Pregnancy week specific reference ranges for thyrotropin and free thyroxine in the North Denmark Region Pregnancy Cohort

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Pregnancy week-specific reference ranges for TSH and free T4 in the North Denmark Region Pregnancy Cohort

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Abstract

Background: Physiological changes in maternal thyroid function during pregnancy necessitate the use of pregnancy specific reference ranges. Dynamic changes in TSH within the first trimester of pregnancy have been reported, but more evidence is needed to substantiate the findings. The objective of this study was to estimate pregnancy week-specific reference ranges for maternal TSH and free T4 (fT4) in early pregnancy.

Methods: We consecutively recruited serum residues from blood samples collected as part of the prenatal screening in the North Denmark Region, 2011-2015. TSH, fT4, thyroid peroxidase antibodies (TPO-Ab) and thyroglobulin antibodies (Tg-Ab) were measured using an ADVIA Centaur XPT immunoassay. The reference cohort included 10,337 pregnant women who had no thyroid disease or other autoimmune diseases and were TPO-Ab and Tg-Ab negative. The main outcome measures were lower and upper reference limits (2.5 and 97.5 percentiles) for TSH and fT4 stratified by week of pregnancy.

Results: Blood samples were drawn in pregnancy week 4-20 (median week 10), and 92% of the pregnancies ended with live birth. TSH varied considerably in the first trimester of pregnancy, and the levels were highest in early pregnancy (week 4-6: 0.6-3.7 mIU/l) followed by a gradual decline to lower levels in week 9-11 (0.1-2.8 mIU/l) and 12-14 (0.03-2.8 mIU/l). Maternal fT4 showed less variation (week 4-6: 12-20 pmol/l; week 9-11: 13-21 pmol/l; week 12-14: 13-20 pmol/l).

Conclusions: The results corroborate dynamic week-specific changes in maternal TSH in early pregnancy. The use of uniform lower and upper reference range for TSH in early pregnancy may be too simple.

Introduction

Thyroid function in pregnant women is of clinical relevance (1). Thyroid hormones in the fetus are solely of maternal origin in early pregnancy, and the crucial role of thyroid hormones in fetal development emphasizes the importance of adequate maternal thyroid hormone levels (2). The physiological changes in maternal thyroid function in early pregnancy are mediated via estrogen, human chorionic gonadotropin (hCG) and alterations in the metabolism of thyroid hormones, and these changes necessitate the use of pregnancy specific reference ranges for evaluation of maternal thyroid function (3). Much emphasis has been on the use of trimester specific reference ranges (1), but recent data indicate that the physiological changes in maternal TSH in early pregnancy are marked, even within the first trimester of pregnancy, and that the use of a uniform reference range may be too simple (4,5).

Thyroid dysfunction in women of reproductive age is predominantly of autoimmune origin (6,7). Thyroid peroxidase antibodies (TPO-Ab) and thyroglobulin antibodies (Tg-Ab) are associated with the presence of hypothyroidism, and TSH is higher in individuals who are positive for thyroid autoantibodies (8). Thus, it is recommended to exclude individuals with TPO-Ab from a reference cohort (1). We previously described the week-specific changes in maternal TSH and free T4 (fT4) in a large cohort of pregnant women from the Danish National Birth Cohort established in 1997-2003 (5). Pregnant women with known thyroid disease were excluded, but the measurement of thyroid autoantibodies was not possible in this cohort. Furthermore, mandatory iodine fortification of salt was introduced in Denmark in the year 2000 (9), and data on the level of TSH in Danish pregnant women after iodine fortification are warranted.

The present study aimed to evaluate the week-specific reference ranges for TSH and fT4 in a recent, large cohort of pregnant women from the North Denmark Region established in 2011-2015. Blood samples were consecutively collected as part of the prenatal screening for chromosomal anomalies in early pregnancy and used for the measurement of maternal thyroid function (TSH and fT4) and thyroid autoantibodies (TPO-Ab and Tg-Ab).

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Materials and Methods

Study population

Nationwide prenatal screening for fetal chromosomal anomalies was implemented in Denmark in 2004 (10). It includes the measurement of biochemical markers in a blood sample from the pregnant woman drawn at the first pregnancy visit in general practice. All serum samples from pregnant women in the North Denmark Region are analyzed at the Department of Clinical Biochemistry, Aalborg University Hospital, Aalborg, Denmark. Serum residues from all samples received in the laboratory from June 28, 2011 to January 5, 2015 were collected for the present study and stored at minus 80° Celsius until the measurement of maternal thyroid function and thyroid autoantibodies. Serum residues from January to March 2012 and January to March 2013 were not available due to logistics in the laboratory, and a total of 18,186 samples were included in the study (Figure 1). The study was approved by the North Denmark Region Committee on Health Research Ethics (N-20150015) and the Danish Data Protection Agency (J.nr. 2008-58-0028).

Laboratory procedures

Serum TSH, fT4, TPO-Ab and Tg-Ab were measured from September 2015 to May 2016 in the Department of Clinical Biochemistry, North Denmark Regional Hospital, Hjørring, Denmark, using an ADVIA Centaur XPT automatic immunoassay (Siemens Healthineers, Germany). For TSH (TSH3-Ultra), the functional sensitivity provided by the manufacturer was 0.008 mIU/l and the analytical measurement range was 0.008-150 mIU/l. Intermediate precision was 3.6-5.2% for six patient serum samples in the range from 1.0-132.8 mIU/l. The reference range for non-pregnant adults was 0.55-4.78 mIU/l. For free T4, the analytical measurement range was 1.3-155 pmol/l, and intermediate precision was 3.4-4.6% for three concentrations in the range from 9.3-38.8 pmol/l. The reference range for non-pregnant adults was 11.5-22.7 pmol/l. For TPO-Ab, the analytical measurement range was 28-1300 U/mL, and intermediate precision was 7.6 and 3.1% for concentrations of 71 and 459 U/ml, respectively. For Tg-Ab, the analytical measurement range was 15-500 U/mL, and intermediate precision 3.9-6.6% for three concentrations in the range from 71-344 U/ml. A cut-off value of 60 U/ml was given by the manufacturer for both TPO-Ab and Tg-Ab, and women with measurements above this cut-off were considered thyroid

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autoantibody positive in the present study. The cut-off for TPO-Ab in pregnant women has previously been evaluated for the ADVIA Centaur assay and was at a comparable level (54 U/ml) (11). We included a sub-analysis in which the cut-off was halved to 30 U/ml to evaluate a possible impact of subtle thyroid autoimmunity.

Data linkage

The date of blood sampling and results of thyroid function parameters and thyroid autoantibodies were linked to information in Danish nationwide registers via Statistics Denmark. All citizens in Denmark are provided a unique personal identification number, which is used in all registers and enables data linkage (12). Data were available in Statistics Denmark in encrypted form so that no individuals could be identified by the researcher. First, pregnancy outcome and the week of blood sampling during pregnancy were established via linkage to the Medical Birth Register (MBR) (13) and the Danish National Hospital Register (DNHR) (14). The MBR holds information on all live- and stillbirths in Denmark including information on whether it was a singleton or multiple birth, and on maternal age, parity, origin (country of birth), smoking in pregnancy, and pre-pregnancy body mass index (BMI). The DNHR holds information on all in- and outpatient visits to Danish hospitals with a diagnosis of pregnancy loss including induced abortion, spontaneous abortion, molar and ectopic pregnancy coded according to the 10th International Classification of Disease (ICD-10). In both registers, information on date and gestational age at the time of pregnancy termination is available, and this information was linked to the date of blood sampling in early pregnancy and used to establish the pregnancy week of blood sampling. Gestational age is established by ultrasound in Denmark (15) and registered in full weeks plus days counted from the first day of the last menstrual period e.g. 8+2, which corresponds to the 9th week of pregnancy. Information on maternal diseases before, during and after the pregnancy was obtained from 1) hospital diagnosis of disease registered from January 1, 1994 to December 31, 2016 in the DNHR and coded according to ICD-10 and from 2) redeemed prescriptions of drugs registered from January 1, 1995 to December 31, 2016 in the Danish National Prescription Register (DNPR) (16) and coded according to the Anatomical Therapeutic Chemical (ATC) Classification System.

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Reference cohort

Among the 18,186 blood samples collected in early pregnancy and used for measurement of maternal thyroid function and thyroid autoantibodies, altogether 17,681 were linked in Statistics Denmark with information on outcome of the pregnancy and gestational age (Figure 1). Some women had more than one blood sample drawn during a pregnancy or consecutive pregnancies in the study period and the first blood sample during the first pregnancy in the study period was selected for each woman (Figure 1). Multiple pregnancies were excluded from the reference cohort as were women with a registration of thyroid or other autoimmune diseases (diabetes, rheumatoid arthritis, and inflammatory bowel disease) before, during or after the pregnancy, women with a registration of thyroid interfering medication (antiepileptic drugs, dopamine agonists, lithium, and prednisolone) in pregnancy defined by redeemed prescriptions in the one-year period prior to pregnancy termination, and women who were positive for TPO-Ab and/or Tg-Ab, leaving 10,337 pregnant women in the reference cohort (Figure 1).

Statistical analyses

Pregnancy week-specific results of TSH and fT4 analyses are reported as median, 2.5 percentile and 97.5 percentile with 95% confidence interval. The early (week 4-6) and the late (week 15-20) pregnancy weeks were collapsed to obtain a sufficient number of individuals in the groups. Within each pregnancy week group, Box-Cox transformation was used to obtain normal distribution prior to the exclusion of outliers using Tukey's fences (17). Analyses were performed both in the full reference cohort (n=10,337) and among women who ended the pregnancy with the birth of a live-born child (n=9515). Predictors of maternal TSH and fT4 were evaluated in pregnancy weeks 9-11, which included the largest number of individuals. This evaluation was performed among women who ended the pregnancy with the birth of a live-born child and had available information on maternal characteristics (n=7438). All predictors (maternal age, parity, origin, smoking and BMI) were assessed as independent categorical variables in multivariate linear regression with Box-Cox transformed TSH or free T4 as dependent variable, and significant predictors were selected for univariate stratified analyses. Statistical analyses were performed using STATA version 15 (Stata Corp).

Results

Reference cohort

Altogether 10,337 pregnant women were included in the reference cohort (Figure 1). Maternal age was at a median of 29 years (range 16-51 years), and 88% of the women were born in Denmark. The blood sample had been drawn in median pregnancy week 10 (9+0 to 9+6) ranging from the 4th week (3+0 to 3+6) to the 20th week (19+0 to 19+6). The birth of a live-born child was the most frequent outcome of pregnancy (n=9515), whereas induced abortion (n=140), spontaneous abortion (n=639), molar and ectopic pregnancy (n=12), and still birth (n=31) accounted for a smaller proportion of the cohort.

Reference ranges

Week-specific median and upper and lower reference limits with 95% CI for TSH and fT4 showed a similar trend when established within the full cohort of 10,337 pregnant women and among the 9515 women who ended the pregnancy with the birth of a live-born child (Figure 2 and Table 1). TSH showed considerable variation in the median and upper and lower reference limits during early pregnancy being high in the early pregnancy weeks followed by a gradual decline to low levels in pregnancy week 9-11 and in week 12-14, which was most pronounced for the lower reference limit. Maternal fT4 showed an opposite pattern, but less pronounced variation with the highest levels in pregnancy week 9-11. A uniform reference range for TSH and fT4 in the first trimester of pregnancy (week 4-12) was 0.14-2.9 mIU/l for TSH and 13.0-20.5 pmol/l for fT4 with similar figures within the full cohort and within the cohort of women with live births. However, the week-specific changes during the first trimester (Figure 2 and Table 1) indicated that shorter distinct intervals could be described (Table 2), particularly for TSH, with an initial high TSH period followed by subsequent lower TSH periods in week 9-11 and 12-14. When the cut-off for TPO-Ab and Tg-Ab was halved to 30 U/ml, the study population was restricted to 6216 pregnant women. Reference limits for maternal thyroid function tests were similar with the same trend during the first trimester of pregnancy (week 4-8: TSH 0.29-3.2 mIU/L, fT4 12.4-19.3 pmol/l; week 9-11: TSH 0.13-2.8 mIU/L, fT4 12.8-20.2 pmol/l; week 12-14: TSH 0.04-2.8 mIU/L, fT4 12.7-19.5 pmol/l). Considering the exclusion of TPO- and/or Tg-Ab positive women, the TSH reference limits prior to exclusion were 0.43-3.8 mIU/L for week

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4-8, 0.17-3.2 mIU/L for week 9-11 and 0.08-3.1 mIU/L for week 12-14. After exclusion of TPO-Ab positive women only, the TSH reference ranges were 0.40-3.4 mIU/L for week 4-8, 0.14-2.9 mIU/L for week 9-11 and 0.03-2.8 mIU/L for week 12-14, and the same after exclusion of Tg-Ab positive women only (week 4-8: 0.40-3.5 mIU/L, week 9-11: 0.15-2.9 mIU/L, week 12-14: 0.03-2.7 mIU/L). Reference limits for fT4 were similar to those presented in Table 2 irrespective of whether TPO- and/or Tg-Ab positive women were excluded (data not shown).

Maternal characteristics

Different maternal characteristics were assessed for women who ended the pregnancy with the birth of a live-born child (Table 3). In multivariate analyses, multiparity and smoking in pregnancy were significant predictors of lower maternal TSH, whereas higher maternal BMI associated with higher TSH. Maternal age and origin were not significant predictors of TSH. For maternal fT4, higher maternal age, higher BMI and smoking in pregnancy were significant predictors of lower fT4, whereas multiparity and origin (not born in Denmark) associated with higher maternal fT4. However, when stratified reference limits for TSH and fT4 in pregnancy week 9-11 were calculated, the impact of individual predictors was modest and confidence limits were overlapping (Table 4). A disparity was most pronounced for maternal BMI, which showed higher TSH and lower fT4 limits in obese women with pre-pregnancy BMI ≥ 30 kg/m^2. Reference limits among overweight women with BMI in the range from 25 to 29.9 kg/m^2 (TSH 0.13-2.73 mIU/l, fT4: 13.1-20.6 pmol/l) were comparable to the reference limits observed among women with BMI below 25 kg/m^2 (TSH 0.11-2.75 mIU/l, fT4: 13.2-20.6 pmol/l).

Discussion

Main findings

The establishment of pregnancy week-specific reference ranges for maternal thyroid function parameters in a large cohort of thyroid autoantibody negative Danish pregnant women corroborate dynamics of TSH during the first trimester of pregnancy. The findings imply that the clinical assessment of smaller time intervals within the first trimester of pregnancy may be preferable to the use of a uniform reference range for evaluation of

maternal thyroid function. Reference limits for maternal fT4 showed opposite, but less marked dynamics during first trimester and a uniform reference range may apply. Maternal characteristics such as age, parity, origin, smoking, and BMI associated with maternal thyroid function parameters, but only small differences were observed in stratified reference limits for TSH and fT4.

Reference ranges

Valid reference ranges are an important part of diagnosis and management in clinical patient care, and as part of exposure and outcome definitions in clinical research. Comprehensive guidance exists on how to establish reference ranges (17), but still many aspects remain debatable on the selection of the reference cohort and it may further pose a challenge to obtain a sufficient number of samples. Whereas some laboratory tests are hardly affected by pregnancy, maternal thyroid function is considerably influenced by physiological changes in pregnancy, and non-pregnant reference ranges should not be used (3). Another challenge related to thyroid function parameters determined by automatic immunoassays is the potential disparity in results obtained with different methods. This is particularly a concern for the measurement of free thyroid hormones, because the determination of free thyroid hormone concentration by an automatic immunoassay is an indirect approach with no complete separation of free and protein-bound hormone (18-20). Thus, clinical guidance recommends the use of method- and pregnancy specific reference ranges (1).

The present study and previous investigations have questioned the use of a uniform reference range in the first trimester of pregnancy (4,5,21-25). We have now observed in two separate, large cohorts of Danish pregnant women that TSH reference limits are highly dynamic in the first trimester of pregnancy with an initially high TSH period followed by gradual decline in week 7-8 to lower levels in week 9 and onwards. The present study suggests that the lower TSH limit continues to be low in the beginning of second trimester, and is lower in week 12-14 compared with week 9-11, whereas no difference was observed in the upper limit between these periods of pregnancy. These findings are in keeping with the clinical experience that TSH may remain low at the beginning of the second trimester. We speculate whether this relates to a prolonged effect of hCG, and if

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women with lower TSH levels are more sensitive to the effect of hCG. This also conforms to the more pronounced dynamics in the lower TSH reference limit within the first trimester observed in the present study and in our previous report (5). Uncertainty may prevail on the exact week of pregnancy in early pregnancy, and the use of pregnancy week-specific reference ranges may not be superior in clinical practice. However, our data suggest that clinical awareness at different time points in early pregnancy may be warranted for the classification of maternal thyroid function with a higher TSH in the time period up to pregnancy week 8 followed by a lower TSH during the period of week 9-11 and by week 12-14.

The mechanisms underlying the dynamic changes in TSH likely relate to the balance between the hCG effect in early pregnancy, which tends to suppress TSH, and the activity of the type 3 deiodinase (DIO3) in the placenta, which tends to increase TSH (3). DIO3 is expressed from the early weeks of pregnancy (26-29), whereas hCG peaks in pregnancy week 9-10 (30). Thus, the initial high TSH period may reflect that the DIO3 effect is predominant in the early pregnancy weeks and the later low TSH period may reflect a dominant hCG effect.

A strength of the present study is the possibility to measure thyroid autoantibodies in a large cohort of pregnant women and to exclude women who were positive for TPO-Ab and/or Tg-Ab from the reference cohort. Such an approach was not possible in our previous investigation of Danish pregnant women (5), but it is noteworthy that similar dynamics of TSH levels in the first trimester were observed in the two Danish investigations. We used the cut-off value of 60 U/ml provided by the manufacturer to identify women with thyroid autoantibodies and 13% of the women were TPO-Ab and/or Tg-Ab positive. For comparison, the frequency was 16% when evaluated in a cohort of Danish pregnant women in East Denmark in 2007-2008 using the same cut-off level with a different immunoassay (31). The selection of reference individuals for the establishment of reference ranges is debatable, and a main focus regarding thyroid function analyses is on how to handle individuals with TPO- and/or Tg-Ab. In the present study, women with known or later diagnosed thyroid disease were excluded, as were outliers of TSH and fT4. We found slightly lower TSH reference limits following the subsequent exclusion of women hosting thyroid autoantibodies. Notably, the reference limits were consistent irrespective

of the cut-off used or whether TPO- and/or Tg-positive women were excluded. Adding to this, a recent study of pregnant women in a Swedish cohort showed that the additional exclusion of Tg-Ab positive women on top of TPO-Ab hardly changed the reference limits (32). More evidence is needed as part of the discussion on how to manage pregnant women who are positive for thyroid autoantibodies and who have no known thyroid disease.

The clinical guidance from the America Thyroid Association on the management of thyroid disease in pregnant women recommends to reduce the upper reference limit with approximately 0.5 mIU/l in the first trimester of pregnancy if local method specific reference ranges are not available (1). We observed that the upper limit of TSH was below this limit throughout pregnancy in this study and in our previous investigation conducted around the time when iodine fortification was implemented in Denmark (5). The uniform upper limit of TSH in first trimester was 3.4 mIU/l in the study of pregnant women in East Denmark in 2008-2009 (33) and therefore slightly higher than the limit of 2.9 mIU/l observed in the present study conducted in West Demark. Methodological differences in thyroid function assays may play a role, but the discrepancy is also compatible with observations in the general Danish population of regional differences in TSH (34). Considering the clinical proposal of a uniform TSH reference limit in the first trimester of pregnancy (1), our results indicate that reference limits vary considerably within the first trimester of pregnancy and suggest that it may be feasible to establish TSH reference limits for shorter time periods during pregnancy.

Predictors

Hyperthyroidism and hypothyroidism in women of reproductive age is mainly of autoimmune origin (6,7). Autoimmune thyroid disease is considered multifactorial, and genetic as well as environmental risk factors have been described (35). For the establishment of reference ranges, it is often relevant to consider if patient characteristics influence the reference limits and whether stratified reference ranges are needed in clinical practice. We were able to evaluate the impact of different maternal characteristics on the reference limits for TSH and fT4 in early pregnancy. In line with our previous investigation of Danish pregnant women (5), we observed that many factors were

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associated with TSH and fT4, but the impact of the individual factors in stratified reference limits was limited. These findings question the clinical usefulness of stratified reference ranges and may favor the use of the pregnancy week-specific reference combined with clinical awareness of maternal characteristics that may affect thyroid function results, particularly in the evaluation of patients with borderline results. Most pronounced was the effect of maternal BMI with higher TSH and lower fT4 limits in obese women, which is compatible with other reports (36-38). The role of other maternal characteristics has been less consistently reported in the literature (39), and our findings are less clear. We observed a trend towards lower TSH levels in multiparous women, which is in line with some studies, whereas others found no effect (39). In our study, ethnicity was not a predictor of maternal TSH. This contrasts with other reports (40,41). These studies had the possibility to stratify by sub-type of maternal ethnic background, whereas the register-based design of our study only provided the possibility to investigate reference ranges according to information on whether the pregnant woman was born in or outside of Denmark. Considering maternal smoking, the effect was smaller, but the results suggested lower TSH and fT4 limits in smokers. Studies of the population in general have shown that smoking tends to be associated with lower TSH levels and an increase the peripheral thyroid hormone concentrations. possibly caused by increased activity in the sympathetic nervous system (42). Our previous Danish investigation (5) and other reports (43) similarly showed lower fT4 levels associated with smoking in pregnancy, whereas other studies found no difference (32). Further studies are needed to corroborate and extend these findings, and to evaluate the clinical impact on maternal and fetal outcomes.

Methodological comments

The present large cohort of pregnant women allowed for the stratification of reference ranges by the week of pregnancy with an appropriate number of individuals in each of the stratified groups. However, the number of samples in the early and late pregnancy weeks was limited and groups were collapsed to obtain sufficient sample size. We excluded women with autoimmune disease from the reference cohort via linkage to information in Danish nationwide registers, and we used a recommended method of outlier detection (17).

Serum samples for the present study were consecutively collected as part of prenatal screening in Denmark, and the rate of participation is high (10). This method of inclusion is expected to minimize the risk of selection bias, and the number of samples obtained was representative for the expected number of pregnant women in the North Denmark Region around the time of inclusion.

Determination of pregnancy outcome was performed via linkage to Danish nationwide registers, and the validity is considered high (44). This provided the possibility to evaluate reference ranges in the full cohort and in pregnancies with live births. Notably, results are consistent in both cohorts, which may apply to the design of future studies and considerations on the selection of reference cohorts. Information was available on pregnancy loss and births including spontaneous and induced abortions as well as live and still births at hospital and at home. However, pregnancies terminating with spontaneous abortion without clinical recognition and hospital contact would not have been identified with an outcome of pregnancy.

Information on a number of maternal characteristics were available for women who terminated the pregnancy with the birth of a live-born child. Pre-pregnancy BMI and smoking status were self-reported by the pregnant women, but any misclassification is expected to be non-differential regarding thyroid function.

All samples were analyzed consecutively in the same laboratory after the last sample had been collected, and storage time until the present analyses ranged from two to six years. The storage time is not expected to influence our results since thyroid function parameters and thyroid autoantibodies in pregnant women have been shown to be stable for more than 20 years of storage at minus 25° Celsius (45).

Conclusion

Reference limits for maternal TSH are dynamic during the first trimester of pregnancy with an initial high TSH period followed by a gradual decline to a low TSH period. A clinical focus on shorter time intervals within the first trimester of pregnancy may be preferable. Future investigations should evaluate the dynamics in maternal thyroid function parameters in other populations using different thyroid function assays. It remains to be addressed

whether clinical awareness of the dynamic changes in maternal TSH in early pregnancy and of maternal characteristics will improve patient care and clinical research.

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Disclosure statement

No competing financial interests exist.

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Table 1 Pregnancy week specific median and reference limits with 95% confidence intervals for maternal TSH and free T4 in all pregnant women from the North Denmark Region and in pregnant women who ended the pregnancy with live birth.

	Maternal TSH (mIU/l)					Maternal free T4 (pmol/l)				
Week	**n[a]**	**2.5p**	**95% CI[b]**	**97.5p**	**95% CI[c]**	**n[a]**	**2.5p**	**95% CI[b]**	**97.5p**	**95% CI[c]**
All										
4-6	171	0.62	0.27-0.66	3.65	3.16-3.78	172	12.4	12.2-13.1	20.0	19.2-21.7
7	251	0.46	0.36-0.59	3.20	2.95-3.51	255	12.3	11.9-12.6	19.9	19.2-21.2
8	739	0.28	0.22-0.36	3.21	3.00-3.69	737	12.7	12.4-12.9	19.9	19.6-20.5
9	2,670	0.15	0.12-0.17	2.94	2.83-3.03	2,649	13.1	13.0-13.1	20.6	20.3-20.8
10	3,404	0.12	0.10-0.14	2.72	2.65-2.80	3,456	13.1	12.9-13.2	20.6	20.4-20.8
11	1,694	0.13	0.10-0.14	2.88	2.74-2.99	1,710	12.9	12.8-13.1	20.3	20.0-20.7
12	597	0.03	0.02-0.04	2.70	2.51-3.00	591	13.1	12.8-13.3	19.8	19.5-20.6

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13	330	0.02	0.01-0.07	3.19	2.64-3.54	321	12.7	12.3-13.1	19.7	19.3-21.2
14	147	0.06	0.01-0.19	2.86	2.38-3.49	148	12.3	11.9-13.0	19.6	19.0-21.1
15-20	135	0.30	0.15-0.39	2.91	2.52-3.27	142	12.8	12.4-13.4	19.0	18.6-20.7
Live births										
4-6	87	0.62	0.59-0.67	3.72	3.29-3.79	85	13.2	12.7-13.7	20.1	19.3-20.3
7	182	0.44	0.36-0.61	3.00	2.73-3.20	186	12.3	12.1-12.7	20.8	19.3-21.4
8	624	0.35	0.29-0.43	3.19	2.99-3.71	626	12.7	12.3-12.9	19.7	19.5-20.2
9	2,504	0.14	0.11-0.17	2.88	2.76-3.02	2,485	13.1	13.0-13.2	20.6	20.3-20.8
10	3,255	0.11	0.09-0.13	2.70	2.60-2.77	3,303	13.1	12.9-13.2	20.7	20.4-20.8
11	1,600	0.12	0.09-0.14	2.83	2.70-	1,607	13.0	12.8-	20.3	20.0-

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					2.97			13.1		20.8
12	530	0.03	0.02-0.04	2.70	2.49-3.00	523	13.1	12.8-13.4	19.6	19.5-20.4
13	293	0.03	0.009-0.10	2.85	2.45-3.50	284	12.6	12.2-12.9	19.5	19.0-20.4
14	135	0.04	0.006-0.17	2.87	2.32-3.49	135	12.3	11.9-13.0	19.7	18.9-21.1
15-20	123	0.31	0.15-0.44	2.93	2.56-3.27	130	12.8	12.4-13.4	19.2	18.6-20.7

Abbreviations: 2.5p: 2.5 percentile, 97.5p: 97.5 percentile, CI: confidence interval

[a]Number of individuals in each group is reported after the exclusion of outliers using Tukey's fences.

[b]Lower reference limit was held at minimum of the sample in week 4-6, 14, and 15-20.

[c]Upper reference limit was held at maximum of the sample in week 4-6, 14, and 15-20.

Table 2 Reference limits of maternal TSH and free T4 with 95% confidence intervals in different periods of early pregnancy.

	Maternal TSH (mIU/l)					Maternal free T4 (pmol/l)				
Week	**n[a]**	**2.5p**	**95% CI**	**97.5p**	**95% CI**	**n[a]**	**2.5p**	**95% CI**	**97.5p**	**95% CI**
All										
4-8	1,155	0.40	0.35-0.45	3.38	3.11-3.55	1,162	12.6	12.4-12.7	19.8	19.6-20.4
9-11	7,761	0.13	0.12-0.14	2.83	2.76-2.89	7,811	13.1	13.0-13.1	20.5	20.3-20.7
12-14	1,072	0.03	0.02-0.05	2.77	2.63-3.01	1,057	12.9	12.7-13.1	19.7	19.5-20.3
Live births										
4-8	898	0.39	0.35-0.46	3.40	3.11-3.61	898	12.6	12.3-12.8	19.8	19.6-20.3
9-11	7,354	0.12	0.11-0.14	2.78	2.72-2.86	7,391	13.1	13.0-13.1	20.6	20.4-20.7
12-14	958	0.03	0.02-0.05	2.71	2.58-3.00	945	12.7	12.5-13.0	19.6	19.5-20.0

Abbreviations: 2.5p: 2.5 percentile, 97.5p: 97.5 percentile, CI: confidence interval

[a]Number of individuals in each group is reported after the exclusion of outliers using Tukey's fences.

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Table 3 Characteristics of the 9,515 pregnant women who gave birth to a live-born singleton child.

	n	%
Maternal age		
< 35 years	8,129	85.4
≥ 35 years	1,386	14.6
Maternal parity[a]		
Nulliparous	4,848	51.2
Multiparous	4,626	48.8
Maternal origin		
Born in Denmark	8,403	88.3
Not born in Denmark	1,112	11.7
Maternal smoking in pregnancy[b]		
No smoking	8,311	88.1
Smoking	1,121	11.9
Maternal pre-pregnancy BMI[c]		
< 30 kg/m^2	8,173	86.3
≥ 30 kg/m^2	1,295	13.7

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Abbreviations: BMI: body mass index

[a]Missing value n = 41 not included.

[b]Missing value n = 83 not included.

[c]Missing value n = 47 not included.

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Table 4 Reference limits with 95% confidence intervals for maternal TSH and free T4 in pregnancy week 9-11 stratified by maternal characteristics.

	Maternal TSH (mIU/l)					Maternal free T4 (pmol/l)				
	n[a]	2.5p	95% CI	97.5p	95% CI	n[a]	2.5p	95% CI	97.5p	95% CI
Maternal age										
< 35 years	6,191	0.13	0.11-0.14	2.79	2.72-2.87	6,223	13.1	13.0-13.2	20.6	20.4-20.7
≥ 35 years	1,097	0.10	0.07-0.14	2.82	2.64-3.06	1,101	13.0	12.8-13.1	20.5	20.0-21.0
Parity										
Nulliparous	3,702	0.15	0.13-0.17	2.86	2.78-2.94	3,734	13.1	12.9-13.1	20.5	20.2-20.7
Multiparous	3,586	0.11	0.09-0.12	2.71	2.59-2.80	3,590	13.1	13.0-13.2	20.6	20.4-20.8
Origin										
Born in Denmark	6,552	0.13	0.12-0.14	2.78	2.71-2.85	6,572	13.1	13.0-13.1	20.5	20.3-20.7
Not born in Denmark	736	0.08	0.07-0.13	2.88	2.70-3.13	752	13.1	12.9-13.3	20.8	20.5-21.3
Smoking in pregnancy										
No	6,483	0.13	0.11-0.14	2.82	2.73-2.88	6,517	13.1	13.0-13.2	20.6	20.4-20.7
Yes	805	0.11	0.08-0.16	2.64	2.46-2.84	807	13.0	12.8-13.1	20.2	19.6-20.8

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Pre-pregnancy BMI										
< 30 kg/m^2	6,320	0.12	0.11-0.13	2.74	2.69-2.83	6,357	13.1	13.1-13.2	20.6	20.4-20.8
≥ 30 kg/m^2	968	0.19	0.15-0.24	3.09	2.85-3.28	967	12.9	12.7-13.0	20.3	19.8-20.6

Abbreviations: 2.5p: 2.5 percentile, 97.5p: 97.5 percentile, CI: confidence interval, BMI: body mass index.

[a]Number of individuals in each group is reported after the exclusion of outliers using Tukey's fences.

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Legends to figures

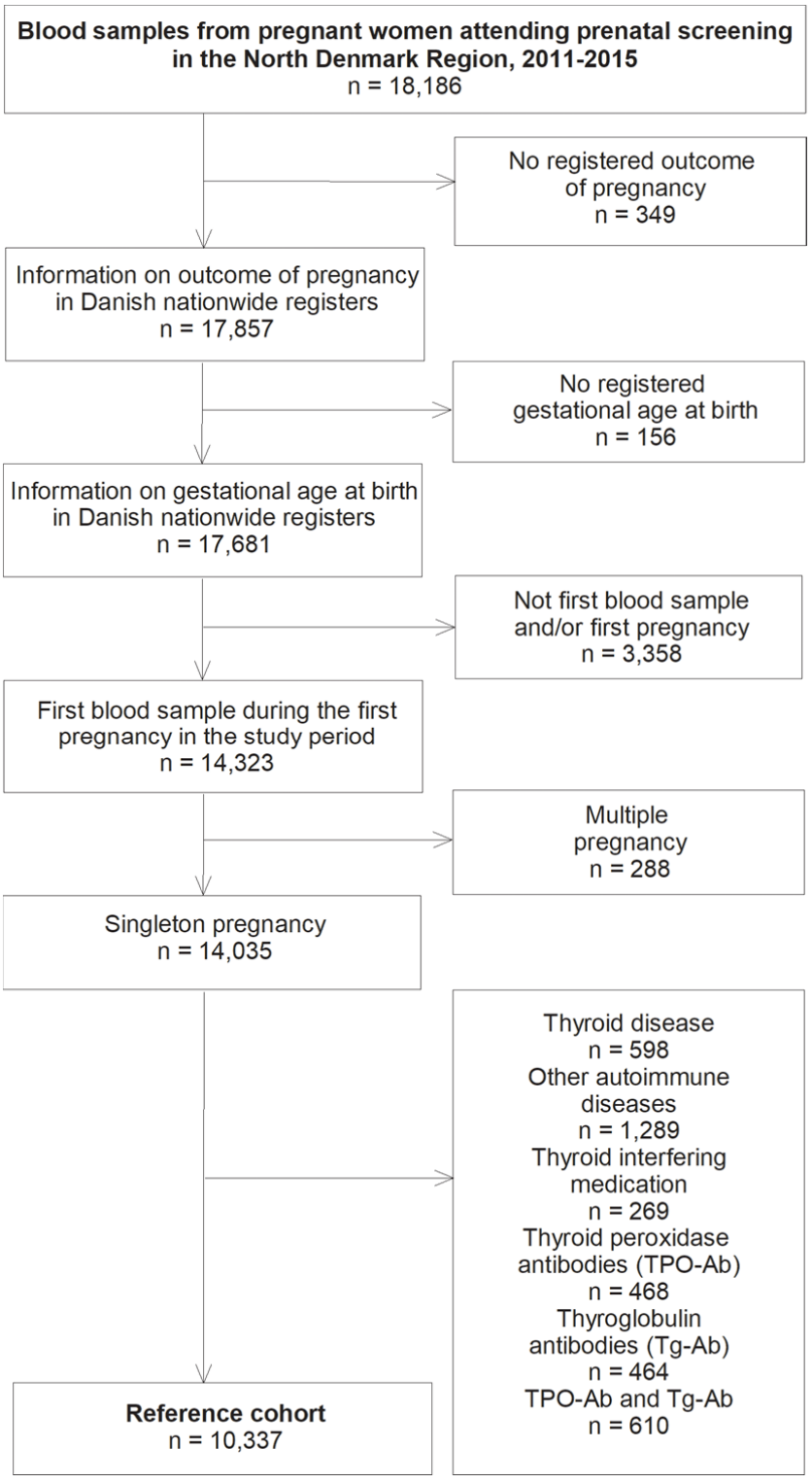

Figure 1

Flowchart illustrating the selection of the reference cohort.

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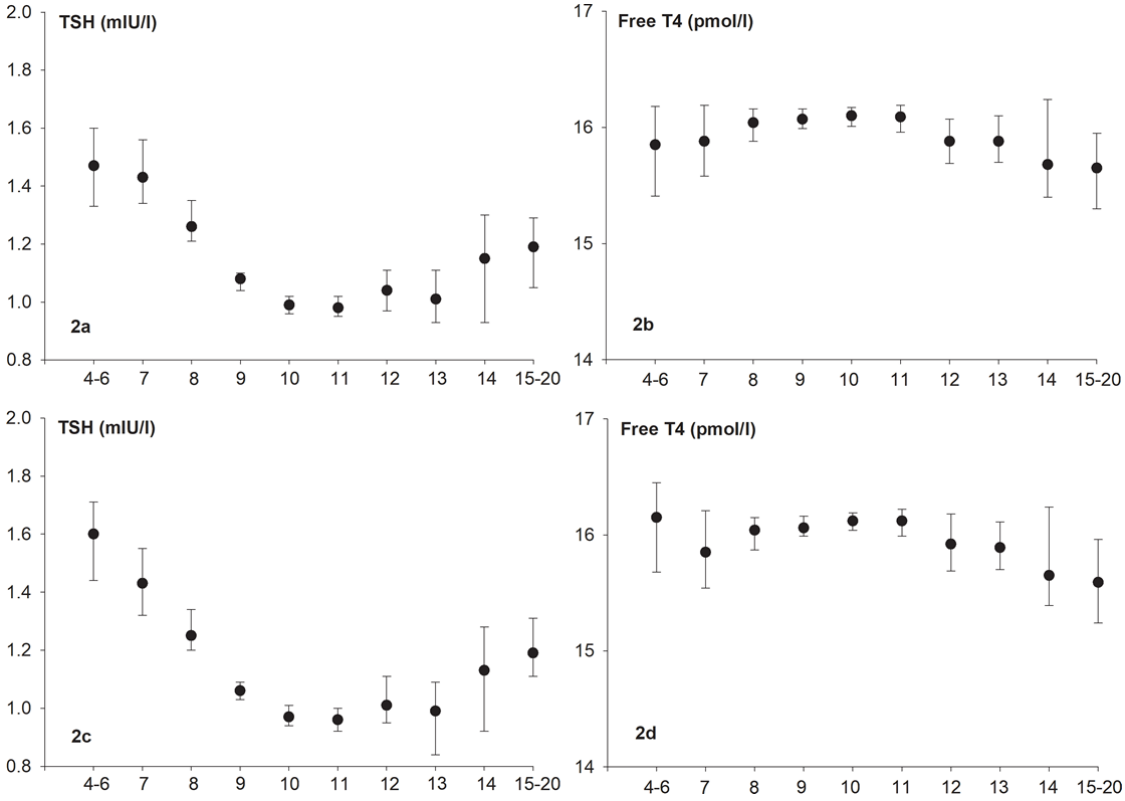

Figure 2

Pregnancy week specific median with 95% confidence intervals for maternal TSH and free T4 in all 10,337 pregnant women from the North Denmark Region (figure 2a and 2b in the upper panel) and in the 9,515 pregnant women who gave birth to a live-born child (figure 2c and 2d in the lower panel).