

TSH and FT4 Reference Intervals in Pregnancy: A Systematic Review and Individual Participant Data Meta-Analysis

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Abstract

Context: Interpretation of thyroid function tests during pregnancy is limited by the generalizability of reference intervals between cohorts due to inconsistent methodology.

Objective: (1) To provide an overview of published reference intervals for thyrotropin (TSH) and free thyroxine (FT4) in pregnancy, (2) to assess the consequences of common methodological between-study differences by combining raw data from different cohorts.

Methods: (1) Ovid MEDLINE, EMBASE, and Web of Science were searched until December 12, 2021. Studies were assessed in duplicate. (2) The individual participant data (IPD) meta-analysis was performed in participating cohorts in the Consortium on Thyroid and Pregnancy.

Results: (1) Large between-study methodological differences were identified, 11 of 102 included studies were in accordance with current guidelines; (2) 22 cohorts involving 63 198 participants were included in the meta-analysis. Not excluding thyroid peroxidase antibody-positive participants led to a rise in the upper limits of TSH in all cohorts, especially in the first (mean +17.4%; range +1.6 to +30.3%) and second trimester (mean +9.8%; range +0.6 to +32.3%). The use of the 95th percentile led to considerable changes in upper limits, varying from -10.8% to -21.8% for TSH and -1.2% to -13.2% for FT4. All other additional exclusion criteria changed reference interval cut-offs by a maximum of 3.5%. Applying these findings to the 102 studies included in the systematic review, 48 studies could be used in a clinical setting.

Conclusion: We provide an overview of clinically relevant reference intervals for TSH and FT4 in pregnancy. The results of the meta-analysis indicate that future studies can adopt a simplified study setup without additional exclusion criteria.

Key Words: thyroid, pregnancy, reference values, thyrotropin (TSH), free thyroxine (FT4)

Abbreviations: ATA, American Thyroid Association; BMI, body mass index; FT4, free thyroxine; IPD, individual patient data; IVF, in vitro fertilization; SGA, small for gestational age; TgAb, thyroglobulin antibody; TPOAb, thyroid peroxidase antibody; TSH, thyrotropin.

Adequate thyroid hormone availability during pregnancy is important for an uncomplicated pregnancy as well as optimal fetal growth and development. Thyroid function test abnormalities during pregnancy are associated with a higher risk of adverse pregnancy and child outcomes (1-4). However, identifying thyroid function abnormalities during pregnancy is complicated by changes in maternal physiology. Furthermore, there is no universal reference interval for thyrotropin (TSH) or free thyroxine (FT4) during pregnancy due to considerable differences between assays as well as population characteristics (5-7). Current guidelines from international thyroid or endocrine societies, including the most recent 2017 guidelines by the American Thyroid Association (ATA), recommend the use of population- and trimester-specific TSH and FT4 reference intervals as the gold standard, calculated in a population with no known thyroid disease, optimal iodine status, and negative thyroid peroxidase antibody (TPOAb) status (4, 8, 9). However, for many laboratories these are unavailable because calculating reference intervals from a local reference population is often not feasible. Another option recently provided in the ATA guidelines either is to use a fixed cut-off for the upper limit of TSH of 4.0 mU/L or to subtract 0.5 mU/L from the nonpregnancy upper reference limit of TSH in the first trimester (4). While the method of using a fixed upper limit

for TSH may lead to considerable under- and overdiagnosis compared with the gold standard because of interpopulation and interassay differences (10), the method of subtracting an absolute number from the nonpregnancy reference interval has not been thoroughly researched.

The most recent addition to the ATA guidelines is the option to adopt reference intervals that were calculated in a center with a similar population and using the same assay, which is a step in between the gold standard and fixed upper TSH limit approach (4). However, identification of adoptable TSH and FT4 reference intervals is cumbersome due to a lack of overview of all published data regarding thyroid hormone reference intervals. Moreover, large methodological differences exist between studies as a result of new insights and changing guidelines (4, 8, 11). One example of this is the use of additional exclusion criteria on top of those recommended by the current ATA guidelines, most of which remain of unknown significance, such as thyroglobulin antibody (TgAb) positivity, conception by in vitro fertilization (IVF), pregnancy complications, and characteristics including pre-existing diabetes mellitus, hypertension, aberrant body mass index (BMI), and active smoking. Although some of these factors are determinants of TSH and FT4 concentrations, only some, but not all, studies show that exclusion of women

according to these determinants affects TSH and FT4 reference intervals (12-17). Another example of between-study differences in methodology is the calculation of the reference intervals. Some studies define reference intervals by the 5th and 95th percentiles, whereas in routine laboratory practice, the 2.5th to 97.5th percentiles are typically used (18). While the methods of many studies are not in line with current international guidelines from thyroid or endocrine societies, it remains unknown to what extent this affects the generalizability of the calculated reference intervals.

To aid clinicians in adopting suitable reference intervals for their center and for the purpose of future research, the aims of our study were (1) to provide an overview of methodologically valid and clinically useful published gestational TSH and FT4 reference intervals; and (2) to perform a meta-analysis of individual participant data (IPD) from a consortium of cohorts to add gestational TSH and FT4 reference intervals to the literature, and utilize these data to assess the validity and clinical relevance of using additional exclusion criteria.

Materials and Methods

For the first study aim, we performed a systematic literature search to identify all available studies on TSH and/or FT4 reference intervals during pregnancy (Fig. 1). To address the second aim, we performed an IPD meta-analysis within the Consortium on Thyroid and Pregnancy.

Systematic Review

We searched Ovid MEDLINE, EMBASE and Web of Science databases from inception to December 12, 2021, without language restrictions (document 1 (24)). All studies with data on TSH and/or FT4 in an unselected (ie, without selection based on health indicators) population of pregnant women were included. For the main figures (Figs. 2-4), we reported reference intervals calculated using the 2.5th to 97.5th percentile and after exclusion of participants with pre-pregnancy thyroid disease, thyroid hormone altering medication use and/or TPOAb-positivity. We then excluded studies in which additional exclusion criteria of unknown significance were applied for selecting the reference population (eg, the exclusion of participants with non-thyroidal autoimmune disease). We also excluded studies in populations with severe iodine

deficiency (defined as urinary iodine secretion <50 µg/L (19)) as assessed in the cohort or, if unavailable, as reported by the WHO or regional studies (20, 21). Mild-to-moderate iodine deficiency (50-149 µg/L) was not a criterion for exclusion, since TSH and FT4 reference intervals do not meaningfully differ from iodine-sufficient reference intervals (22, 23). Finally, studies in which reference intervals were not reported or in which the exact methods for reference interval determinations could not be extracted were excluded. Studies which published reference intervals which covered less than 2 gestational weeks were not included in the final overview, as these were deemed to be less useful to clinicians. If additional exclusion criteria for the reference population were used that could be assessed in the meta-analyses, studies were included if the additional exclusion criteria led to a maximum of 5% variability around the reference limit as compared to the gold standard. If additional exclusion criteria could not be assessed in the meta-analysis, or if the criteria led to more than 5% variability around the reference limits, the studies were excluded from the final overview (Figs. 2-4).

Possible studies for inclusion were independently assessed for suitability in duplicate (title and abstracts: A.D. and T.K.; full texts: A.D. and J.O.), and any disagreement was resolved by discussion with a third author (J.O. or T.K., respectively). Additional records were retrieved through citation searching. The study protocol was preregistered which can be found elsewhere along with protocol violations.

Individual Participant Data Meta-analysis

Eligible studies were those that participated in the Consortium on Thyroid and Pregnancy (<https://www.consortiumthyroidpregnancy.org>), an international research collaboration that aims to study gestational thyroid (dys) function physiology, determinants, and clinical risk profiles. Information on regional iodine status and assays used in each cohort can be found elsewhere (Tables 1 and 2 (24)). Reference intervals were calculated as the 2.5th to 97.5th interval after excluding participants with known thyroid disease, use of thyroid (interfering) medication, multiple gestation, and/or TPOAb positivity. To quantify the effects of between-study methodological differences of TSH and FT4 reference interval calculations, reference intervals were also calculated using 9 different methodologies in addition to the methodology described

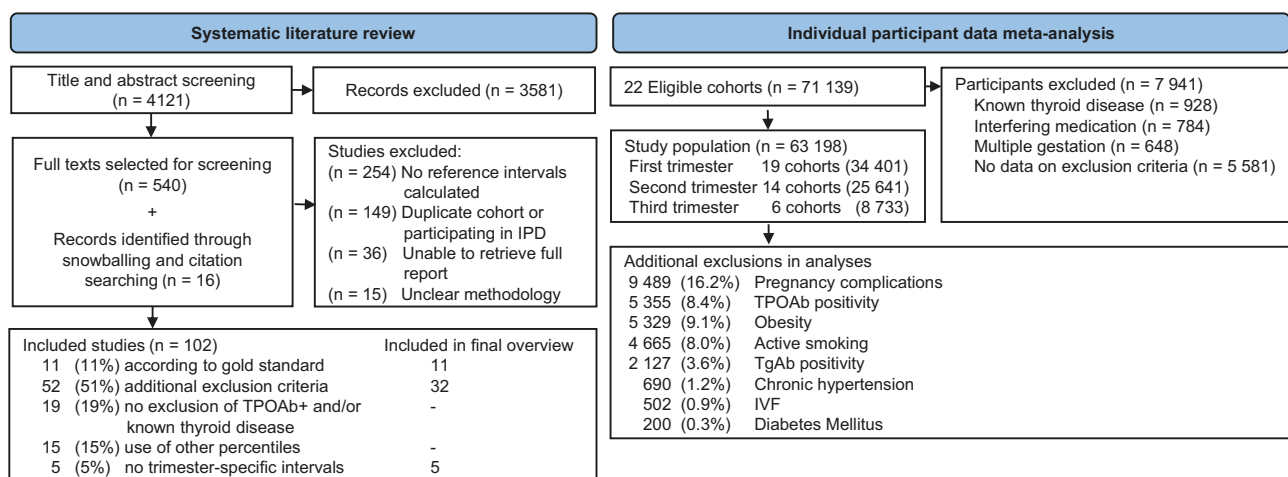


Figure 1. Study selection flowchart.

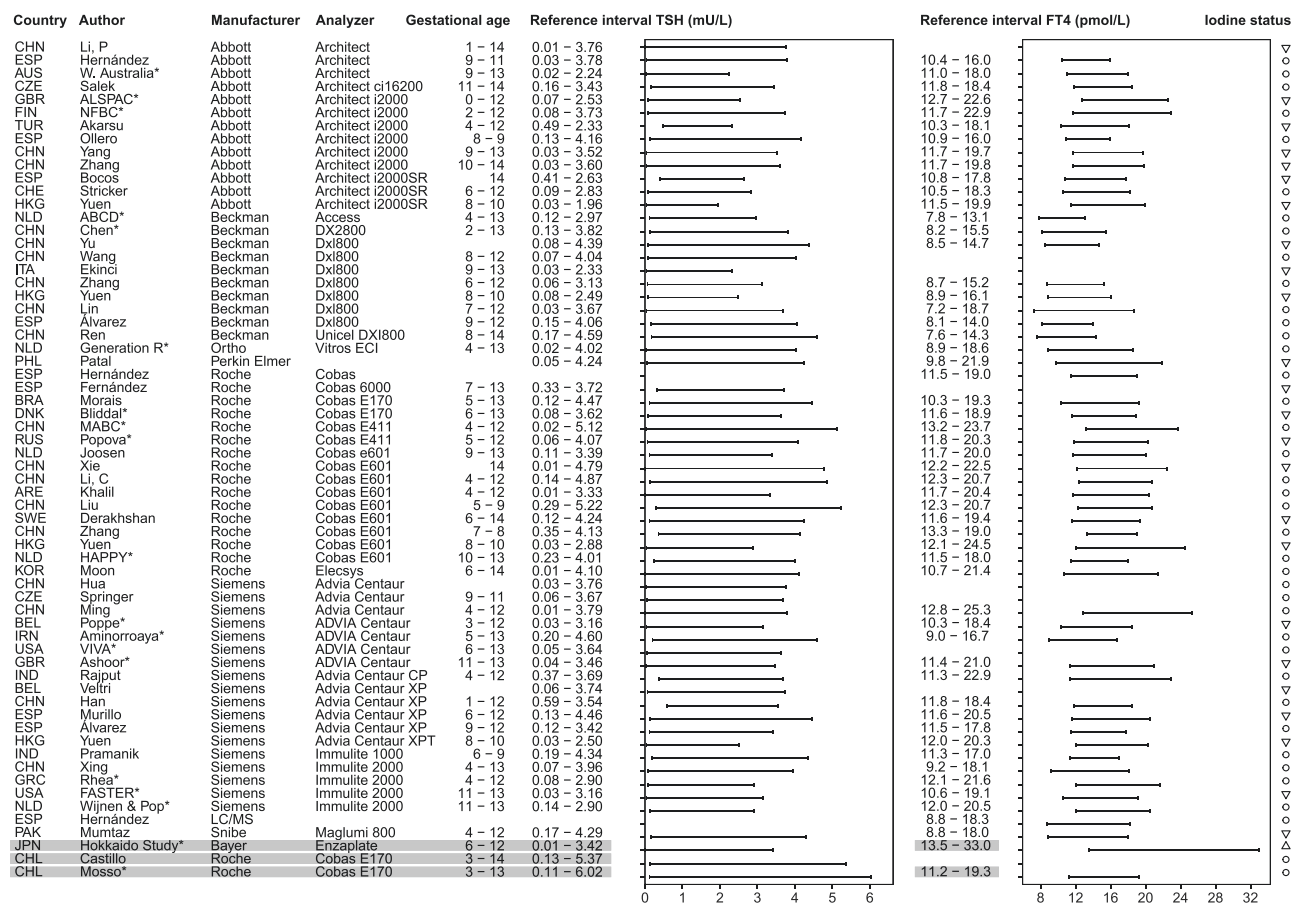


Figure 2. Overview studies systematic review trimester 1. *Reference intervals calculated with individual participant data in consortium. O, iodine sufficiency; ∇, mild-to-moderate iodine deficiency; Δ, excessive iodine status. Reference intervals calculated in cohorts with fluctuating or excessive iodine status are listed in grey.

above: (1) without excluding TPOAb-positive participants, (2) using the 5th to 95th percentiles, and using additional exclusion criteria defined as (3) prepregnancy diabetes mellitus, (4) essential hypertension, (5) obesity, defined as a BMI > 30 kg/m² at time of the first study visit, (6) active smoking, (7) conception by IVF, (8) exclusion of TgAb positivity, or (9) pregnancy complications, defined as gestational diabetes, gestational hypertension, pre-eclampsia, preterm birth, and/or small for gestational age (SGA). We defined the trimesters as 0 to 13 weeks, 13 to 27 weeks, and >27 weeks of gestation.

Statistical Analyses

Trimester-specific reference intervals were calculated per cohort including participants with data on exclusion criteria. For cohorts containing participants with repeated measurements, we used the first available sample for each trimester. Outliers were removed if values were inaccurate (eg, TSH or FT4 above or below detection limit). All analyses were performed using IBM SPSS Statistics for Windows (version 25.0) and R 4.1.1 for Windows.

Results

Systematic Review (Aim 1)

We identified 4121 published reports, of which 540 were eligible for inclusion based on title and abstract screening. After reading full texts and adding 16 articles identified through

snowballing, 102 articles were included in the systematic review (Fig. 1). Out of all included studies, 11 (11%) reported reference intervals calculated in accordance with the current ATA guidelines, 52 (51%) studies used additional exclusion criteria for the selection of the reference population (ie, participants with acute or chronic illnesses, pre-existing hypertension or diabetes mellitus, autoimmune disease, and some or all pregnancy complications defined above), 15 (15%) used percentile cut-offs other than the 2.5th to 97.5th percentiles (mostly the 5th to 95th percentiles (8)) (Fig. 1). An overview of selected TSH and FT4 reference intervals is presented in Figs. 2-4, which either adhere to the ATA guidelines (11 studies), or use additional exclusion criteria which had less than 5% effect on the reference limits according to our meta-analysis (32 studies, see below). Data (including future updates) can be downloaded from <https://www.consortiumthyroidpregnancy.org/referenceintervals>.

Individual Participant Data Meta-analysis (Aim 2)

At time of analysis, 71 139 participants in 22 cohorts participating in the Consortium on Thyroid and Pregnancy were eligible for inclusion. After exclusions, the final study population comprised 63 198 women (Fig. 1). Between all included cohorts, the upper limit for TSH ranged from 2.24 to 6.02 mU/L in the first trimester, from 2.67 to 6.15 mU/L in the second trimester and from 3.03 to 6.13 mU/L in the third trimester (third trimester data from n = 2 cohorts). The lower FT4 limit ranged from 7.8 to 13.2 pmol/L (0.61-1.03 ng/dL)

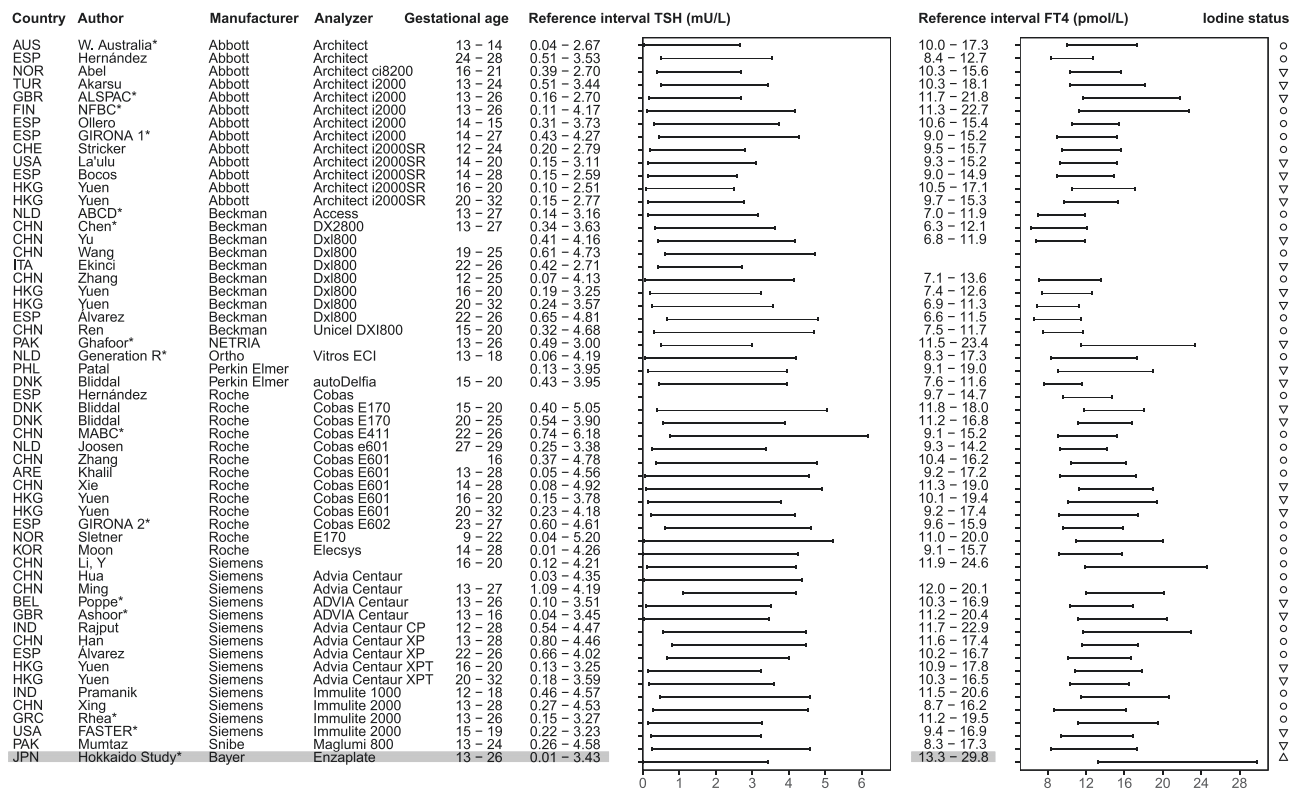


Figure 3. Overview studies systematic review trimester 2. *Reference intervals calculated with individual participant data in consortium. ○, iodine sufficiency; ▽, mild-to-moderate iodine deficiency; △, excessive iodine status. Reference intervals calculated in cohorts with fluctuating or excessive iodine status are listed in gray.

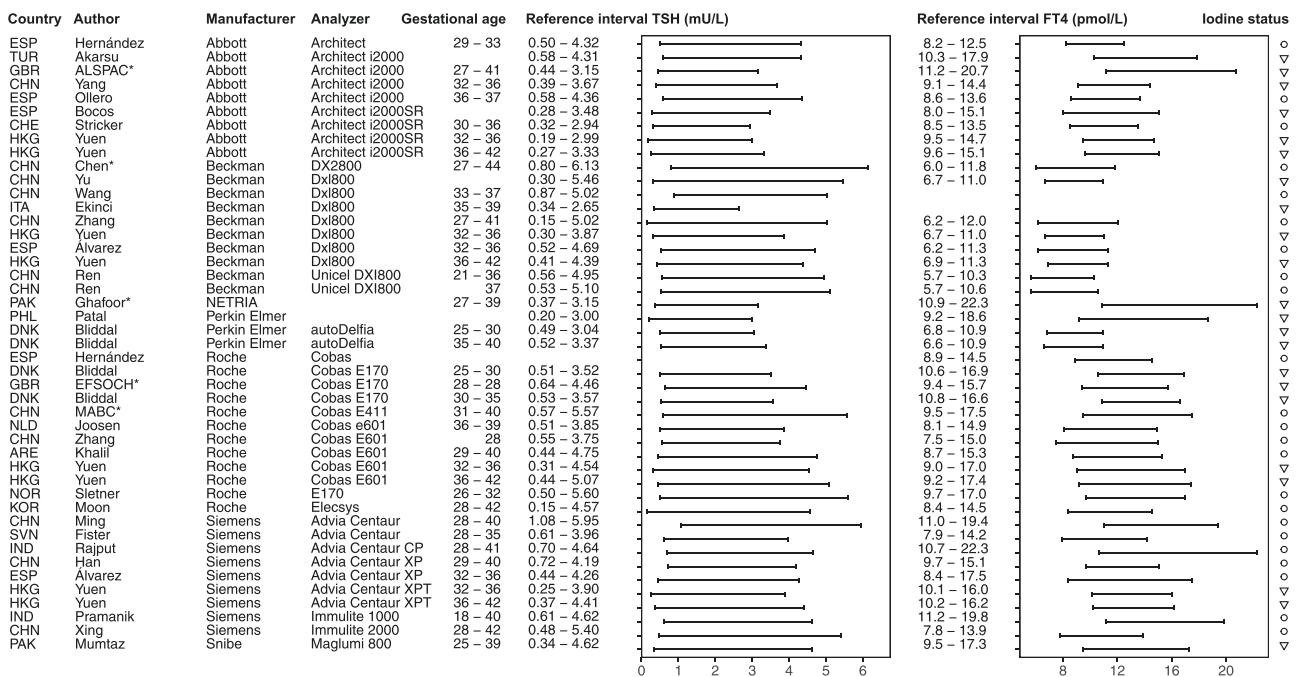


Figure 4. Overview studies systematic review trimester 3. *Reference intervals calculated in individual participant data in consortium; ○, iodine sufficiency; ▽, mild-to-moderate iodine deficiency.

in the first trimester, from 7.1 to 11.5 pmol/L (0.55-0.89 ng/dL) in the second trimester, and from 9.5 to 11.1 pmol/L (0.74-0.86 ng/dL) in the third trimester (third trimester data from n = 2 cohorts). The upper limit of FT4 ranged from 13.2 to 23.8 pmol/L (1.03-1.85 ng/dL) in the first trimester,

from 11.8 to 22.7 pmol/L (0.92-1.76 ng/dL) in the second trimester, and 17.5 to 19.6 pmol/L (1.36-1.52 ng/dL) in the third trimester (third trimester data from n = 2 cohorts). Cohort-specific reference intervals can be found elsewhere (Tables 4-9 (24)).

Compared with the 2.5th to 97.5th population-based reference intervals, the use of the 5th to 95th percentiles resulted in an upper limit of TSH that was 0.63 mU/L (range 0.35-1.22) lower in the first trimester, 0.65 mU/L (range 0.29-0.83) lower in the second trimester, and 0.73 mU/L (range 0.70-0.73) lower in the third trimester (Fig. 5; Tables 4-6 (24)). For FT4, the lower limits were 0.3 to 1.3 pmol/L (0.02-0.10 ng/dL) higher in the first trimester, 0.0 to +1.0 pmol/L (0.0-0.08 ng/dL) in the second trimester, and +0.5 to +1.2 pmol/L (0.04-0.09 ng/dL) in the third trimester (Fig. 6). The upper limit of FT4 was -0.4 to -2.2 pmol/L lower among cohorts in the first trimester, -0.2 to -3.0 pmol/L in the second trimester, and -0.4 to -0.7 pmol/L in the third trimester (Fig. 7; Tables 7-9 (24)).

Not excluding TPOAb-positive participants led to an increase in the upper limit of TSH in all cohorts, with a mean increase of 0.65 mU/L (range 0.05-1.34) in the first trimester, 0.42 mU/L (range 0.02-0.83) in the second trimester, and 0.14 mU/L (range 0.08-0.65) in the third trimester (Fig. 5; Tables 4-6 (24)). No meaningful changes were observed in the lower and upper reference limits of FT4 (Figs. 6 and 7; Tables 7-9 (24)).

All other additional exclusion criteria, namely pre-existing diabetes mellitus or essential hypertension, obesity, active smoking, conception by IVF, TgAb positivity or any pregnancy complication, led to less than 3.5% variation around the upper limits of TSH and the lower and upper limits of FT4, without a clear trend toward an increase or decrease in the reference limits (Figs. 5-7).

Discussion

In this study, we present an overview of reference intervals for TSH and FT4 during pregnancy, exhibiting widely differing absolute values for TSH and FT4 reference intervals and considerable heterogeneity in the methods used for reference interval calculations. Furthermore, in an IPD meta-analysis, we showed that inclusion of TPOAb-positive women was associated with a considerable increase in the upper limits for TSH but not FT4 reference intervals, while the use of a 5th to 95th percentile range led to a considerable decrease in the upper limit for both TSH and FT4 reference intervals as well as an increase in the lower limit for the FT4 interval. On the other hand, none of the studied additional reference population selection criteria meaningfully affected TSH and FT4 reference intervals.

Systematic Review (Aim 1)

In our systematic review, we identified that out of all published studies reporting reference intervals in the literature, at least 48 out of 102 studies can be implemented in clinical settings. These studies published reference intervals which either adhere to the most recent ATA guidelines or used additional exclusion criteria which resulted in less than 5% change of the reference limit, as assessed in our meta-analysis. The considerable variation in methods is partly explained by progressive insight and changing guidelines over the years. However, well before this time, guidelines by various clinical chemistry societies for reference interval determinations were already

Additional exclusions	First trimester			Second trimester			Third trimester		
	N cohorts (participants)	Pooled difference in upper limit ¹ (mU/L)	Range of difference in cohorts	N cohorts (participants)	Pooled difference in upper limit ¹ (mU/L)	Range of difference in cohorts	N cohorts (participants)	Pooled difference in upper limit ¹ (mU/L)	Range of difference in cohorts
Gold standard ²	11 (17,924)			9 (10,929)			2 (2,957)		
Without TPOAb+ exclusion	11 (19,723)	0.6	+0.05 to +1.34	9 (11,726)	0.4	+0.02 to +0.83	2 (3,216)	0.1	+0.08 to +0.65
5th - 95th percentile	11 (17,924)	-0.6	-1.22 to -0.35	9 (10,929)	-0.7	-0.83 to -0.29	2 (2,957)	-0.7	-0.73 to -0.70
Diabetes mellitus ³	11 (17,795)	0.0	-0.01 to +0.01	9 (10,829)	0.0	-0.06 to +0.02	2 (2,950)	0.0	0 to 0
Essential hypertension	11 (17,521)	0.0	-0.08 to +0.07	9 (10,775)	0.0	-0.02 to +0.03	2 (2,943)	0.0	0 to +0.02
Obesity ⁴	11 (16,467)	0.0	-0.15 to +0.11	9 (10,016)	0.0	-0.08 to +0.08	2 (2,918)	0.0	0 to +0.02
Active smoking	11 (16,078)	0.0	-0.06 to +0.11	9 (9,858)	0.0	-0.24 to +0.14	2 (2,880)	0.0	0 to +0.06
Gold standard ²	17 (20,121)			13 (19,002)					
IVF ⁵	17 (19,811)	0.0	-0.03 to +0.05	13 (18,826)	0.0	-0.02 to +0.04			
Gold standard ²	10 (21,306)			8 (16,555)			2 (7,044)		
TgAb+	10 (20,264)	-0.1	-0.21 to +0.08	8 (15,855)	-0.1	-0.19 to +0.10	2 (6,808)	0.0	-0.08 to 0
Gold standard ²	17 (22,533)			13 (20,306)			4 (7,260)		
Pregnancy complications ⁶	17 (18,986)	0.0	-0.51 to +0.29	13 (17,303)	0.0	-0.02 to +0.18	4 (6,370)	0.0	-0.04 to +0.01

Figure 5. Results meta-analyses trimester 1. ¹Defined as the weighted average change in reference limits across cohorts. ²Calculated using 2.5th to 97.5th percentiles, excluding prepregnancy thyroid disease, use of thyroid hormone-altering medication, and TPOAb positivity; the total number of participants in subgroups differs based on availability of data on additional exclusion criteria. ³Defined as prepregnancy diabetes mellitus. ⁴Defined as BMI > 30 kg/m² at time of intake. ⁵Pregnancy by in vitro fertilization. ⁶Defined as gestational diabetes, gestational hypertension, pre-eclampsia, preterm birth, and/or small for gestational age.

Additional exclusions	First trimester			Second trimester			Third trimester		
	N cohorts (participants)	Pooled difference in lower limit ¹ (pmol/L)	Range of difference in cohorts	N cohorts (participants)	Pooled difference in lower limit ¹ (pmol/L)	Range of difference in cohorts	N cohorts (participants)	Pooled difference in lower limit ¹ (pmol/L)	Range of difference in cohorts
Gold standard ²	10 (17,435)			9 (10,962)			2 (2,955)		
Without TPOAb+ exclusion	10 (19,153)	-0.1	-0.3 to 0	9 (11,768)	0.0	-0.1 to 0	2 (3,215)	0.0	0 to 0
5th - 95th percentile	10 (17,435)	0.5	+0.3 to +1.3	9 (10,962)	0.5	0 to +1.0	2 (2,955)	0.6	+0.5 to +1.2
Diabetes mellitus ³	10 (17,305)	0.0	0 to 0	9 (10,862)	0.0	0 to 0	2 (2,948)	0.0	0 to 0
Essential hypertension	10 (17,043)	0.0	0 to 0	9 (10,807)	0.0	-0.1 to +0.1	2 (2,942)	0.0	0 to 0
Obesity ⁴	10 (16,038)	0.0	0 to +0.5	9 (10,046)	0.1	-0.1 to +0.2	2 (2,916)	0.0	0 to 0
Active smoking	10 (15,681)	0.0	0 to +0.1	9 (9,889)	0.0	-0.1 to +0.1	2 (2,879)	0.0	-0.2 to 0
Gold standard ²	10 (20,238)			8 (19,051)					
IVF ⁵	10 (19,927)	0.0	-0.3 to 0	8 (18,875)	0.0	-0.1 to 0			
Gold standard ²	10 (21,370)			8 (16,753)			2 (7,177)		
TgAb+	10 (20,325)	0.0	-0.1 to +0.1	8 (15,969)	0.0	-0.1 to +0.1	2 (6,884)	0.0	0 to 0
Gold standard ²	11 (21,956)			8 (20,320)			2 (7,260)		
Pregnancy complications ⁶	11 (18,512)	0.0	0 to +0.3	8 (17,312)	0.1	-0.1 to +0.1	2 (6,370)	0.0	0 to 0

Figure 6. Results meta-analyses trimester 2. ¹Defined as the weighted average pooled change in reference limits across cohorts. ²Calculated using 2.5th to 97.5th percentiles, excluding pre-pregnancy thyroid disease, use of thyroid hormone altering medication, and TPOAb-positivity; the total number of participants in subgroups differs based on availability of data on additional exclusion criteria. ³Defined as prepregnancy diabetes mellitus. ⁴Defined as BMI > 30 kg/m² at time of intake. ⁵Pregnancy by in vitro fertilization. ⁶Defined as gestational diabetes, gestational hypertension, pre-eclampsia, preterm birth, and/or small for gestational age.

Additional exclusions	First trimester			Second trimester			Third trimester		
	N cohorts (participants)	Pooled difference in upper limit ¹ (pmol/L)	Range of difference in cohorts	N cohorts (participants)	Pooled difference in upper limit ¹ (pmol/L)	Range of difference in cohorts	N cohorts (participants)	Pooled difference in upper limit ¹ (pmol/L)	Range of difference in cohorts
Gold standard ²	10 (17,435)			9 (10,962)			2 (2,955)		
Without TPOAb+ exclusion	10 (19,153)	0.1	-0.3 to +0.8	9 (11,768)	0.0	-0.2 to +0.2	2 (3,215)	0.1	+0.1 to +0.1
5th - 95th percentile	10 (17,435)	-1.6	-2.2 to -0.4	9 (10,962)	-1.2	-3.0 to -0.2	2 (2,955)	-0.7	-0.7 to -0.4
Diabetes mellitus ³	10 (17,305)	0.0	-0.1 to 0	9 (10,862)	0.0	0 to +0.1	2 (2,948)	0.0	0 to 0
Essential hypertension	10 (17,043)	0.0	0 to +0.2	9 (10,807)	0.0	-0.3 to +0.2	2 (2,942)	0.0	0 to +0.1
Obesity ⁴	10 (16,038)	0.1	0 to +0.4	9 (10,046)	0.2	-0.2 to +1.1	2 (2,916)	0.0	0 to 0
Active smoking	10 (15,681)	0.0	-0.1 to +0.9	9 (9,889)	0.0	-0.9 to +0.2	2 (2,879)	0.0	0 to 0
Gold standard ²	10 (20,238)			8 (19,051)					
IVF ⁵	10 (19,927)	0.0	-0.1 to +0.6	8 (18,875)	0.0	-0.2 to +0.1			
Gold standard ²	10 (21,370)			8 (16,753)			2 (7,177)		
TgAb+	10 (20,325)	0.0	-0.2 to +0.4	8 (15,969)	0.0	-0.3 to +0.1	2 (6,884)	0.0	0 to 0
Gold standard ²	11 (21,956)			8 (20,320)			2 (7,260)		
Pregnancy complications ⁶	11 (18,512)	0.1	-0.2 to +0.9	8 (17,312)	0.0	-0.2 to +1.1	2 (6,370)	0.1	0 to +0.1

Figure 7. Results meta-analyses trimester 3. ¹Defined as the weighted average change in reference limits across cohorts. ²Calculated using 2.5th to 97.5th percentiles, excluding prepregnancy thyroid disease, use of thyroid hormone altering medication, and TPOAb-positivity; the total number of participants in subgroups differs based on availability of data on additional exclusion criteria. ³Defined as prepregnancy diabetes mellitus. ⁴Defined as BMI >30 kg/m² at time of intake. ⁵Pregnancy by in vitro fertilization. ⁶Defined as gestational diabetes, gestational hypertension, pre-eclampsia, preterm birth, and/or small for gestational age.

advising similar selection criteria, including but not limited to excluding TPOAb positivity, prepregnancy thyroid disease, thyroid-interfering medication use, and the use of a 2.5th to 97.5th percentile range (11). We identified that these key selection criteria were not met for 37% (20% lack of advised exclusion criteria +17% use of different percentiles) of all published studies, potentially causing relevant under- and overdiagnosis of gestational thyroid disease. This is particularly important in light of the recommendation in the current ATA guidelines that TSH and FT4 reference intervals can be adopted from similar patient populations using similar TSH assays. Interestingly, we also identified that 54% of all studies in the published literature used additional exclusion criteria for the selection of a reference population. To our knowledge, this is the first study to systematically quantify the effects of such exclusions on TSH and FT4 reference intervals during pregnancy in a multicohort setting, showing no meaningful effects besides decreasing the study population sample size. On the one hand, these results validate currently available studies that have used these additional exclusion criteria. On the other hand, our results also caution against the use of such methods because a decrease in the size of a study population due to unnecessary exclusions can affect the precision of reference interval calculations.

A notable observation from the results of the systematic review is the large variation in reference limits within the same assays, as it can be observed in Figs. 2-4. Furthermore, while no formal test for differences between reference intervals has been carried out, it can be observed that the Beckman assay has relatively lower FT4 reference intervals than the other assays. This demonstrates the impact of preanalytical factors as well as determinants of thyroid function tests on the variation between populations, and the importance of locally derived reference intervals for TSH and FT4 in pregnancy.

Regarding iodine status, iodine deficiency is associated with a higher risk of thyroid dysfunction, and inclusion of participants with iodine deficiency might lead to unreliable reference intervals. However, 2 recently published studies show no significant differences in reference limits when including mild to moderate iodine-insufficient participants (urinary iodine secretion 50-149 µg/L), which calls for a more lenient recommendation regarding the iodine status of the reference population (22, 23). On the other hand, chronic excessive iodine intake may lead to hypothyroidism, while a sudden increase in iodine intake may lead to transient hyperthyroidism (25). For instance, the Chilean population has a history of chronic high iodine intake following the iodine fortification program

in 1979, which resulted in a reduction of fortification in 2000 (26). These fluctuations might explain the relatively high upper reference limits for TSH in the Chilean cohort in the IPD meta-analysis and the Chilean study included in the systematic review, although the inclusion periods were well over a decade past the reduction of the fortification dose. One Japanese cohort in the consortium had excessive iodine intake at the time of inclusion, which may explain the relatively high reference limits of FT4. Iodine status, in particular severe deficiency or excessive intake, influences TSH and FT4 concentrations considerably, and thyroid function test measurements are highly dependent on the timing and amount of iodine intake. Reference intervals calculated in a region known for an excessive or fluctuating iodine status are listed in gray in the final overview (Figs. 2-4) and should be interpreted with care. In addition, it is also important to realize that geographic determinants of thyroid function tests other than iodine status, such as ethnicity or genetics, exposure to endocrine disrupting chemicals, and harmonization of local laboratories, affect the variation in TSH and FT4 reference intervals. These should be taken into account when adopting reference intervals, most pragmatically by using locally derived reference intervals.

Individual Participant Data Meta-analysis (Aim 2)

One of the most prominent quantifications of the IPD analyses we performed is that not excluding TPOAb-positive women when calculating reference intervals considerably increases the upper reference limit of TSH, especially in the first (+0.65 mU/L) and second trimester (+0.42 mU/L). The larger effect in the first trimester coincides with the physiological peak of human chorionic gonadotropin and the higher prevalence of TPOAb positivity in early pregnancy. Between the cohorts in this study, 3% to 18% of all participants are TPOAb-positive, which is associated with an impaired thyroidal response to human chorionic gonadotropin stimulation and a higher risk of adverse pregnancy outcomes (27-29). It is noteworthy that 18% of all studies in the literature did not exclude TPOAb-positive participants. This specifically leads to underdiagnosis of TPOAb-positive subclinical hypothyroidism, for which levothyroxine treatment is recommended (4). The subsequent potential risk of under treatment emphasizes the importance of excluding TPOAb-positive women from the reference population. The additional exclusion of TgAb positivity on top of TPOAb positivity only led to a 3.5% mean decrease of the upper reference limit of TSH. Studies on the risk of adverse pregnancy outcomes in TgAb-positive women have been inconsistent (30-32), but the

lack of a relevant effect (defined as at least 5% variability around the reference limit) of excluding this group on the reference limits is in line with the results of a recent prospective Swedish cohort study (12). This was further established in a recent study by the Consortium on Thyroid and Pregnancy wherein TPOAbs were shown to have a stronger association with higher TSH concentrations than TgAbs, and where the association with TgAb concentrations lost significance when adjusting for TPOAb concentration (33). The results of the current meta-analysis confirm the validity of reference intervals from previous studies in which TgAb-positive women were additionally excluded, but also implicate no added value from the additional efforts and costs related to measurement of TgAbs for studies with the purpose of defining TSH and FT4 reference intervals during pregnancy.

Another important finding of the IPD is the substantial change in reference limits when using the 5th to 95th percentile range rather than the internationally advised 2.5th to 97.5th percentile range (18). This effect was consistent through all trimesters for the upper limit of TSH (for the first, second, and third trimester: -0.63 , -0.65 , and -0.73 mU/L, respectively), the lower limit of FT4 ($+0.5$, $+0.5$, $+0.6$ pmol/L, or $+0.04$, $+0.04$, $+0.05$ ng/dL) and in a declining trend for the upper limit of FT4 (-1.6 , -1.2 , -0.7 pmol/L, or -0.12 , -0.09 , -0.05 ng/dL). Especially TSH, and to a lesser extent FT4, concentrations in pregnancy follow a right skewed distribution, which explains the relatively larger absolute effect of percentile shifts on the upper reference limit. Narrowing the reference intervals by using reference cut-offs above the 2.5th and below the 97.5th percentile (eg, the 5th to 95th percentile as assessed in this study) will result in more women diagnosed with thyroid disease in clinical settings purely from a mathematical point of view. Moreover, since predominantly the upper rather than the lower reference limits of TSH and FT4 are affected, specifically more women will be diagnosed with subclinical hypothyroidism and overt hyperthyroidism. To our knowledge, no studies have systematically assessed whether women with TSH or FT4 concentrations between the 95th and 97.5th percentile are also at increased risk of adverse pregnancy events and whether they might benefit from treatment. Implementing reference intervals based on the 5th to 95th percentiles may therefore lead to considerable overdiagnosis and overtreatment in the clinical setting.

Interestingly, all other additional exclusion criteria (eg, pre-existing diabetes mellitus, essential hypertension, obesity, active smoking, IVF, and all pregnancy complications) assessed in the IPD did not meaningfully change the reference limits. This is despite opposite results in some single-center studies and considering that most factors are known determinants of mean TSH and/or FT4 concentrations in a nonpregnant population (34). One interesting example of this is BMI. While a higher BMI is positively associated with TSH, and negatively with FT4 in nonpregnant individuals (35), the results in pregnancy are inconclusive (36–38), and the effect of excluding obese participants for calculating reference intervals is largely unknown. Two cohort studies from Finland and China demonstrated a positive correlation between BMI and TSH in pregnancy (16, 38); however, while a higher BMI was associated with a higher upper limit of TSH in the Finnish cohort, in the Chinese cohort a higher BMI was associated with a lower reference limit of TSH. The results of the current study show that additional exclusion of women with pregnancy complications on average led to less than 1.5% (range between cohorts -8% to $+7\%$) change of TSH and

FT4 reference limits. While the lack of clinically meaningful changes with these additional exclusions does not affect the validity of studies that incorporated them in the past, future studies should avoid these exclusion criteria as they may limit precision of study results by decreasing the total number of participants in a study population.

For this study, we were able to summarize key features of TSH and FT4 reference intervals in pregnant women and further elucidate and quantify the relevance of commonly encountered deviations from guideline methodology standards using a large dataset consisting of worldwide prospective cohort studies. The interpretation of the results of our systematic literature overview is limited by the details communicated in the original reports. Underreporting of relevant details in original work may have affected the generalizability of our results to the literature as a whole and may have resulted in exclusion of studies which published valid reference intervals. Furthermore, analyses focusing on exclusion criteria which are uncommon in a population, such as pre-existing diabetes in young women, are not likely to meaningfully change the reference limits, because only a small number of participants are excluded. These results may not be directly generalizable to populations which have a substantially higher prevalence of the analyzed exclusion criteria. Moreover, this study was limited by data available in the included cohorts and therefore it was not possible to assess the impact of all additional exclusion criteria found in the literature, for instance the additional exclusion of participants with a nonthyroidal autoimmune disease.

In conclusion, this systematic review and IPD meta-analysis provides an overview with available reference intervals which can be adopted in clinical settings, taking population and assay similarity into account. The importance of excluding TPOAb-positive participants and the use of proper percentiles as defined by international guidelines is emphasized, and future studies aiming to calculate reference intervals can adopt a simplified study setup in terms of exclusion criteria for the reference population.

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Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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