects of iodine excess both temporarily after an increase in iodine intake and in the long run (16). Iodine-induced thyrotoxicosis has been described following many iodization programmes (269). Further, it has now been demonstrated that a close, positive association exists between iodine intake and the incidence of hypothyroidism over the entire spectre of iodine intake from moderate iodine deficiency to iodine excess.

The goal on the other hand is to prevent iodine deficiency disorders (IDD). Previously, goitre prevalence among children and adolescent has been the preferred parameter for monitoring of IDD, but this may not be a sufficiently sensitive parameter. A high occurrence of multinodular goitre among the elderly in a population is seen as a consequence of iodine deficiency even in populations where goitre is not endemic among children, and the level of iodine intake sufficient to prevent the high prevalence of thyroid multinodularity among the elderly is not known today. Suggestions of 120-150 μg/day as sufficient to prevent IDD have been made with a target of iodization programmes at 120-220 μg total daily iodine intake (123).

The delicate balance is thus between prevention of goitre and multinodular goitre with hyperthyroidism in iodine deficiency on one hand and prevention of hypothyroidism in iodine excess on the other hand. The balance has large implications also for the occurrence of the possible complications to thyroid dysfunction such as heart disease and osteoporosis in hyperthyroidism and cardiovascular disease and dementia in hypothyroidism. These complications are even associated with mild and unrecognised thyroid dysfunction (270-272), which is found with a high prevalence in many populations (Table 7).

Monitoring of IDD should accordingly include different groups in the society. Children are often easily accessible for logistic reasons, but in areas with mild iodine deficiency also groups with maximal risk should be investigated such as elderly subjects and pregnant women. Measurements of iodine excretion and thyroid volume at ultrasonography are standard methods today, but serum thyroglobulin may prove to be a valid supplement or alternative.

We demonstrated a population attributable fraction of 48% of tobacco smoking on the prevalence of clinically detectable goitre (IX). Thus, smoking is a major risk factor with a large potential for prevention. Still, goitre will not be the major argument against smoking in population-based initiatives. But for subjects with beginning goitres or subjects otherwise disposed for goitre, smoking cessation could be of great benefit, and individual counselling should be given. This is hopefully already the case in relation to subjects with Graves' disease or predisposition for the disease.

The social imbalances in goitre prevalence underline the impact of life-style on thyroid disease. Smoking habits explained some of the difference between educational groups, and iodine intake and thereby choice of food explained another part of the variation. Still, some of the association with education has not been accounted for, and other life-style interventions yet to be described might be of benefit.

As the association between goitre and parity is mediated by unmet iodine demands during pregnancy, the increased risk during and after pregnancy is preventable. Successful iodization programmes may eradicate the risk of goitre associated with pregnancy, but until this is accomplished, and possibly also in a population with an optimal but not excessive iodine intake, other initiatives should be taken to ensure adequate iodine supply to pregnant women. This is for the sake of the mother, but even more important for prevention of IDD in the foetus. Administration of 150 μg iodine daily to pregnant women does not increase the risk of post partum thyroiditis, though this could theoretically be apprehended (273).

The association between alcohol intake and goitre is less established. Under all circumstances, the value of alcohol in prevention of goitre is limited, as the amounts required to obtain a clinically relevant reduction in risk of goitre is of a magnitude that may be associated with increased morbidity from other diseases. However, the beneficial effect of a low to moderate alcohol consumption that has been described on the risk of cardiovascular disease also seem to apply for the risk of goitre, but at higher doses.

Goitre prevalence was markedly reduced among users of oral contraceptives, and the impact of oral contraceptives was illustrated by the preventive fraction of 29% (assuming causal relationship), indicating that goitre prevalence would have been almost 50% higher without use of oral contraceptives in our cohort. Due to the other effects and side effects of oral contraceptives, goitre prevention can only be a minor point in the choice of contraception. The increased risk of goitre with use of oral contraceptives that could have been anticipated from experimental studies was, however, effectively excluded by our study.

OVERALL CONCLUSION

The major risk factor for thyroid disease is iodine deficiency. Iodine status influences the overall prevalence of benign thyroid disease, but iodine intake also influences the relationship between different types of thyroid disease. Non-toxic goitre and multinodular toxic goitre have a close, positive association with iodine deficiency, whereas the incidence of hypothyroidism has a positive association with iodine intake, and Graves disease probably also has a positive association with iodine intake though less pronounced. The benefits of iodine supplementation in areas with iodine deficiency are undisputable, but iodine intake should only be increased to a level sufficient to prevent iodine deficiency disorders, probably around 150 µg/day. As it is a delicate balance to reach to optimal level of iodine intake, monitoring of iodization programmes is mandatory, and the "DanThyr" project provides such monitoring of effects and possible side effects in Denmark with validated methods.

Other important risk factors have been identified. Smoking is probably the most important life-style factor with impact on the occurrence of thyroid disease. As risk factor for goitre, the importance of smoking is dependent on iodine status, and the importance should diminish with implementation of successful iodization programmes. Parity is another risk factor that may be of less importance after eradication of iodine deficiency. Moderate alcohol consumption as preventive factor needs confirmation from other studies and elucidation of possible mechanisms, but has no place in prevention of goitre. Oral contraceptives seemed to have a prominent preventive effect against goitre. This may inspire for further experimental research on the interplay between sex steroids and the thyroid, and give clues to an explanation of the gender difference in the occurrence of thyroid disease.

Further research should be encouraged in the field of thyroid epidemiology and interplay between life-style and thyroid disease, as the aetiology of thyroid diseases is still only briefly described. A special challenge will be investigation of interactions between different environmental factors, and the investigation of gene-environment interactions. Considerable morbidity and costs for the health care system is still preventable through prevention of thyroid disease. What is the optimal level of iodine intake, and how is this level best achieved? Which life-style interventions could supplement the optimisation of iodine intake?

SUMMARY

METHODOLOGY

In thyroid epidemiology, the most widely used estimate of iodine intake is iodine concentration in spot urine samples. This estimate is only valid at the population level, and if individualisation of the estimate is required as in regression models, stratified analyses, or with investigation of selected groups of the population, we suggested the use of an age- and sex adjusted iodine/creatinine ratio. This is still not a precise estimate of the individual's habitual iodine intake, but the correlation with 24- hour iodine excretion and other markers of iodine status was satisfactory.

Thyroid volume measured with ultrasonography has been the preferred marker of iodine deficiency in epidemiological studies in the last decades. It is, however, a subjective parameter, which hampers comparisons from different investigators. Marked differences between observers were recently demonstrated even if similar equipment was used. We suggested standardized measuring procedures to improve the comparability of studies, and we demonstrated that results from different investigators were unbiased for thyroid volume and number of thyroid nodules but not for echogenecity and echo pattern.

It appeared that serum thyroglobulin was a better indicator of iodine status in a population than thyroid volume. It could be even better than iodine excretion in spot urine samples – also with the use of the adjusted iodine/creatinine ratio – and in some cases easier to apply. Serum thyroglobulin is closely related to thyroid volume, thyroid nodules and thyroid function, but it may also have a direct association with iodine status independent from the structural and functional differences associated with iodine deficiency. Thus, serum thyroglobulin measurements may deserve a more prominent position in thyroid epidemiology, especially when focus is on measuring and monitoring iodine deficiency.

IODINE DEFICIENCY

Severe iodine deficiency is associated with increased goitre prevalence especially among adolescents and young adults. In areas with mild to moderate iodine deficiency, an increase in thyroid volume is seen particularly in the age between 30 and 40 years, but the goitre prevalence continues to increase at least up to the age around 70 years. This is accompanied by an increase in the occurrence of multiple thyroid nodules, a decrease in serum TSH levels, and an increase in the incidence of toxic multinodular goitre in older ages. This is in accordance with the conception of the natural history of iodine-deficiency goitre as an initial diffuse goitre followed by development of nodular hyperplasia and later thyroid autonomy of these nodules, eventually leading to toxic multinodular goitre. The molecular background for these macroscopic changes is not known in detail. Somatic mutations and unspecific growth stimulation whether mediated by TSH or other growth promoters seem involved. We could demonstrate a high occurrence of goitre in Denmark compared with iodine-sufficient areas. Even a modest difference in iodine excretion the two regions in our DanThyr study was associated with a 50% difference in the occurrence of goitre or thyroid enlargement.

A low prevalence of primary hypothyroidism is observed in iodine-deficient areas. The tendency also seems to be slightly lower incidence of Graves' disease and possibly a later age of onset. We found high prevalences and incidences of hyperthyroidism, but low occurrence of hypothyroidism in Denmark compared to iodine-sufficient areas, and these differences were most pronounced in the region with the most pronounced iodine deficiency. Thus, the association between hypothyroidism and iodine status is present already in moderate to mild iodine deficiency. This association may be caused by the direct inhibitory effect of iodine on thyroid function. The interplay between thyroid autoimmunity and iodine intake has not been settled in detail.

OTHER RISK FACTORS

From previous studies, conflicting results have been published concerning the association between tobacco smoking and goitre. Apart from methodological differences it is now clear that iodine status of the investigated cohorts explain a major part of the difference. In iodine-deficient areas, a clear, positive association between smoking and goitre is seen, whereas the association is less pronounced or even absent in iodine-sufficient areas. This synergistic effect of smoking and iodine deficiency was confirmed by the interactions in our study. Also thyroid multinodularity was associated with smoking.

In a recent Danish study, a positive association between overt hypothyroidism and smoking was found, whereas conflicting results have been reported previously. In our study, mild hypothyroidism was negatively associated with smoking whereas we found no association between smoking and hyperthyroidism. Most of these associations could be explained by the blocking of iodine uptake and -organification by thiocyanate generated from cyanide in tobacco smoke, as the effects mimic the effects of low iodine intake. The conflicting results concerning hypothyroidism could be caused by competing causes of hypothyroidism; the blocking of iodine uptake by tobacco smoke may protect against mild hypothyroidism, whereas severe hypothyroidism is increased among smokers due to induction of autoimmunity. Correspondingly, a close association between smoking and Graves' disease and especially ophthalmopathy has previously been described. The mechanism behind this association

is not known in detail but it probably involves modulation of endogenous immunosuppression and cytokine production.

Previously two smaller studies found a low occurrence of goitre among alcoholics. A moderate to high alcohol intake was associated with a low prevalence of goitre and solitary thyroid nodules in our study. This association did not seem to be driven by different iodine status or different life style in the alcohol intake groups. No other study with a sufficient number of observations has reported on this association. Among alcoholics, thyroid fibrosis was found, but with low serum Tg levels among alcohol consumers in our study, the mechanism may be different, and the association within low to moderate alcohol consumption remains to be further elucidated.

In two previous epidemiological studies a low occurrence of goitre among users of oral contraceptives was found, and recently also low occurrence of hyperthyroidism was found among users of OC. We found similarly that the odds ratio for goitre was 0.25 for users of OC compared to non-users. Multiple pathogenetic pathways for such an association are possible. Experimental studies could support a goitrogenic effect of particular metabolites of the genuine 17-β estradiol, metabolites suppressed with administration of the artificial ethinyl estradiol. Also suppression of a thyroid stimulating effect of LH or FSH, or impaired binding of TSH to thyrocytes under estrogen administration may be involved.

An increased prevalence of goitre and increased thyroid volumes during pregnancy have previously been reported from iodine-deficient areas, whereas no association has been found in iodine-sufficient areas. The few previous studies with data on the long-term association between pregnancies and thyroid volume confirm this synergy between iodine status and parity in the association with goitre. Previous pregnancies were associated with increased goitre prevalence and increased thyroid volumes in the DanThyr cohort but only when combined with iodine deficiency or tobacco smoking. This is all compatible with a goitrogenic effect of pregnancies due to increased iodine demands during pregnancy, demands that are not met in iodine-deficient areas.

Social imbalances in the occurrence of goitre have been investigated in a few studies but with conflicting results, possibly because of imprecise methods. We found goitre and thyroid nodules with higher prevalence in groups with a low educational level. This could in part be explained by a higher prevalence of tobacco smoking and lower iodine intake in the groups with short education.

CONCLUSION

Iodine is a major risk factor for goitre also in Denmark, and even modest regional differences in iodine excretion seemed associated with marked differences in the occurrence of thyroid disease. An iodization programme seems justified, but due to possible side effects, monitoring is mandatory, and the optimal level of iodine intake still needs to be established. Other risk factors also play an important role, some in interaction with iodine deficiency. Still, knowledge of some of these other factors and possible mechanisms is limited, and further research in risk factors, interactions, and interplay with genetic factors is needed.

REFERENCES

I Knudsen N, Jørgensen T, Rasmussen S, Christiansen E, Perrild H 1999 The prevalence of thyroid dysfunction in a population with borderline iodine deficiency. Clin Endocrinol (Oxf) 51 (3):361-367.

II Knudsen N, Bols B, Bulow I, Jorgensen T, Perrild H, Ovesen L, Laurberg P 1999 Validation of ultrasonography of the thyroid gland for epidemiological purposes. Thyroid 9 (11):1069-1074.

III Knudsen N, Perrild H, Christiansen E, Rasmussen S, Dige-Petersen H, Jorgensen T 2000 Thyroid structure and size and two-year follow-up of solitary cold thyroid nodules in an unselected population with borderline iodine deficiency. Eur J Endocrinol 142 (3):224-230.

IV Knudsen N, Christiansen E, Brandt-Christensen M, Nygaard B, Perrild H 2000 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. Eur J Clin Nutr 54 (4):361-363.

V Knudsen N, Bulow I, Jorgensen T, Laurberg P, Ovesen L, Perrild H 2000 Goitre prevalence and thyroid abnormalities at ultrasonography. A comparative epidemiological study in two regions with slightly different iodine status. Clin Endocrinol (Oxf) 53 (4):479-485.

VI Knudsen N, Bulow I, Jørgensen T, Laurberg P, Ovesen L, Perrild H 2000 Comparative study of thyroid function and types of thyroid dysfunction in two areas in Denmark with slightly different iodine status. Eur J Endocrinol 143 (4):485-491.

VII Knudsen N, Bulow I, Jorgensen T, Perrild H, Ovesen L, Laurberg P 2001 Serum tg-a sensitive marker of thyroid abnormalities and iodine deficiency in epidemiological studies. J Clin Endocrinol Metab 86 (8):3599-3603.

VIII Knudsen N, Bulow I, Laurberg P, Perrild H, Ovesen L, Jorgensen T 2001 Alcohol consumption is associated with reduced prevalence of goitre and solitary thyroid nodules. Clin Endocrinol (Oxf) 55 (1):41-46.

IX Knudsen N, Bulow I, Laurberg P, Ovesen L, Perrild H, Jørgensen T 2002 Association of Tobacco Smoking with Goiter in a Low-Iodine-Intake Area. Arch Intern Med 162 (439-443.

X Knudsen N, Bulow I, Laurberg P, Perrild H, Ovesen L, Jorgensen 2002 Low goitre prevalence among users of oral contraceptives in a population sample of 3712 women. Clin Endocrinol (Oxf) 57 (1):71-76.

XI Knudsen N, Bulow I, Laurberg P, Ovesen L, Perrild H, Jorgensen T 2002 Parity is associated with increased thyroid volumes solely among smokers in an area with moderate to mild iodine deficiency. Eur J Endocrinol 146 (1):39-43.

XII Knudsen N, Bulow I, Laurberg P, Perrild H, Ovesen L, Jorgensen T 2002 High occurrence of thyroid multinodularity and low occurrence of subclinical hypothyroidism among tobacco smokers in a large population study. J Endocrinol 175 (3):571-576.

XIII Knudsen N, Bulow I, Laurberg P, Ovesen L, Perrild H, Jorgensen T 2003 Low socio-economic status and familial occurrence of goitre are associated with a high prevalence of goitre. Eur J Epidemiol 18 (2):175-181.

14. Burgi H, Supersaxo Z, Selz B 1990 Iodine deficiency diseases in Switzerland one hundred years after Theodor Kocher's survey: a historical review with some new goitre prevalence data. Acta Endocrinol (Copenh) 123 (6):577-590.
15. Delange F 1994 The disorders induced by iodine deficiency. Thyroid 4 (1):107-128.
16. Laurberg P 1994 Iodine Intake – What Are We Aiming At? J Clin Endocrinol Metab 79 (1):17-19.
17. Laurberg P, Nohr SB, Pedersen KM, Hreidarsson AB, Andersen S, Bulow P, I, Knudsen N, Perrild H, Jorgensen T, Ovesen L 2000 Thyroid disorders in mild iodine deficiency. Thyroid 10 (11):951-963.
18. Gaitan E 1990 Goitrogens in food and water. Annu Rev Nutr 10:21-39.
19. Bertelsen JB and Hegedus L 1994 Cigarette smoking and the thyroid. Thyroid 4 (3):327-331.
20. Bartalena L, Bogazzi F, Tanda ML, Manetti L, Dell'Unto E, Martino E 1995 Cigarette smoking and the thyroid. Eur J Endocrinol 133 (5):507-512.
21. Brix TH, Kyvik KO, Hegedus L 1999 Major role of genes in the etiology of simple goiter in females: a population-based twin study. J Clin Endocrinol Metab 84 (9):3071-3075.
22. Brix TH, Kyvik KO, Hegedus L 2000 A population-based study of chronic autoimmune hypothyroidism in Danish twins. J Clin Endocrinol Metab 85 (2):536-539.
23. Brix TH, Kyvik KO, Hegedus L 1998 What is the evidence of genetic factors in the etiology of Graves' disease? A brief review. Thyroid 8 (8):727-734.
24. Delange F 1995 Correction of iodine deficiency: benefits and possible side effects. Eur J Endocrinol 132 (5):542-543.
25. Delange F, de Benoist B, Pretell E, Dunn JT 2001 Iodine deficiency in the world: where do we stand at the turn of the century? Thyroid 11 (5):437-447.
26. Pfannenstiel P. Schilddrüsenreport. 1997. Darmstadt, Germany, Merck KGaA.
27. Pedersen KM, Nohr SB, Laurberg P 1997 Iodine intake in Denmark. Ugeskr Laeger 159 (15):2201-2206.
28. Munkner T, Hummer L, Sorensen SS 1977 Nuclear medical studies in congenital thyroid defects. Ugeskr Laeger 139 (41):2435-2439.
29. Knudsen N 2001 Regional variations in goitre prevalence in Denmark – and the impact of tobacco smoking. PhD thesis, available from the author at nils.knudsen@dadlnet.dk
30. Bulow Pedersen I, Knudsen N, Jorgensen T, Perrild H, Ovesen L, Laurberg P 2002 Large differences in incidences of overt hyper- and hypothyroidism associated with a small difference in iodine intake. A prospective comparative register based population survey. J Clin Endocrinol Metab 87 (10):4462-4469.
31. Lendvay J, Kuulasma J, Evans A, Molarius A, Ruokokoski E 1993 Qual-

ity assesment of smoking data in the second survey of the WHO MONICA project. MONICA Memo 247A
32. Molarius A, Kuulasma K, Evans A, McCrum E, Tolonen H 1999 Quality assessment of data on smoking behaviour in the WHO MONICA project. http://www.ktl.fi/publications/monica/smoking/qa30.htm
33. Bland JM and Altman DG 1999 Measuring agreement in method comparison studies. Stat Methods Med Res 8 (2):135-160.
34. Gutekunst R, Becker W, Hehrmann R, Olbricht T, Pfannenstiel P 1988 Ultraschalldiagnostik der Schilddruse. Dtsch Med Wochenschr 113 (27):1109-1112.
35. Hegedus L, Karstrup S, Rasmussen N 1986 Evidence of cyclic alterations of thyroid size during the menstrual cycle in healthy women. Am J Obstet Gynecol 155 (1):142-145.
36. Hill AB 1965 The environment and disease: association or causation? Proc R Soc Med 58:295-300.
37. Olsen J 2003 What characterises a useful concept of causation in epidemiology? J Epidemiol Community Health 57 (2):86-88.
38. Weed DL 1997 On the use of causal criteria. Int J Epidemiol 26 (6): 1137-1141.
39. Rasmussen LB, Ovesen L, Bulow I, Jorgensen T, Knudsen N, Laurberg P, Perrild H 2002 The relation between various measures of iodine intake and thyroid volume, thyroid nodularity and serum thyroglobulin. Am J Clin Nutr 76 (5):1069-1076.
40. Hegedus L, Rasmussen N, Ravn V, Kastrup J, Krogsgaard K, Aldershvile J 1988 Independent effects of liver disease and chronic alcoholism on thyroid function and size: the possibility of a toxic effect of alcohol on the thyroid gland. Metabolism 37 (3):229-233.
41. Greenland S. Applications of Stratified Analysis Methods. Rothman, J and Greenland, S. Modern Epidemiology. 2.[16], 281-300. 1998. Philadelphia, Lippincott-Raven Publishers.
42. Miettinen OS 1974 Proportion of disease caused or prevented by a given exposure, trait or intervention. Am J Epidemiol 99 (5):325-332.
43. Vought RL, London WT, Lutwak L, Dublin TD 1963 Reliability of Estimates of Serum Inorganic Iodine and Daily Fecal and Urinary Iodine Excretion from Single Casual Specimens. Clin Endocrinol Metab 23:1218-1228.
44. Rasmussen LB, Ovesen L, Bulow I, Jorgensen T, Knudsen N, Laurberg P, Perrild H 2001 Evaluation of a semi-quantitative food frequency questionnaire to estimate iodine intake. Eur J Clin Nutr 55 (4):287-292.
45. Rasmussen LB, Ovesen L, Christiansen E 1999 Day-to-day and within-day variation in urinary iodine excretion. Eur J Clin Nutr 53 (5):401-407.
46. Bourdoux P. Biochemical Evaluation of Iodine Status. Delange, F. Iodine Deficiency in Europe. 119-124. 1993. New York, Plenum Press.
47. Thomson CD, Smith TE, Butler KA, Packer MA 1996 An Evaluation of Urinary Measures of Iodine and Selenium Status. J Trace Elements med Biol 10:214-222.
48. Als C, Keller A, Minder C, Haldimann M, Gerber H 2000 Age- and gender-dependent urinary iodine concentrations in an area-covering population sample from the bernese region in switzerland [In Process Citation]. Eur J Endocrinol 143 (5):629-637.
49. Simpson FO, Nye ER, Bolli P, Waal MH, Goulding AW, Phelan EL, deHamel FA, Stewart RD, Spears GF, Leek GM, Stewart AC 1978 The Milton survey: Part 1, General methods, height, weight and 24-hour excretion of sodium, potassium, calcium, magnesium and creatinine. N Z Med J 87 (613):379-382.
50. Bleiler RE and Schedl HP 1962 Creatinine excretion: Variability and relationships to diet and body size. J Lab & Clin Med 59 (6):945-955.
51. Scott PJ and Hurley PJ 1968 Demonstration of individual variation in constancy of 24-hour urinary creatinine excretion. Clin Chim Acta 21 (3):411-414.
52. Greenblatt DJ, Ransil BJ, Harmatz JS, Smith TW, Duhme DW, Koch-Weser J 1976 Variability of 24-hour Urinary Creatinine by Normal Subjects. J Clin Pharmacol 16:321-328.
53. Kesteloot H and Joossens JV 1996 On the determinants of the creatinine clearance: a population study. J Hum Hypertens 10 (4):245-249.
54. Frey H 1986 The prevalence of hypothyroidism in the elderly [editorial]. Acta Med Scand 219 (2):145-146.
55. Konno N, Yuri K, Taguchi H, Miura K, Taguchi S, Hagiwara K, Murakami S 1993 Screening for thyroid diseases in an iodine sufficient area with sensitive thyrotrophin assays, and serum thyroid autoantibody and urinary iodide determinations. Clin Endocrinol Oxf 38 (3):273-281.
56. Bourdoux P 1998 Evaluation of the iodine intake: problems of the iodine/creatinine ratio--comparison with iodine excretion and daily fluctuations of iodine concentration. Exp Clin Endocrinol Diabetes 106 Suppl 3 (S17-S20.
57. Jolin T and Escobar del Ray F 1965 Evaluation of iodine/creatinine ratios of casual samples as indices of daily urinary iodine output during field studies. J Clin Endocrinol Metab 25:540-542.
58. Sackett-Lundeen L, Nicolau GY, Lakatua DJ, Ehresman DJ, Dumitriu L, Plinga L, Petrescu E, Haus E 1990 Circadian and seasonal variation in iodine excretion in children in an endemic goitre area. Chronobiology: Its Role in Clinical Medicine, General Biology, and Agriculture A:689-698.
59. Als C, Helbling A, Peter K, Haldimann M, Zimmerli B, Gerber H 2000 Urinary iodine concentration follows a circadian rhythm: a study with 3023 spot urine samples in adults and children. J Clin Endocrinol Metab 85 (4):1367-1369.
60. Brunn J, Block U, Ruf G, Bos I, Kunze WP, Scriba PC 1981 Volumetrie der Schilddrusenlappen mittels Real-time-Sonographie. Dtsch Med Wochenschr 106 (41):1338-1340.
61. Peterson S, Sanga A, Eklof H, Bunga B, Taube A, Gebre-Medhin M, Rosling H 2000 Classification of thyroid size by palpation and ultrasonography in field surveys. Lancet 355 (9198):106-110.
62. Siminoski K 1995 Does this patient have a goiter? JAMA 273 (10):813-817.
63. Zimmermann M, Saad A, Hess S, Torresani T, Chaouki N 2000 Thyroid ultrasound compared with World Health Organization 1960 and 1994 palpation criteria for determination of goiter prevalence in regions of mild and severe iodine deficiency. Eur J Endocrinol 143 (6):727-731.
64. Jarlov AE, Hegedus L, Gjorup T, Hansen JE 1991 Accuracy of the clinical assessment of thyroid size. Dan Med Bull 38 (1):87-89.
65. Tannahill AJ, Hooper MJ, England M, Ferriss JB, Wilson GM 1978 Measurement of thyroid size by ultrasound, palpation and scintiscan. Clin Endocrinol Oxf 8 (6):483-486.
66. Berghout A, Wiersinga WM, Smits NJ, Touber JL 1988 The value of thyroid volume measured by ultrasonography in the diagnosis of goitre. Clin Endocrinol Oxf 28 (4):409-414.
67. Gutekunst R, Smolarek H, Hasenpusch U, Stubbe P, Friedrich HJ, Wood WG, Scriba PC 1986 Goitre epidemiology: thyroid volume, iodine excretion, thyroglobulin and thyrotropin in Germany and Sweden. Acta Endocrinol Copenh 112 (4):494-501.
68. Smyth PP, Darke C, Parkes AB, Smith DF, John R, Hetherton AM, Lazarus JH 1999 Assessment of goiter in an area of endemic iodine deficiency. Thyroid 9 (9):895-901.
69. Brander A, Viikinkoski P, Tuuhea J, Voutilainen L, Kivisaari L 1992 Clinical versus ultrasound examination of the thyroid gland in common clinical practice. J Clin Ultrasound 20 (1):37-42.
70. Brown MC and Spencer R 1978 Thyroid gland volume estimated by use of ultrasound in addition to scintigraphy. Acta Radiol Oncol Radiat Phys Biol 17 (4):337-341.
71. Rasmussen SN and Hjorth L 1974 Determination of thyroid volume by ultrasonic scanning. J Clin Ultrasound 2 (2):143-147.
72. Hegedus L, Perrild H, Poulsen LR, Andersen JR, Holm B, Schnohr P, Jensen G, Hansen JM 1983 The determination of thyroid volume by ultrasound and its relationship to body weight, age, and sex in normal subjects. J Clin Endocrinol Metab 56 (2):260-263.
73. Yokoyama N, Nagayama Y, Kakezono F, Kiriyama T, Morita S, Ohtakara S, Okamoto S, Morimoto I, Izumi M, Ishikawa N, et a 1986 Determination of the Volume of the Thyroid Gland by a High Resolutional Ultrasonic Scanner. J Nucl Med 27 (9):1475-1479.
74. Nygaard B, Nygaard T, Court-Payen M, Jensen LI, Soe-Jensen P, Gerhard NK, Fugl M, Hegedus L 2002 Thyroid volume measured by ultrasonography and CT. Acta Radiol 43 (3):269-274.
75. Schlogl S, Werner E, Lassmann M, Terekhova J, Muffert S, Seybold S, Reiners C 2001 The use of three-dimensional ultrasound for thyroid volumetry. Thyroid 11 (6):569-574.
76. Szebeni A and Beleznay E 1992 New simple method for thyroid volume determination by ultrasonography. J Clin Ultrasound 20 (5):329-337.
77. Hampel R, Kulberg T, Klein K, Jerichow JU, Pichmann EG, Clausen V, Schmidt I 1995 Strumapravalenz in Deutschland grosser als bisher angenommen. Med Klin 90 (6):324-329.
78. Zimmermann MB, Molinari L, Spehl M, Weidinger-Toth J, Podoba J, Hess S, Delange F 2001 Toward a consensus on reference values for thyroid volume in iodine-replete schoolchildren: results of a workshop on inter-observer and inter-equipment variation in sonographic measurement of thyroid volume. Eur J Endocrinol 144 (3):213-220.
79. Scheler S, Becker W, Reiners C, Börner W 1986 Physiological Variations and Technical Variability in the Sonographic Measurement of Thyroid Volume. Akt Endokr Stoffw 7:40-42.
80. Vitti P, Martino E, Aghini LF, Rago T, Antonangeli L, Maccherini D, Nanni P, Loviselli A, Balestrieri A, Araneo G, et a 1994 Thyroid volume measurement by ultrasound in children as a tool for the assessment of mild iodine deficiency. J Clin Endocrinol Metab 79 (2):600-603.
81. Pedersen KM, Borlum KG, Knudsen PR, Hansen ES, Johannesen PL, Laurberg P 1988 Urinary iodine excretion is low and serum thyroglobulin high in pregnant women in parts of Denmark. Acta Obstet Gynecol Scand 67 (5):413-416.
82. Berghout A, Wiersinga WM, Smits NJ, Touber JL 1987 Determinants of thyroid volume as measured by ultrasonography in healthy adults in a non-iodine deficient area. Clin Endocrinol Oxf 26 (3):273-280.
83. Jarlov AE, Nygard B, Hegedus L, Karstrup S, Hansen JM 1993 Observer variation in ultrasound assessment of the thyroid gland. Br J Radiol 66 (787):625-627.

84. Pedersen OM, Aardal NP, Larssen TB, Varhaug JE, Myking O, Vik-Mo H 2000 The value of ultrasonography in predicting autoimmune thyroid disease. Thyroid 10 (3):251-259.
85. Hayashi N, Tamaki N, Konishi J, Yonekura Y, Senda M, Kasagi K, Yamamoto K, Iida Y, Misaki T, Endo K 1986 Sonography of Hashimoto's thyroiditis. J Clin Ultrasound 14 (2):123-126.
86. Vitti P, Lampis M, Piga M, Loviselli A, Brogioni S, Rago T, Pinchera A, Martino E 1994 Diagnostic usefulness of thyroid ultrasonography in atrophic thyroiditis. J Clin Ultrasound 22 (6):375-379.
87. Bennedbaek FN and Hegedus L 1997 The value of ultrasonography in the diagnosis and follow-up of subacute thyroiditis. Thyroid 7 (1):45-50.
88. Spencer CA, LoPresti JS, Fatemi S, Nicoloff JT 1999 Detection of residual and recurrent differentiated thyroid carcinoma by serum thyroglobulin measurement. Thyroid 9 (5):435-441.
89. Hidaka Y, Nishi I, Tamaki H, Takeoka K, Tada H, Mitsuda N, Amino N 1994 Differentiation of postpartum thyrotoxicosis by serum thyroglobulin: usefulness of a new multisite immunoradiometric assay. Thyroid 4 (3):275-278.
90. Mariotti S, Martino E, Cupini C, Lari R, Giani C, Baschieri L, Pinchera A 1982 Low serum thyroglobulin as a clue to the diagnosis of thyrotoxicosis factitia. N Engl J Med 307 (7):410-412.
91. Matsubara S, Inoh M, Tarumi Y, Sato M, Takahara J 1995 An outbreak (159 cases) of transient thyrotoxicosis without hyperthyroidism in Japan. Intern Med 34 (6):514-519.
92. Date J, Feldt-Rasmussen U, Blichert-Toft M, Hegedus L, Graversen HP 1996 Long-term observation of serum thyroglobulin after resection of nontoxic goiter and relation to ultrasonographically demonstrated relapse. World J Surg 20 (3):351-356.
93. Burgi U, Scazziga BR, Rosselet PO, Burgi H, Studer H 1988 Can serum thyroglobulin predict the effect of thyroid hormone therapy on goitre growth? Acta Endocrinol (Copenh) 119 (1):118-124.
94. Feldt-Rasmussen U, Hegedus L, Hansen JM, Perrild H 1984 Relationship between thyroid volume and serum thyroglobulin during long-term suppression with triiodothyronine in patients with diffuse nontoxic goitre. Acta Endocrinol (Copenh) 105 (2):184-189.
95. Pacini F, Pinchera A, Giani C, Grasso L, Doveri F, Baschieri L 1980 Serum thyroglobulin in thyroid carcinoma and other thyroid disorders. J Endocrinol Invest 3 (3):283-292.
96. Feldt-Rasmussen U, Bech K, Date J 1979 Serum thyroglobulin in patients with toxic and non-toxic goitres compared to sex- and age-matched control subjects. Acta Endocrinol (Copenh) 91 (2):264-270.
97. Berghout A, Wiersinga WM, Smits NJ, Touber JL 1990 Interrelationships Between Age, Thyroid Volume, Thyroid Nodularity, and Thyroid Function in Patients with Sporadic Nontoxic Goiter. Am J Med 89 (5):602-608.
98. Pezzino V, Vigneri R, Squatrito S, Filetti S, Camus M, Polosa P 1978 Increased serum thyroglobulin levels in patients with nontoxic goiter. J Clin Endocrinol Metab 46 (4):653-657.
99. Szabolcs I, Bernard W, Horster FA 1994 Serum thyroglobulin in hospitalized chronic geriatric patients: its relationship to age, non-thyroidal illness, goitre and thyroid dysfunction in a follow-up study. Eur J Endocrinol 131 (5):462-466.
100. Shlossberg AH, Jacobson JC, Ibbertson HK 1979 Serum thyroglobulin in the diagnosis and management of thyroid carcinoma. Clin Endocrinol (Oxf) 10 (1):17-27.
101. Gartner R, Hainzinger A, Horn K, Pickardt RC 1983 Evidence for autonomous thyroglobulin release from euthyroid and hyperthyroid nodular goitre – thyroglobulin, a possible helpful parameter in diagnosis of non-malignant thyroid disorders. Klin Wochenschr 61 (15):737-741.
102. Gerfo PL, Colacchio T, Colacchio D, Feind C 1979 Thyroglobulin in benign and malignant thyroid disease. JAMA 241 (9):923-924.
103. Torrigiani G, Doniach D, Roitt IM 1969 Serum thyroglobulin levels in healthy subjects and in patients with thyroid disease. J Clin Endocrinol Metab 29 (3):305-314.
104. Tomimori E, Pedrinola F, Cavaliere H, Knobel M, Medeiros-Neto G. 1995 Prevalence of incidental thyroid disease in a relatively low iodine intake area. Thyroid 5 (4):273-276.
105. Feldt-Rasmussen U, Hegedus L, Perrild H, Rasmussen N, Hansen JM 1989 Relationship between serum thyroglobulin, thyroid volume and serum TSH in healthy non-goitrous subjects and the relationship to seasonal variations in iodine intake. Thyroidology 1 (3):115-118.
106. Hintze G, Windeler J, Baumert J, Stein H, Kobberling J 1991 Thyroid volume and goitre prevalence in the elderly as determined by ultrasound and their relationships to laboratory indices. Acta Endocrinol Copenh 124 (1):12-18.
107. Feldt-Rasmussen U 1983 Serum thyroglobulin and thyroglobulin autoantibodies in thyroid diseases. Pathogenic and diagnostic aspects. Allergy 38 (6):369-387.
108. Van Herle AJ, Vassart G, Dumont JE 1979 Control of thyroglobulin synthesis and secretion (second of two parts). N Engl J Med 301 (6):307-314.
109. Madeddu G, Casu AR, Marrosu A, Marras G, Langer M 1984 Serum thyroglobulin in patients with autonomous thyroid nodules. Clin Endocrinol (Oxf) 21 (4):377-382.
110. Fenzi GF, Ceccarelli C, Macchia E, Monzani F, Bartalena L, Giani C, Ceccarelli P, Lippi F, Baschieri L, Pinchera A 1985 Reciprocal changes of serum thyroglobulin and TSH in residents of a moderate endemic goitre area. Clin Endocrinol (Oxf) 23 (2):115-122.
111. Nakamura S, Sakata S, Minamori Y, Komaki T, Kojima N, Kamikubo K, Yasuda K, Miura K 1984 Serum thyroglobulin (Tg) concentration in healthy subjects: absence of age- and sex-related differences. Endocrinol Jpn 31 (1):93-98.
112. Van Herle AJ, Vassart G, Dumont JE 1979 Control of thyroglobulin synthesis and secretion. (First of two parts). N Engl J Med 301 (5):239-249.
113. Torres MS, Ramirez L, Simkin PH, Braverman LE, Emerson CH 2001 Effect of various doses of recombinant human thyrotropin on the thyroid radioactive iodine uptake and serum levels of thyroid hormones and thyroglobulin in normal subjects. J Clin Endocrinol Metab 86 (4):1660-1664.
114. Van Herle AJ, Hershman JM, Hornabrook RW, Chopra IJ 1976 Serum thyroglobulin in inhabitants of an endemic goiter region of New Guinea. J Clin Endocrinol Metab 43 (3):512-516.
115. Lima N, Knobel M, Medeiros-Neto G 1986 Long-term effect of iodized oil on serum thyroglobulin levels in endemic goitre patients. Clin Endocrinol (Oxf) 24 (6):635-641.
116. Missler U, Gutekunst R, Wood WG 1994 Thyroglobulin is a more sensitive indicator of iodine deficiency than thyrotropin: development and evaluation of dry blood spot assays for thyrotropin and thyroglobulin in iodine-deficient geographical areas. Eur J Clin Chem Clin Biochem 32 (3):137-143.
117. Premawardhana LD, Lo SS, Phillips DI, Prentice LM, Rees SB 1994 Variability of serum thyroglobulin levels is determined by a major gene. Clin Endocrinol (Oxf) 41 (6):725-729.
118. Fukayama H, Nasu M, Murakami S, Sugawara M 1992 Examination of antithyroid effects of smoking products in cultured thyroid follicles: only thiocyanate is a potent antithyroid agent. Acta Endocrinol (Copenh) 127 (6):520-525.
119. Dunn JT 1996 Seven deadly sins in confronting endemic iodine deficiency, and how to avoid them. J Clin Endocrinol Metab 81 (4):1332-1335.
120. Buchinger W, Lorenz-Wawschinek O, Semlitsch G, Langsteger W, Binter G, Bonelli RM, Eber O 1997 Thyrotropin and thyroglobulin as an index of optimal iodine intake: correlation with iodine excretion of 39, 913 euthyroid patients. Thyroid 7 (4):593-597.
121. Laurberg P, Pedersen KM, Hreidarsson A, Sigfusson N, Iversen E, Knudsen PR 1998 Iodine intake and the pattern of thyroid disorders: a comparative epidemiological study of thyroid abnormalities in the elderly in Iceland and in Jutland, Denmark. J Clin Endocrinol Metab 83 (3):765-769.
122. Szabolcs I, Podoba J, Feldkamp J, Dohan O, Farkas I, Sajgo M, Takats KI, Goth M, Kovacs L, Kressinszky K, Hnilica P, Szilagyi G 1997 Comparative screening for thyroid disorders in old age in areas of iodine deficiency, long-term iodine prophylaxis and abundant iodine intake. Clin Endocrinol Oxf 47 (1):87-92.
123. Laurberg P, Bulow P, I, Knudsen N, Ovesen L, Andersen S 2001 Environmental iodine intake affects the type of nonmalignant thyroid disease. Thyroid 11 (5):457-469.
124. Suzuki H, Higuchi T, Sawa K, Ohtaki S, Horiuchi Y 1965 "Endemic coast goitre" in Hokkaido, Japan. Acta Endocrinol (Copenh) 50(2):161-176.
125. Zhao J, Wang P, Shang L, Sullivan KM, van der HF, Maberly G 2000 Endemic goiter associated with high iodine intake. Am J Public Health 90 (10):1633-1635.
126. Li M, Liu DR, Qu CY, Zhang PY, Qian QD, Zhang CD, Jia QZ, Wang HX, Eastman CJ, Boyages SC 1987 Endemic goitre in central China caused by excessive iodine intake. Lancet 2 (8553):257-259.
127. Olbricht T and Hoff HG 1988 Factors affecting thyroid gland volume. A contribution to the epidemiology of goiter. Med Klin 83 (8-9):279-284.
128. Barrere X, Valeix P, Preziosi P, Bensimon M, Pelletier B, Galan P, Hercberg S 2000 Determinants of thyroid volume in healthy French adults participating in the SU.VI.MAX cohort. Clin Endocrinol (Oxf) 52 (3):273-278.
129. Gomez JM, Maravall FJ, Gomez N, Guma A, Soler J 2000 Determinants of thyroid volume as measured by ultrasonography in healthy adults randomly selected. Clin Endocrinol (Oxf) 53 (5):629-634.
130. Olbricht T, Schmitka T, Mellinghoff U, Benker G, Reinwein D 1983 Sonographische Bestimmung von Schilddrusenvolumina bei Schilddrusengesunden. Dtsch Med Wochenschr 108 (36):1355-1358.
131. Nygaard B, Gideon P, Dige-Petersen H., Jespersen N, Solling K, Veje A 1993 Thyroid volume and morphology and urinary iodine excretion in a Danish municipality. Acta Endocrinol Copenh 129 (6):505-510.
132. Schaaf L, Pohl T, Schmidt R, Vardali I, Teuber J, Schlote SB, Nowotny B, Schiebeler H, Zober A, Usadel KH 1993 Screening for thyroid disorders in a working population. Clin Investig 71 (2):126-131.

133. Wesche MF, Wiersinga WM, Smits NJ 1998 Lean body mass as a determinant of thyroid size. Clin Endocrinol (Oxf) 48 (6):701-706.
134. Aghini-Lombardi F, Antonangeli L, Martino E, Vitti P, Maccherini D, Leoli F, Rago T, Grasso L, Valeriano R, Balestrieri A, Pinchera A 1999 The spectrum of thyroid disorders in an iodine-deficient community: the Pescopagano survey. J Clin Endocrinol Metab 84 (2):561-566.
135. Tunbridge WM, Evered DC, Hall R, Appleton D, Brewis M, Clark F, Evans JG, Young E, Bird T, Smith PA 1977 The spectrum of thyroid disease in a community: the Whickham survey. Clin Endocrinol Oxf 7 (6):481-493.
136. Matovinovich J, Hayner NS, Epstein FH, Kjelsberg MO 1965 Goiter and Other Thyroid Diseases in Tecumseh, Michigan. JAMA 192 (3):134-140.
137. Ericsson UB and Lindgarde F 1990 The epidemiology of thyroid disorders in a seaport community in southern Sweden. J Clin Epidemiol 43 (7):645-650.
138. Struve C and Hinrichs J 1989 Schilddrusenvolumina und Haufigkeit herdformiger Veranderungen bei schilddrusengesunden Mannern und Frauen verschiedener Altersklassen. Dtsch Med Wochenschr 114 (8):283-287.
139. Andersen-Ranberg K, Jeune B, Hoier-Madsen M, Hegedus L 1999 Thyroid function, morphology and prevalence of thyroid disease in a population-based study of Danish centenarians. J Am Geriatr Soc 47 (10):1238-1243.
140. Trowbridge FL, Hand KE, Nichaman MZ 1975 Findings relating to goiter and iodine in the Ten-State Nutrition Survey. Am J Clin Nutr 28 (7):712-716.
141. Woestyn J, Afschrift M, Schelstraete K, Vermeulen A 1985 Demonstration of nodules in the normal thyroid by echography. Br J Radiol 58:1179-1182.
142. Miki H, Oshimo K, Inoue H, Kawano M, Tanaka K, Komaki K, Uyama T, Morimoto T, Monden Y 1993 Incidence of ultrasonographically-detected thyroid nodules in healthy adults. Tokushima J Exp Med 40 (1-2):43-46.
143. Brander A, Viikinkoski P, Nickels J, Kivisaari L 1989 Thyroid Gland: US Screening in Middle-aged Women with No Previous Thyroid Disease. Radiology 173 (2):507-510.
144. Ezzat S, Sarti DA, Cain DR, Braunstein GD 1994 Thyroid incidentalomas. Prevalence by palpation and ultrasonography. Arch Intern Med 154 (16):1838-1840.
145. Carroll BA 1982 Asymptomatic Thyroid Nodules: Incidental Sonographic Detection. AJR Am J Roentgenol 138 (3):499-501.
146. Mazzaferri EL, de-los-Santos ET, Rofagha KS 1988 Solitary thyroid nodule: diagnosis and management. Med Clin North Am 72 (5):1177-1211.
147. Stark DD, Clark OH, Gooding GA, Moss AA 1983 High-resolution ultrasonography and computed tomography of thyroid lesons in patients with hyperparathyroidism. Surgery 94 (6):863-868.
148. Brander AE, Viikinkoski VP, Nickels JI, Kivisaari LM 2000 Importance of thyroid abnormalities detected at US screening: a 5-year follow-up. Radiology 215 (3):801-806.
149. Papini E, Guglielmi R, Bianchini A, Crescenzi A, Taccogna S, Nardi F, Panunzi C, Rinaldi R, Toscano V, Pacella CM 2002 Risk of malignancy in nonpalpable thyroid nodules: predictive value of ultrasound and color-Doppler features. J Clin Endocrinol Metab 87 (5):1941-1946.
150. Tan GH and Gharib H 1997 Thyroid incidentalomas: management approaches to nonpalpable nodules discovered incidentally on thyroid imaging. Ann Intern Med 126 (3):226-231.
151. Leenhardt L, Hejblum G, Franc B, Fediaevsky LD, Delbot T, Le Guillouzic D, Menegaux F, Guillausseau C, Hoang C, Turpin G, Aurengo A 1999 Indications and limits of ultrasound-guided cytology in the management of nonpalpable thyroid nodules. J Clin Endocrinol Metab 84 (1):24-28.
152. Belfiore A, La Rosa GL, Padova G, Sava L, Ippolito O, Vigneri R 1987 The frequency of cold thyroid nodules and thyroid malignancies in patients from an iodine-deficient area. Cancer 60 (12):3096-3102.
153. Sprogel P and Storm HH 1989 Thyroid cancer: incidence, mortality and histological pattern in Denmark. Int J Epidemiol 18 (4):990-992.
154. Chopra IJ, Hershman JM, Hornabrook RW 1975 Serum thyroid hormone and thyrotropin levels in subjects from endemic goiter regions of New Guinea. J Clin Endocrinol Metab 40 (2):326-333.
155. Hansen PS, Brix TH, Sorensen TI, Kyvik KO, Hegedus L 2004 Major genetic influence on the regulation of the pituitary-thyroid axis: a study of healthy Danish twins. J Clin Endocrinol Metab 89 (3):1181-1187.
156. Brix TH, Kyvik KO, Christensen K, Hegedus L 2001 Evidence for a major role of heredity in Graves' disease: a population-based study of two Danish twin cohorts. J Clin Endocrinol Metab 86 (2):930-934.
157. Seck T, Scheidt NC, Ziegler R, Pfeilschifter J 1997 Prevalence of thyroid gland dysfunctions in 50- to 80-year-old patients. An epidemiologic cross-sectional study in a southwestern community. Med Klin 92 (11):642-646.
158. Eggertsen R, Petersen K, Lundberg PA, Nystrom E, Lindstedt G 1988 Screening for thyroid disease in a primary care unit with a thyroid stimulating hormone assay with a low detection limit. BMJ 297 (6663):1586-1592.
159. Sawin CT, Castelli WP, Hershman JM, McNamara P, Bacharach P 1985 The aging thyroid. Thyroid deficiency in the Framingham Study. Arch Intern Med 145 (8):1386-1388.
160. Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, Braverman LE 2002 Serum TSH, T (4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab 87 (2):489-499.
161. Okamura K, Nakashima T, Ueda K, Inoue K, Omae T, Fujishima M 1987 Thyroid Disorders in the General Population of Hisayama Japan, with Special Reference to Prevalence and Sex Differences. Int J Epidemiol 16 (4):545-549.
162. Vanderpump MP, Tunbridge WM, French JM, Appleton D, Bates D, Clark F, Grimley EJ, Hasan DM, Rodgers H, Tunbridge F, et a 1995 The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey. Clin Endocrinol Oxf 43 (1):55-68.
163. Demers LM and Spencer CA (Editors) 2003 Laboratory Medicine Practice Guidelines for the Diagnosis and Monitoring of Thyroid Disease. Thyroid 13 (1):36-
164. Roberts CG and Ladenson PW 2004 Hypothyroidism. Lancet 363 (9411):793-803.
165. Bjoro T, Holmen J, Kruger O, Midthjell K, Hunstad K, Schreiner T, Sandnes L, Brochmann H 2000 Prevalence of thyroid disease, thyroid dysfunction and thyroid peroxidase antibodies in a large, unselected population. The health study of nord-trondelag (HUNT). Eur J Endocrinol 143 (5):639-647.
166. Laurberg P, Bulow P, I, Pedersen KM, Vestergaard H 1999 Low incidence rate of overt hypothyroidism compared with hyperthyroidism in an area with moderately low iodine intake. Thyroid 9 (1):33-38.
167. Laurberg P, Pedersen KM, Vestergaard H, Sigurdsson G 1991 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. J Intern Med 229 (5):415-420.
168. Petersen K, Lindstedt G, Lundberg PA, Bengtsson C, Lapidus L, Nystrom E 1991 Thyroid disease in middle-aged and elderly Swedish women: thyroid-related hormones, thyroid dysfunction and goitre in relation to age and smoking. J Intern Med 229 (5):407-413.
169. Roti E, Gardini E, D'Amato L, Salvi M, Robuschi G, Manfredi A, Dallara G, Pino S, Guazzi AM, Gnudi A 1986 Goiter size and thyroid function in an endemic goiter area in northern Italy. J Clin Endocrinol Metab 63 (3):558-563.
170. Laurberg P, Pedersen KM, Vestergaard P, Vestergaard H 1991 Hyperfunctioning thyroid nodules. Thyroidology 3 (1):1-6.
171. Tonacchera M and Pinchera A 2000 Thyrotropin receptor polymorphisms and thyroid diseases. J Clin Endocrinol Metab 85 (8):2637-2639.
172. Derwahl M and Studer H 2001 Nodular goiter and goiter nodules: Where iodine deficiency falls short of explaining the facts. Exp Clin Endocrinol Diabetes 109 (5):250-260.
173. Tonacchera M, Agretti P, Chiovato L, Rosellini V, Ceccarini G, Perri A, Viacava P, Naccarato AG, Miccoli P, Pinchera A, Vitti P 2000 Activating thyrotropin receptor mutations are present in nonadenomatous hyperfunctioning nodules of toxic or autonomous multinodular goiter. J Clin Endocrinol Metab 85 (6):2270-2274.
174. Tonacchera M, Vitti P, Agretti P, Giulianetti B, Mazzi B, Cavaliere R, Ceccarini G, Fiore E, Viacava P, Naccarato A, Pinchera A, Chiovato L 1998 Activating thyrotropin receptor mutations in histologically heterogeneous hyperfunctioning nodules of multinodular goiter. Thyroid 8 (7):559-564.
175. Tonacchera M, Chiovato L, Pinchera A, Agretti P, Fiore E, Cetani F, Rocchi R, Viacava P, Miccoli P, Vitti P 1998 Hyperfunctioning thyroid nodules in toxic multinodular goiter share activating thyrotropin receptor mutations with solitary toxic adenoma. J Clin Endocrinol Metab 83 (2):492-498.
176. Derwahl M, Hamacher C, Russo D, Broecker M, Manole D, Schatz H, Kopp P, Filetti S 1996 Constitutive activation of the Gs alpha protein-adenylate cyclase pathway may not be sufficient to generate toxic thyroid adenomas. J Clin Endocrinol Metab 81 (5):1898-1904.
177. Corvilain B, Van Sande J, Dumont JE, Bourdoux P, Ermans AM 1998 Autonomy in endemic goiter. Thyroid 8 (1):107-113.
178. Derwahl M and Studer H 2001 Nodular goiter and goiter nodules: Where iodine deficiency falls short of explaining the facts. Exp Clin Endocrinol Diabetes 109 (5):250-260.
179. Robuschi G, Safran M, Braverman LE, Gnudi A, Roti E 1987 Hypothyroidism in the elderly. Endocr Rev 8 (2):142-153.
180. Bulow Pedersen I, Knudsen N, Jorgensen T, Perrild H, Ovesen L, Laurberg P 2002 Thyroid peroxidase and thyroglobulin autoantibodies in a large survey of populations with mild and moderate iodine deficiency. Clin Endocrinol (Oxf) 58 (1):36-42.

181. Konno N, Makita H, Yuri K, Iizuka N, Kawasaki K 1994 Association between dietary iodine intake and prevalence of subclinical hypothyroidism in the coastal regions of Japan. J Clin Endocrinol Metab 78 (2):393-397.
182. Tajiri J, Higashi K, Morita M, Umeda T, Sato T 1986 Studies of hypothyroidism in patients with high iodine intake. J Clin Endocrinol Metab 63 (2):412-417.
183. Prummel MF and Wiersinga WM 1993 Smoking and risk of Graves' disease. JAMA 269 (4):479-482.
184. Bartalena L, Martino E, Marcocci C, Bogazzi F, Panicucci M, Velluzzi F, Loviselli A, Pinchera A 1989 More on smoking habits and Graves' ophthalmopathy. J Endocrinol Invest 12 (10):733-737.
185. Hegedus L, Karstrup S, Veiergang D, Jacobsen B, Skovsted L, Feldt-Rasmussen U 1985 High frequency of goitre in cigarette smokers. Clin Endocrinol (Oxf) 22 (3):287-292.
186. Ericsson UB and Lindgarde F 1991 Effects of cigarette smoking on thyroid function and the prevalence of goitre, thyrotoxicosis and autoimmune thyroiditis. J Intern Med 229 (1):67-71.
187. Christensen SB, Ericsson UB, Janzon L, Tibblin S, Melander A 1984 Influence of Cigarette Smoking on Goiter Formation, Thyroglobulin, and Thyroid Hormone Levels in Women. J Clin Endocrinol Metab 58 (4):615-618.
188. Brix TH, Hansen PS, Kyvik KO, Hegedus L 2000 Cigarette smoking and risk of clinically overt thyroid disease: a population-based twin case-control study. Arch Intern Med 160 (5):661-666.
189. Georgiadis E, Papapostolou C, Korakis T, Evagelopoulou K, Mantzoros C, Batrinos M 1997 The influence of smoking habits on thyroid gland volume: an ultrasonic approach. J R Soc Health 117 (6):355-358.
190. Chen YL, Chang TC, Chen CJ 1994 Influence of smoking on Graves' disease with or without ophthalmopathy and nontoxic nodular goiter in Taiwan. J Formos Med Assoc 93 (1):40-44.
191. Lio S, Napolitano G, Marinuzzi G, Monaco F 1989 Role of smoking in goiter morphology and thyrotropin response to TRH in untreated goitrous women. J Endocrinol Invest 12 (2):93-97.
192. Rossing MA, Cushing KL, Voigt LF, Wicklund KG, Daling JR 2000 Risk of papillary thyroid cancer in women in relation to smoking and alcohol consumption. Epidemiology 11 (1):49-54.
193. Galanti MR, Hansson L, Lund E, Bergstrom R, Grimelius L, Stalsberg H, Carlsen E, Baron JA, Persson I, Ekbom A 1996 Reproductive history and cigarette smoking as risk factors for thyroid cancer in women: a population-based case-control study. Cancer Epidemiol Biomarkers Prev 5 (6):425-431.
194. Eden S, Jagenburg R, Lindstedt G, Lundberg PA, Mellstrom D 1984 Thyroregulatory changes associated with smoking in 70-year-old men. Clin Endocrinol (Oxf) 21 (6):605-610.
195. Melander A, Nordenskjold E, Lundh B, Thorell J 1981 Influence of smoking on thyroid activity. Acta Med Scand 209 (1-2):41-43.
196. Fisher CL, Mannino DM, Herman WH, Frumkin H 1997 Cigarette smoking and thyroid hormone levels in males. Int J Epidemiol 26 (5):972-977.
197. Karakaya A, Tuncel N, Alptuna G, Kocer Z, Erbay G 1987 Influence of cigarette smoking on thyroid hormone levels. Hum Toxicol 6 (6):507-509.
198. Sepkovic DW, Haley NJ, Wynder EL 1984 Thyroid activity in cigarette smokers. Arch Intern Med 144 (3):501-503.
199. Vestergaard P, Rejnmark L, Weeke J, Hoeck HC, Nielsen HK, Rungby J, Laurberg P, Mosekilde L 2002 Smoking as a risk factor for Graves' disease, toxic nodular goiter, and autoimmune hypothyroidism. Thyroid 12 (1):69-75.
200. Vestergaard P 2002 Smoking and thyroid disorders--a meta-analysis. Eur J Endocrinol 146 (2):153-161.
201. Williams ED, Doniach I, Bjarnason O, Michie W 1977 Thyroid cancer in an iodide rich area: a histopathological study. Cancer 39 (1):215-222.
202. Colzani R, Fang SL, Alex S, Braverman LE 1998 The effect of nicotine on thyroid function in rats. Metabolism 47 (2):154-157.
203. Melander A, Westgren U, Ericson LE, Sundler F 1977 Influence of the sympathetic nervous system on the secretion and metabolism of thyroid hormone. Endocrinology 101 (4):1228-1237.
204. Hart P, Farrell GC, Cooksley WG, Powell LW 1976 Enhanced drug metabolism in cigarette smokers. Br Med J 2 (6028):147-149.
205. Metcalfe RA and Weetman AP 1994 Stimulation of extraocular muscle fibroblasts by cytokines and hypoxia: possible role in thyroid-associated ophthalmopathy. Clin Endocrinol (Oxf) 40 (1):67-72.
206. Hegedus L 1984 Decreased thyroid gland volume in alcoholic cirrhosis of the liver. J Clin Endocrinol Metab 58 (5):930-933.
207. Burke V, Puddey IB, Beilin LJ 1995 Mortality associated with wines, beers, and spirits. Australian data suggest that choice of beverage relates to lifestyle and personality. BMJ 311 (7013):1166-
208. Tjonneland A, Gronbaek M, Stripp C, Overvad K 1999 Wine intake and diet in a random sample of 48763 Danish men and women. Am J Clin Nutr 69 (1):49-54.
209. Klatsky AL, Armstrong MA, Kipp H 1990 Correlates of alcoholic beverage preference: traits of persons who choose wine, liquor or beer. Br J Addict 85 (10):1279-1289.
210. Rasmussen LB, Larsen EH, Ovesen L 2000 Iodine content in drinking water and other beverages in Denmark. Eur J Clin Nutr 54 (1):57-60.
211. Clark OH and Gerend PL 1986 Effect of ethyl alcohol on the TSH-receptor-cyclase system in thyroid and nonthyroid tissues. World J Surg 10 (5):787-796.
212. Hegedus L, Kastrup J, Feldt-Rasmussen U, Petersen PH 1983 Serum thyroglobulin in acute and chronic liver disease. Clin Endocrinol (Oxf) 19 (2):231-237.
213. Noach EL 1955 Influence of oestrogens on thyroid function (1). Acta Endocrinol (Copenh) 19:127-138.
214. Furlanetto TW, Nguyen LQ, Jameson JL 1999 Estradiol increases proliferation and down-regulates the sodium/iodide symporter gene in FRTL-5 cells. Endocrinology 140 (12):5705-5711.
215. del Senno L, degli UE, Hanau S, Piva R, Rossi R, Trasforini G 1989 In vitro effects of estrogen on tgb and c-myc gene expression in normal and neoplastic human thyroids. Mol Cell Endocrinol 63 (1-2):67-74.
216. Rumsey TS, Elsasser TH, Kahl S 1997 Roasted soybeans and an estrogenic growth promoter affect the thyroid status of beef steers. J Nutr 127 (2):352-358.
217. Kuhl H, Gahn G, Romberg G, Althoff PH, Taubert HD 1985 A randomized cross-over comparison of two low-dose oral contraceptives upon hormonal and metabolic serum parameters: II. Effects upon thyroid function, gastrin, STH, and glucose tolerance. Contraception 32 (1):97-107.
218. Zaninovich AA, Boado R, Ulloa E, Bromage NR, Matty AJ 1982 Inhibition of thyroidal iodine release by oestrogens in euthyroid subjects. Acta Endocrinol (Copenh) 99 (3):386-392.
219. Weeke J and Hansen AP 1975 Serum TSH and serum T3 levels during normal menstrual cycles and during cycles on oral contraceptives. Acta Endocrinol (Copenh) 79 (3):431-438.
220. Muller AF, Verhoeff A, Mantel MJ, De Jong FH, Berghout A 2000 Decrease of free thyroxine levels after controlled ovarian hyperstimulation. J Clin Endocrinol Metab 85 (2):545-548.
221. Frank P and Kay CR 1978 Incidence of thyroid disease associated with oral contraceptives. Br Med J 2 (6151):1531.
222. Wong FL, Ron E, Gierlowski T, Schneider AB 1996 Benign thyroid tumors: general risk factors and their effects on radiation risk estimation. Am J Epidemiol 144 (8):728-733.
223. Strieder TG, Prummel MF, Tijssen JG, Endert E, Wiersinga WM 2003 Risk factors for and prevalence of thyroid disorders in a cross-sectional study among healthy female relatives of patients with autoimmune thyroid disease. Clin Endocrinol (Oxf) 59 (3):396-401.
224. Sorger D, Schenk S, Schneider G 1992 Effects of various contraceptives on laboratory parameters in diagnosis of thyroid gland function with special reference to the free hormones FT4 and FT3. Z Gesamte Inn Med 47 (2):58-64.
225. Wang R, Nelson JC, Weiss RM, Wilcox RB 2000 Accuracy of free thyroxine measurements across natural ranges of thyroxine binding to serum proteins. Thyroid 10 (1):31-39.
226. Lisboa PC, Curty FH, Moreira RM, Pazos-Moura CC 1997 Effects of estradiol benzoate on 5'-iodothyronine deiodinase activities in female rat anterior pituitary gland, liver and thyroid gland. Braz J Med Biol Res 30 (12):1479-1484.
227. Rossi R, Zatelli MC, Franceschetti P, Maestri I, Magri E, Aguiari G, Cavazzini P, degli Uberti EC, del Senno L 1996 Inhibitory effect of dihydrotestosterone on human thyroid cell growth. J Endocrinol 151 (2):185-194.
228. Casson PR, Elkind-Hirsch KE, Buster JE, Hornsby PJ, Carson SA, Snabes MC 1997 Effect of postmenopausal estrogen replacement on circulating androgens. Obstet Gynecol 90 (6):995-998.
229. Furlanetto TW, Nunes RB, Sopelsa AM, Maciel RM 2001 Estradiol decreases iodide uptake by rat thyroid follicular FRTL-5 cells. Braz J Med Biol Res 34 (2):259-263.
230. Ramey JN, Burrow GN, Polackwich RJ, Donabedian RK 1975 The effect of oral contraceptive steroids on the response of thyroid-stimulating hormone to thyrotropin-releasing hormone. J Clin Endocrinol Metab 40 (4):712-714.
231. Rey-Stocker I, Zufferey MM, Lemarchand MT, Rais M 1981 The sensibility of the hypophysis, the gonads and the thyroid of adolescents before and after the administration of oral contraceptives. A resume. Pediatr Ann 10 (12):15-20.
232. Arafah BM 2001 Increased need for thyroxine in women with hypothyroidism during estrogen therapy. N Engl J Med 344 (23):1743-1749.
233. Banu KS, Govindarajulu P, Aruldhas MM 2001 Testosterone and estradiol modulate TSH-binding in the thyrocytes of Wistar rats: influence of age and sex. J Steroid Biochem Mol Biol 78 (4):329-342.
234. Wang SH, Myc A, Koenig RJ, Bretz JD, Arscott PL, Baker JR 2000 2-Methoxyestradiol, an endogenous estrogen metabolite, induces thyroid cell apoptosis. Mol Cell Endocrinol 165 (1-2):163-172.
235. Carayon P, Lefort G, Nisula B 1980 Interaction of human chorionic go-

nadotropin and human luteinizing hormone with human thyroid membranes. Endocrinology 106 (6):1907-1916.
236. Williams JF, Davies TF, Catt KJ, Pierce JG 1980 Receptor-binding activity of highly purified bovine luteinizing hormone and thyrotropin, and their subunits. Endocrinology 106 (5):1353-1359.
237. Taliadouros GS, Canfield RE, Nisula BC 1978 Thyroid-stimulating activity of chorionic gonadotropin and luteinizing hormone. J Clin Endocrinol Metab 47 (4):855-860.
238. Glinoer D 1999 What happens to the normal thyroid during pregnancy? Thyroid 9 (7):631-635.
239. Balen AH, Tan SL, Jacobs HS 1993 Hypersecretion of luteinising hormone: a significant cause of infertility and miscarriage. Br J Obstet Gynaecol 100 (12):1082-1089.
240. Fleury Y, Van Melle GG, Woringer V, Gaillard RC, Portmann L 2001 Sex-Dependent Variations and Timing of Thyroid Growth during Puberty. J Clin Endocrinol Metab 86 (2):750-754.
241. Glinoer D, De Nayer P, Bourdoux P, Lemone M, Robyn C, van Steirteghem A, Kinthaert J, Lejeune B 1990 Regulation of maternal thyroid during pregnancy. J Clin Endocrinol Metab 71 (2):276-287.
242. Smyth PP, Hetherton AM, Smith DF, Radcliff M, O'Herlihy C 1997 Maternal iodine status and thyroid volume during pregnancy: correlation with neonatal iodine intake. J Clin Endocrinol Metab 82 (9):2840-2843.
243. Rasmussen NG, Hornnes PJ, Hegedus L 1989 Ultrasonographically determined thyroid size in pregnancy and post partum: the goitrogenic effect of pregnancy. Am J Obstet Gynecol 160 (5 Pt 1):1216-1220.
244. Romano R, Jannini EA, Pepe M, Grimaldi A, Olivieri M, Spennati P, Cappa F, D'Armiento M 1991 The effects of iodoprophylaxis on thyroid size during pregnancy. Am J Obstet Gynecol 164 (2):482-485.
245. Glinoer D, De Nayer P, Delange F, Lemone M, Toppet V, Spehl M, Grun JP, Kinthaert J, Lejeune B 1995 A randomized trial for the treatment of mild iodine deficiency during pregnancy: maternal and neonatal effects. J Clin Endocrinol Metab 80 (1):258-269.
246. Pedersen KM, Laurberg P, Iversen E, Knudsen PR, Gregersen HE, Rasmussen OS, Larsen KR, Eriksen GM, Johannesen PL 1993 Amelioration of some pregnancy-associated variations in thyroid function by iodine supplementation. J Clin Endocrinol Metab 77 (4):1078-1083.
247. Kung AW, Lao TT, Chau MT, Tam SC, Low LC 2000 Goitrogenesis during pregnancy and neonatal hypothyroxinaemia in a borderline iodine sufficient area. Clin Endocrinol (Oxf) 53 (6):725-731.
248. Nelson M, Wickus GG, Caplan RH, Beguin EA 1987 Thyroid gland size in pregnancy. An ultrasound and clinical study. J Reprod Med 32 (12):888-890.
249. Berghout A, Endert E, Ross A, Hogerzeil HV, Smits NJ, Wiersinga WM 1994 Thyroid function and thyroid size in normal pregnant women living in an iodine replete area. Clin Endocrinol (Oxf) 41 (3):375-379.
250. Long TJ, Felice ME, Hollingsworth DR 1985 Goiter in pregnant teenagers. Am J Obstet Gynecol 152 (6 Pt 1):670-674.
251. Levy RP, Newman DM, Rejali LS, Barford DA 1980 The myth of goiter in pregnancy. Am J Obstet Gynecol 137 (6):701-703.
252. Crooks J, Tulloch MI, Turnbull AC, Davidsson D, Skulason T, Snaedal G 1967 Comparative incidence of goitre in pregnancy in Iceland and Scotland. Lancet 2 (7517):625-627.
253. Rotondi M, Amato G, Biondi B, Mazziotti G, Del Buono A, Rotonda NM, Balzano S, Bellastella A, Glinoer D, Carella C 2000 Parity as a Thyroid Size-Determining Factor in Areas with Moderate Iodine Deficiency. J Clin Endocrinol Metab 85 (12):4534-4537.
254. Struve CW, Haupt S, Ohlen S 1993 Influence of frequency of previous pregnancies on the prevalence of thyroid nodules in women without clinical evidence of thyroid disease. Thyroid 3 (1):7-9.
255. Kung AW, Chau MT, Lao TT, Tam SC, Low LC 2002 The effect of pregnancy on thyroid nodule formation. J Clin Endocrinol Metab 87 (3):1010-1014.
256. Chaouki ML and Benmiloud M 1994 Prevention of iodine deficiency disorders by oral administration of lipiodol during pregnancy. Eur J Endocrinol 130 (6):547-551.
257. Liesenkotter KP, Gopel W, Bogner U, Stach B, Gruters A 1996 Earliest prevention of endemic goiter by iodine supplementation during pregnancy. Eur J Endocrinol 134 (4):443-448.
258. Aboul-Khair SA, Crooks J, Turnbull AC, Hytten FE 1964 The physiological changes in thyroid function during pregnancy. Clin Sci 27:195-207.
259. Muzzo S, Burgueno M, Carvajal F, Moreno R, Leiva L 1994 Endemic goiter in three census areas of Chile. Arch Latinoam Nutr 44 (2):82-86.
260. Freire-Maia DV and Freire-Maia A 1981 Endemic goitre in whites and negroes: racial or social effect? Trop Geogr Med 33 (4):387-392.
261. Mertz DP and Tomaras K 1976 The importance of sociomedical factors for the regression of endemic goiter in South Baden. MMW Munch Med Wochenschr 118 (44):1419-1422.
262. Stone T 1984 Endemic goitre, nutrition, and landholding in Bangladesh. Int J Epidemiol 13 (1):58-64.
263. Joshi DC, Mishra VN, Bhatnagar M, Singh RB, Garg SK, Chopra H 1993 Socioeconomic factors and prevalence of endemic goitre. Indian J Public Health 37 (2):48-53.
264. Freire-Maia DV, Freire-Maia A, Schull WJ, Morton NE, Quelce-Salgado A, Lobo LC 1983 Epidemiology and genetics of endemic goiter. I. Epidemiological aspects. Isr J Med Sci 19 (1):11-16.
265. Gibson HB, Howeler JF, Clements FW 1960 Seasonal epidemics of endemic goitre in Tasmania. Med J Aust June 4:875-880.
266. Koutras DA, Katsouyanni EK, Livadas DP, Piperingos GD, Tzonou A, Trichopoulos D 1982 An epidemiologic survey of thyroid enlargement among schoolchildren in a non-endemic area. Endokrinologie 79 (3):349-354.
267. Hess SY, Zimmermann MB, Adou P, Torresani T, Hurrell RF 2002 Treatment of iron deficiency in goitrous children improves the efficacy of iodized salt in Cote d'Ivoire. Am J Clin Nutr 75 (4):743-748.
268. Hess SY, Zimmermann MB, Arnold M, Langhans W, Hurrell RF 2002 Iron deficiency anemia reduces thyroid peroxidase activity in rats. J Nutr 132 (7):1951-1955.
269. Delange F 1998 Risks and benefits of iodine supplementation. Lancet 351 (9107):923-924.
270. Hak AE, Pols HA, Visser TJ, Drexhage HA, Hofman A, Witteman JC 2000 Subclinical hypothyroidism is an independent risk factor for atherosclerosis and myocardial infarction in elderly women: the Rotterdam Study. Ann Intern Med 132 (4):270-278.
271. Kalmijn S, Mehta KM, Pols HA, Hofman A, Drexhage HA, Breteler MM 2000 Subclinical hyperthyroidism and the risk of dementia. The Rotterdam study. Clin Endocrinol (Oxf) 53 (6):733-737.
272. Toft AD 2001 Clinical practice. Subclinical hyperthyroidism. N Engl J Med 345 (7):512-516.
273. Nohr SB, Jorgensen A, Pedersen KM, Laurberg P 2000 Postpartum thyroid dysfunction in pregnant thyroid peroxidase antibody-positive women living in an area with mild to moderate iodine deficiency: is iodine supplementation safe? J Clin Endocrinol Metab 85 (9):3191-3198.