Gestational age-specific reference ranges from different laboratories misclassify pregnant women’s thyroid status: comparison of two longitudinal prospective cohort studies

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Abstract

Objectives: Correct interpretation of thyroid status during pregnancy is vital to secure fetal development. Pregnancy-related changes in maternal thyroid status necessitate the use of gestational age-specific reference ranges. In this study, we investigated between-laboratory reproducibility of thyroid reference ranges in pregnant women. Design: Comparison of two longitudinal prospective cohort studies including 255 (cohort 1) and 101 (cohort 2) healthy antibody-negative Danish pregnant women attending prenatal care at Copenhagen University Hospital. Methods: Different immunoassays were used to measure thyroid hormone levels in the two cohorts. Thyroid hormone reference ranges were established for every 5 weeks of gestation. Differences between cohorts were explored through mixed-model repeated measures regression analyses. By applying reference ranges from one cohort to the other, the proportion of women who would be misclassified by doing so was investigated. Results: TSH increased and free thyroxine (FT4) decreased as pregnancy progressed. Results indicated highly significant differences between cohorts in free triiodothyronine (F=21.3, P<0.001) and FT4 (F=941, P<0.001). TSH levels were comparable (P=0.09). Up to 90.3% of the women had FT4 levels outside their laboratory’s nonpregnant reference range, and up to 100% outside the other cohort’s gestational-age-specific reference ranges. Z-score-based reference ranges markedly improved comparison between cohorts. Conclusion: Even in the same region, the use of gestational-age-specific reference ranges from different laboratories led to misclassification. Up to 100% of maternal FT4 levels fell outside the other cohort’s reference range despite similar TSH levels. In clinical practice, thyroid testing of pregnant women without adding method specificity to gestational age-dependent reference ranges will compromise patient safety.
Introduction

Thyroid dysfunction during pregnancy is a risk factor to both mother and child (1, 2, 3). The high prevalence of thyroid dysfunction in young women has led to increased support among endocrinologists for thyroid screening of all pregnant women (4, 5). Evaluation of thyroid function in pregnant women, without taking into account the pregnancy-related changes in maternal thyroid status, has been shown to lead to misclassification and misdiagnosis of thyroid disease of risk to obstetric outcome and fetal development (6).

During pregnancy, maternal thyroid status changes according to gestational age (7). Especially in the first trimester, the rise in the thyrotropic-acting pregnancy-related hormone, human chorionic gonadotropin, leads to a rise in free thyroxine (FT4) and free triiodothyronine (FT3), and by negative feedback to a suppression of thyroid-stimulating hormone (TSH). Furthermore, the maternal thyroid status is affected by an estrogen-induced increase in thyroxine-binding globulin, an altered iodine clearance in the kidneys, as well as a placental metabolism of thyroid hormones (7). These unequivocal changes in maternal thyroid status necessitate the need for gestational age-specific reference ranges.

In recent years, several studies have been published with the aim of providing thyroid hormone reference ranges for use in pregnant women (8, 9, 10, 11) (reviewed in (11, 12)). Most of the studies were retrospective, cross-sectional studies of maternal thyroid status in the first trimester. The use of such studies entirely depends on the reproducibility of reference ranges in different populations, especially when using different laboratory methods.

In this study, we examined the maternal thyroid status throughout pregnancy based on two prospective longitudinal cohort studies on Danish pregnant women. The aim of the study was to establish two independent sets of longitudinal gestational age-specific reference ranges, and subsequently evaluate the use of these when applied to another comparable cohort.

Subjects and methods

Subjects

This study was based on results from two prospective longitudinal cohort studies on 466 pregnant Danish women at Copenhagen University Hospital (Rigshospitalet and Herlev Hospital), Copenhagen, Denmark (Fig. 1).

Cohort 1 ▶ From August 1996 to May 1997, all Danish-speaking pregnant women (n = 2526) attending prenatal care at Copenhagen University Hospital (Rigshospitalet) were invited to join a project regarding stress factors and pregnancy. A total of 1605 women (1623 pregnancies) filled in a health questionnaire including information regarding socioeconomic status, smoking habits, prescription medications, and parity. A random subset of 315 women (316 pregnancies) with no regular cigarette smoking or alcohol consumption nor drug abuse, no psychiatric illness, and no known chronic diseases requiring daily prescription medications, was consecutively enrolled. They were followed with questionnaires, ultrasound scans, and sequential blood sampling once each trimester and upon birth (including umbilical cord blood sampling of the newborn). Within 1 week post partum, neurological examinations of their neonates were performed. The examining doctors had no knowledge of the women’s background or thyroid status.

Cohort 2 ▶ During the period of May 1999 to October 2001, women referred to prenatal care at Copenhagen University Hospital (Herlev) were invited to participate in a study regarding fetal growth during pregnancy (13). Among the women who actively volunteered to participate in the study, 151 pregnant women with no risk factors for preeclampsia were consecutively recruited. After informed consent, all the women were scheduled to have three ultrasound examinations and questionnaires, and blood samples drawn approximately every 4–6 weeks throughout pregnancy. Placental growth hormone and insulin-like growth factor 1 levels were analyzed (13). Subsequently, thyroid hormone levels were analyzed to compare intra-individual changes with inter-individual changes during pregnancy (10).

In both cohorts, informed consent was obtained from each woman, and the projects were approved by the Danish Data Protection Agency as well as the local branch of the Danish Ethics Committee (reference numbers 01-077/96 and KF 01 276357 respectively).

In this, only antibody-negative women with singleton pregnancies, without known thyroid disease and without other significant disease (preeclampsia, hypertension, and asthma treated with moderate/high-dose steroid) were included. Women who had achieved pregnancy through assisted reproductive technologies (n = 14), or had taken hormone supplements during pregnancy (n = 4), were excluded. Antibody positivity was defined as a thyroid peroxidase antibody level ≥30 U/ml and/or
a thyroglobulin antibody level ≥ 25 U/ml corresponding to the functional assay sensitivities. Although lower than most cutoffs used in clinical practice, we found this cutoff to be reasonable when attempting to establish normal reference ranges given that the clinical impact of antibody presence was still unresolved even in euthyroid pregnant women (14). Women in whom thyroid antibody analysis (thyroid peroxidase antibody and/or thyroglobulin antibody) had not been performed at least once during their pregnancy were excluded (n = 18). Twenty-one (7.0%) women in cohort 1 and 15 (10.1%) women in cohort 2 were antibody positive and excluded from the calculation of reference ranges.

In both cohorts, intake of vitamins, prepregnancy height and weight, and smoking status were self-reported in questionnaires. Women who had reported smoking at any time during pregnancy were considered to be smoking.

**Laboratory methods**

In both cohorts, blood was drawn as nonfasting peripheral venous samples between midmorning and early afternoon. Samples were centrifuged and sera were stored until hormone analysis at −80 °C in cohort 1 and −20 °C in cohort 2.

In cohort 1, the 1235 AutoDelfia automatic fluoroimmunoassay system (Wallac, Turku, Finland) was used to analyze the following: TSH, FT₃, total T₃(TT₃), and FT₄, and total T₄(TT₄). The inter-assay coefficients of variance (CV) were 4.8, 2.2, and 2.2% at the concentrations of 0.05, 0.9, and 17.6 mU/l respectively for TSH; 10.7, 4.4, 3.2, and 1.6% at concentrations of 2.8, 4.7, 6.5, and 9.7 pmol/l respectively for FT₃; and 5.3, 3.7, and 3.1% for 9.3, 15.9, and 19.5 pmol/l respectively for FT₄. Thyroid peroxidase antibodies (functional assay sensitivity 30 U/ml) were analyzed by RIA (DYNOtest, Brahms, Hennigsdorf, Germany).

In cohort 2, electrochemiluminescense immunoassays on the Roche Modular E170 (Roche) were used to analyze the following: TSH, FT₃, TT₃, and FT₄, and TT₄. The inter-assay CV were 8.7 and 8.4% at concentrations of 0.9 and 4.9 mU/l respectively for TSH; 10.7, 4.4, 3.2, and 1.6% at concentrations of 2.8, 4.7, 6.5, and 9.7 pmol/l respectively for FT₃; and 5.3, 3.7, and 3.1% for 9.3, 15.9, and 19.5 pmol/l respectively for FT₄. Thyroid peroxidase antibodies (functional assay sensitivity 28 U/ml) and thyroglobulin antibodies (functional assay sensitivity 25 U/ml) were analyzed by immunofluorescent assays on the Kryptor instrument (Brahms).
Statistical analysis

We used SPSS Statistics, version 20.0 (IBM) for all transformations, independent-sample t-tests, χ² tests, and mixed model analyses. TSH, FT₃, and FT₄ variables were log transformed to approach normal distributions. No outliers were deleted as these were considered true values rather than analytical errors. Differences in demographics were tested with independent-sample t-tests rather than analytical errors. Differences in demographic differences and the varying number of blood samples from each participant, we entered data into a model that controlled for the nonindependence of the participants’ repeated visits. TSH, FT₃, and FT₄ levels, respectively, were predicted in a mixed model repeated measures regression analysis using SPSS Software as described in the Methods section. In order to minimize estimation bias, we did not replace missing data with dummy variables or indicators, leaving a total of 311 women in the model. As predicted, the difference in cohort (laboratory method) did not have a significant relationship with those in cohort 2 showing higher hormone levels than those in cohort 1, Previous births (%), n 87 (34.3), 60 (60.6)* Smoking (%), n 2 (0.8), 23 (30.3)* Vitamin intake (%), n 244 (96.8), 82 (85.4)*.were all highly significant (P<0.001) in the mixed model analysis (described in detail below).

We calculated longitudinal gestational age-specific reference ranges for each cohort and compared the ranges with those of the other cohort (Table 2). To adjust for demographic differences and the varying number of blood samples from each participant, we entered data into a model that controlled for the nonindependence of the participants’ repeated visits. TSH, FT₃, and FT₄ levels, respectively, were predicted in a mixed model repeated measures regression analysis using SPSS Software as described in the Methods section. In order to minimize estimation bias, we did not replace missing data with dummy variables or indicators, leaving a total of 311 women in the model. As predicted, the difference in cohort (laboratory method) did not have a significant relationship with TSH (F(1,297)=2.9, P=0.09). As pregnancy progressed, there was a small but highly significant increase (P<0.001) in TSH levels. None of the other covariates entered into the model had a significant effect. In FT₃, there was a significant effect of cohort F(1,276)=21.3, P<0.001 after adjustment of covariates, of which gestational week group, maternal prepregnancy BMI, and maternal age had a significant effect on the model. Finally, there was a highly significant difference between cohorts (laboratory methods) in FT₄ F(1,271)=941, P<0.001, with those in cohort 2 showing higher hormone levels than those in cohort 1, β=-0.21, S.E.M.=0.01. This effect was found regardless of the significant influence of TSH and gestational week group. Maternal prepregnancy BMI and smoking had borderline significant effects on FT₄ (P=0.06 and P=0.09 respectively).

In cohort 1, the average amount of women who fell outside their own laboratory’s nonpregnant reference range (Fig. 3A) were for TSH 9.4% (median 8.2%), FT₃ 77.4% (median 81.2), and FT₄ 87.8% (median

Results

We examined maternal thyroid status throughout pregnancy based on two prospective longitudinal studies on Danish pregnant women (Fig. 1). In cohort 1, a total of 722 blood samples were obtained from 254 women (255 pregnancies) between 14 and 38 weeks of pregnancy. In cohort 2, 715 blood samples from 101 women were taken between 4 and 42 weeks of pregnancy. The demographics of the two cohorts differed significantly in terms of age, smoking, parity, prepregnancy BMI, prepregnancy weight, and self-reported intake of vitamins (Table 1).

In both cohorts, there was a small increase in TSH as pregnancy progressed (Fig. 2A). FT₃ (Fig. 2B) and FT₄ (Fig. 2C) decreased slightly after the first trimester. The changes in hormone levels with increasing gestational age were highly significant (P<0.001) in the mixed model analysis (described in detail below).
90.1%). The corresponding values for cohort 2 (Fig. 3B) include TSH 2.1% (median 2.2%), FT₃ 30.1% (median 34.2%), and FT₄ 58.4% (median 62.8%).

When applying the reference ranges from one cohort to the other, the number of women with abnormal FT₄ concentrations increased markedly (Fig. 3C and D). Across gestational age-groups, the average percentage of women in cohort 1 who would fall outside of the cohort 2 reference ranges were as follows: TSH 7.0% (median 5.1%), FT₃ 3.5% (median 4.1%), and FT₄ 96.3% (median 95.5%). When applying the cohort 2 reference range to cohort 1, the corresponding numbers were TSH 5.7% (median 5.8%), FT₃ 16.5% (median 17.4%), and FT₄ 95.6% (median 94.6%). As much as 100% of maternal FT₄ values within a gestational age-group were outside of the nonmethod specific reference range.

The two cohorts had rather large proportions of women with a TSH above 3.0 mU/l in all three trimesters (Fig. 2A). To account for this, we recalculated free thyroid hormone reference ranges including only women with a TSH of 0.1–2.5 mIU/l in the first trimester, 0.2–3.0 mIU/l in the second trimester, and 0.3–3.0 mIU/l in the third trimester. These TSH reference ranges were suggested by the American Thyroid Association (ATA) as standardized ranges applicable to all pregnant women (14). Doing so, there was still a significant difference between FT₃ and FT₄ for the two cohorts in all gestational age groups. By use of the reference ranges based on women with a TSH within the ATA-ranges, up to 18.2% (mean 10.6%) of the FT₃ values and 100% (mean 95.7%) of the FT₄ values were outside the other cohort’s reference range. When applying the ATA-guideline range for TSH, a mean of 1.9% (up to 4.0%) in cohort 1 and 4.2% (up to 8.2%) in cohort 2 had abnormal TSH-values, despite the fact that these women were within the limits of their own cohort-specific reference range. These proportions were similar to the ones found by applying the TSH-reference range from the other cohort (2.9% (up to 8.0%) and 3.1% (up to 5.1%) respectively). In both cohorts, the TSH reference ranges

**Figure 2**

Thyroid status in two cohorts of Danish pregnant women according to gestational age. Box plots of (A) TSH, (B) FT₃, and (C) FT₄ concentrations in cohorts 1 and 2 according to 5 week periods of gestation. Boxes represent the 25th–75th percentiles. Horizontal dark line represent the median. Whiskers indicate the 95% CI of the mean. Outliers are illustrated by squares (values > 1.5 interquartile ranges away from the 25th and 75th percentiles) and dots (values > 3 interquartile ranges away from the 25th and 75th percentiles). Horizontal lines across plots represent the calculated mean values of the laboratories’ non-pregnant reference ranges. *P < 0.05. TSH, thyroid-stimulating hormone; FT₃, free triiodothyronine; FT₄, free thyroxine.
had both higher lower and upper limits (Table 2) than the ones suggested by the ATA.

Z-scores were calculated for each woman based upon her own laboratory’s nonpregnant-reference range. The mean and S.D. were calculated assuming that the reference ranges were based on 95% CIs of the mean. This was only done for the free hormone values, as such an approach would not be suitable for a skewed distribution like the one for TSH values. The women outside the other cohorts’ z-score based FT4 reference range fell from a mean of 96.3 to 14.3% in cohort 1 and 95.6 to 24.3% in cohort 2 (Fig. 3E and F). The Z-score reference ranges for FT4 are provided in Table 3.

### Discussion

Consistent with other studies, we found that thyroid status changes with gestational age. While the development in thyroid hormone levels was similar between cohorts, the actual hormone levels were not. Using the gestational age-specific reference ranges obtained from another comparable cohort to interpret pregnant women’s thyroid status misclassified up to 100% of FT4 values. Although this study was not a methodological study comparing two assays applied to one set of blood samples (diagnostic accuracy study), it does provide an insight into the limited use of previously published reference ranges for pregnant women.

The two cohorts in this study consisted of women living in the Copenhagen area of Denmark, which is traditionally a mildly iodine-deficient area (17). The cohorts were gathered only 2 years apart, although with the latter cohort overlapping the transition of the Danish iodine fortification program from voluntary to mandatory (17). Although no urine samples were collected in either cohort to test for urinary iodine, a study by Ristic-Medic et al. (18) showed that the most reliable biomarker of iodine status in pregnancy is TSH. TSH levels did not differ

### Table 2

95% reference ranges for TSH, FT3, and FT4 in Danish pregnant women according to gestational age (weeks). Analyses were performed in two different laboratories using different methods: in cohort 1, EEG Wallac 1235 AutoDelfia automatic fluoroimmunoassay system, and in cohort 2, Roche Modular E170 electrochemiluminescence immunoassays. Results were based on log-transformed values, which were then inversed to yield the displayed results.

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Cohort 1</th>
<th>Cohort 2</th>
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<tr>
<td></td>
<td>2.5%</td>
<td>97.5%</td>
</tr>
<tr>
<td>TSH (mU/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–10</td>
<td>–</td>
<td>–</td>
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<tr>
<td>10–15</td>
<td>–</td>
<td>–</td>
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<tr>
<td>15–20</td>
<td>0.43</td>
<td>3.95</td>
</tr>
<tr>
<td>20–25</td>
<td>0.49</td>
<td>3.37</td>
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<tr>
<td>25–30</td>
<td>0.49</td>
<td>3.04</td>
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<tr>
<td>30–35</td>
<td>0.39</td>
<td>4.31</td>
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<tr>
<td>35–40</td>
<td>0.52</td>
<td>3.37</td>
</tr>
<tr>
<td>40+</td>
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<td>–</td>
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<tr>
<td>FT3 (pmol/l)</td>
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<tr>
<td>0–10</td>
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<tr>
<td>10–15</td>
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<tr>
<td>15–20</td>
<td>3.44</td>
<td>5.09</td>
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<tr>
<td>20–25</td>
<td>3.47</td>
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<td>30–35</td>
<td>3.28</td>
<td>4.86</td>
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<td>35–40</td>
<td>3.27</td>
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<tr>
<td>40+</td>
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<td>FT4 (pmol/l)</td>
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<td>0–10</td>
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<td>10–15</td>
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<tr>
<td>15–20</td>
<td>7.62</td>
<td>11.56</td>
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<tr>
<td>30–35</td>
<td>6.87</td>
<td>10.52</td>
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<tr>
<td>40+</td>
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TSH, thyroid-stimulating hormone; FT3, free triiodothyronine; FT4, free thyroxine.
Figure 3

Percentages of women whose thyroid status (TSH, FT₃, and FT₄ respectively) would be misclassified by use of a predetermined reference range. (A) Women in cohort 1 outside of own laboratory’s nonpregnant reference range. (B) Women in cohort 2 outside of own laboratory’s nonpregnant reference range. (C) Women in cohort 1 outside of cohort 2 gestational age-specific reference range. (D) Women in cohort 2 outside of cohort 1 gestational age-specific reference range. (E) Women in cohort 1 outside of cohort 2 Z-score-reference range based on cohort 2 nonpregnant reference range. (F) Women in cohort 2 outside of cohort 1 Z-score-reference range based upon cohort 1 nonpregnant reference range. TSH, thyroid-stimulating hormone; FT₃, free triiodothyronine; FT₄, free thyroxine.
Table 3 95% Z-score reference ranges for free T4 (FT4) levels in Danish pregnant women according to gestational age (weeks). Z-scores were based on the calculated mean and s.d. of the respective laboratories’ non-pregnant reference range for FT4.

<table>
<thead>
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<th>Weeks</th>
<th>Cohort 1</th>
<th>Cohort 2</th>
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<tr>
<td></td>
<td>2.5%</td>
<td>97.5%</td>
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<tr>
<td>FT4 (Z-score)</td>
<td></td>
<td></td>
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<tr>
<td>10–15</td>
<td>–</td>
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<tr>
<td>15–20</td>
<td>–3.6</td>
<td>–1.3</td>
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<tr>
<td>20–25</td>
<td>–4.0</td>
<td>–1.3</td>
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<tr>
<td>25–30</td>
<td>–4.1</td>
<td>–1.7</td>
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<tr>
<td>30–35</td>
<td>–4.0</td>
<td>–1.9</td>
</tr>
<tr>
<td>35–40</td>
<td>–4.2</td>
<td>–1.7</td>
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<tr>
<td>40+</td>
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significantly between cohorts, and the differences in FT4 concentrations remained the same when only including women with a TSH-level within the narrow range recommended in the ATA-guidelines. We therefore believe that it is reasonable to assume that the differences in FT4 concentrations could not be attributed to differences in iodine status.

Differences between the cohorts in demographics did not alter the conclusions when included in the mixed model analysis. Also, while the cohorts differed markedly in smoking status, Shields et al. (19) showed that smoking status did not affect FT4 concentrations. We therefore believe our results to show that even in women from the same geographical area, using the same methodological approach to calculating reference ranges, such reference ranges were highly laboratory-dependent and not applicable outside of its own clinical setting. This was especially true with regards to the analysis of free thyroid hormones.

Although TSH remains the gold standard for thyroid status monitoring during pregnancy (20), it is far from sufficient in most clinical settings (21), and the clinician must often rely on free hormone analyses to monitor a pregnant woman’s thyroid status. In a systematic review (22), the present authors showed that in pregnant women treated with antithyroid drugs, FT4 was a better indicator of maternal and fetal hypothyroidism than TSH. Also, recent guidelines from ATA (14) recommends that in hyperthyroid pregnant women, FT4 should be kept in the upper normal reference range of FT4 (regardless of the knowledge of pregnancy-related changes in both thyroid physiology and FT4-assay results). Further, the importance of free thyroid hormones for fetal development and obstetric outcome has been demonstrated in recent years (23, 24, 25). Henrichs et al. (26) found a significant delay in communication skills in children of women who had a low FT4 during the first trimester of pregnancy. Making the important distinction between maternal subclinical and clinical hypothyroidism, as well as diagnosing hypothyroxinemia, requires reliable testing of free thyroid hormones.

Regardless of the method used to estimate FT4 in pregnant women, this is susceptible to the extreme rise in thyroxine-binding globulin and thus the altered serum T4 binding (22, 27). As recently reviewed by Thienpont et al. (28), the gold standard methodology for determination of thyroid hormones is liquid chromatography (LC)/tandem mass spectrometry. However, due to the high costs of this method, RIA is still the most commonly used method in thyroid hormone analysis (28). The difficulty in obtaining consistent RIA-based measures of free thyroid hormones between laboratories has led to a current discussion of whether to recommend analysis of total T4 or FT4 index instead of FT4 (4, 29, 30, 31). The main benefit of total T4 analyses is a lower inter assay variability, which in turn facilitates the use of universal guidelines and reference ranges. As mentioned by Thienpont et al. (28), one can use the nonpregnant reference range for total T4 multiplied by 1.5 throughout pregnancy. However, the inter individual variability in total T4 is high, resulting in a broader reference range with greater diagnostic imprecision (10, 32). The latter can partly be overcome by comparing test results with former results from the same woman (10).

On the basis of our results, we suggest to work towards a standardization of FT4 levels. Standardization of highly method-dependent analyses is known from, i.e. the evaluation of prothrombin time by use of the international normalized ratio (33). With Z-scores calculated for each woman based on her own laboratory’s nonpregnant reference range, we showed a marked improvement in the comparability of FT4 levels between
the two cohorts. In our cohorts, the proportionate reaction of each method to the pregnancy-related changes seemed to be of equal size. Therefore, the use of Z-scores enabled a comparison between otherwise completely different absolute FT₄ values in women with similar TSH-levels, from the same region, and at an equal stage of pregnancy.

This study has great implications for the use of thyroid reference ranges in pregnant women. Universal thyroid function screening during pregnancy has recently been officially endorsed by members of the Endocrine Society (4). Little doubt remains that such screening will detect many women with unknown thyroid dysfunction (5, 34, 35). However, our results show that implementing screening programs will have a grave impact if doing so without prior establishment of method- and region-specific gestational age-related reference ranges.

Conclusion

Incorrect interpretation of maternal thyroid status is a great risk factor in the care of pregnant women. Such medical errors can alter treatment strategies to negatively impact the fetal development and obstetrical outcome. Maternal thyroid status must be interpreted in the context of the dynamic pregnancy-related changes in thyroid hormone production and metabolism. The impracticality and costs of establishing method-specific reference ranges for pregnant women make the use of pre-established reference ranges highly appealing. This study provided longitudinal gestational age-specific reference ranges from two cohorts based on different immunooassays. We demonstrated that while this provides a reliable option in the interpretation of TSH, using predetermined reference ranges to interpret free thyroid hormone values will misclassify up to 100% of pregnant women – even within populations from the same region and using the same methodological approach to establish the reference ranges. However, our results showed that a feasible approach to overcome such differences could be to implement Z-score-standardization of FT₄-levels across laboratories. While the importance of trimester-specific reference ranges has been widely stressed, the importance of method specificity of such reference ranges is largely neglected when publishing reference ranges from different populations – this study illustrates the problems of doing so. Hopefully, in the future, FT₄ methods will be compared with and calibrated against the ‘gold standard’ method, LC/tandem mass spectrometry.

Regardless of the purpose of thyroid function testing during pregnancy (implementation of screening strategies, monitoring of treatment for thyroid disorders, etc.), the care taker must be aware of the analytical pitfalls when doing so. In the care of pregnant women, patient safety will be compromised if method specificity is not added to gestational age-dependent reference ranges.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

D H Precht was in charge of the study design, clinical examinations, and data gathering in cohort 1, and supervised the statistical analyses. A Juul, T Larsen, and M Boas were in charge of data gathering in cohort 2. J Faber was in charge of the analyses of the thyroid autoantibodies in cohort 1. S Bliddal did the statistical analyses and prepared the manuscript. All authors contributed to the discussion and review of the manuscript.

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