Longitudinal Trajectories of Gestational Thyroid Function: A New Approach to Better Understand Changes in Thyroid Function

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Context: Most studies of thyroid function changes during pregnancy use a cross-sectional design comparing means between groups rather than similarities within groups.

Objective: Latent class growth analysis (LCGA) is a novel approach to investigate longitudinal changes that provide dynamic understanding of the relationship between thyroid status and advancing pregnancy.

Design: Prospective observational study with repeated assessments.

Setting: General community.

Patients: Eleven hundred healthy women were included at 12 weeks' gestation.

Main Outcome Measures: The existence of both free T4 (fT4) and TSH trajectories throughout pregnancy determined by LCGA.

Results: LCGA revealed three trajectory classes. Class 1 (n = 1019; 92.4%), a *low increasing TSH* reference group, had a gradual increase in TSH throughout gestation (from 1.1 to 1.3 IU/L). Class 2 (n = 30; 2.8%), a *high increasing TSH* group, displayed the largest increase in TSH (from 1.9 to 3.3 IU/L). Class 3 (n = 51; 4.6%), a *decreasing TSH* group, had the largest fall in TSH (from 3.2 to 2.4 IU/L). Subclinical hypothyroidism at 12 weeks occurred in up to 60% of class 3 women and was accompanied by elevated thyroid peroxidase antibodies (TPO-Ab) titers (50%) and a parental history of thyroid dysfunction (23%). In class 2, 70% of women were nulliparous compared with 46% in class 1 and 49% in class 3.

Conclusions: LCGA revealed distinct trajectories of longitudinal changes in fT4 and TSH levels during pregnancy in 7.4% of women. These trajectories were correlated with parity and TPO-Ab status and followed patterns that might reflect differences in pregnancy-specific immune tolerance between nulliparous and multiparous women. **(*J Clin Endocrinol Metab* 103: 2889–2900, 2018)**

Pregnancy causes profound changes in regulation of the hypothalamus-pituitary-thyroid (HPT) axis and extrathyroidal thyroid hormone metabolism (1). Consequently, results of thyroid function tests differ in pregnant women compared with nonpregnant women, and trimester-specific reference ranges of TSH and free T4 (fT4) have been proposed (2). Usually, reference ranges are obtained using blood samples taken from groups of thyroid

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Abbreviations: BIC, Bayesian information criterion; BLRT, bootstrapped likelihood ratio test; BP, blood pressure; fT4, free T4; GHT, gestational hypertension; hCG, human chorionic gonadotropin; HPT, hypothalamus-pituitary-thyroid; LCGA, latent class growth analysis; LMR-LRT, Lo-Mendell-Rubin likelihood ratio test; PTB, preterm birth; SGA, small-for-gestational age; TPO-Ab, thyroid peroxidase antibody; Treg, regulatory T-cell.

peroxidase antibodies (TPO-Ab)–negative women in their first, second, or third trimester rather than by sampling the same women in each trimester (2). Although this cross-sectional approach succeeds in obtaining reliable trimester-specific reference ranges for the population, it obscures longitudinal changes in thyroid function that occur in individual women during the course of their pregnancy.

Each individual has a unique set point of the HPT axis, and genetic studies demonstrate that thyroid status is inherited as a complex trait (3, 4). Thus, circulating TSH and fT4 concentrations within an individual vary across a rather narrow range, which is smaller than 50% of the population-based reference range (5). Moreover, a large study in >150,000 subjects showed that the TSH-fT4 relationship is not inverse log-linear and is also age and sex dependent (6). Cross-sectional measurements of TSH and fT4 levels have been related in many studies to various obstetric outcomes, including gestational diabetes, gestational hypertension (GHT), and abnormal fetal position, as reviewed elsewhere (2, 7). Some studies observed an association between outcomes and both TSH and fT4 levels, whereas others reported a relationship either with TSH but not fT4 or *vice versa* (2, 7). Similarly, studies investigating the consequences of maternal thyroid dysfunction on infant neurodevelopment have reported disparate findings (2, 7). Such inconsistencies have always been difficult to reconcile but may result from the use of thyroid function reference ranges derived from cross-sectional data. Also, as recently summarized, the reliability of fT4 assessments during pregnancy is still subject to debate, especially during late gestation, because of the physiological changes related to pregnancy (increase in thyroid-binding-globulin and decreased albumin concentrations) (2). It is therefore likely that more accurate information can be obtained when longitudinal TSH and fT4 measurements from the same women during each trimester are used in epidemiological studies.

Longitudinal mixture models, including latent class growth analysis (LCGA), have been advocated as sophisticated statistical approaches that can accommodate temporal changes within heterogeneous population samples (8). For example, studies in perinatal psychiatry reported various trajectories of perinatal depressive symptoms (9).

Therefore, we studied whether LCGA can be used as an alternative approach to mitigate the limitations of cross-sectional studies on thyroid function by classifying individuals into groups according to their differing longitudinal trajectories of TSH and fT4 during pregnancy. We also investigated whether these longitudinal trajectories shared different obstetric complications. Finally, we executed *post hoc* analyses to validate the findings of the LCGA. In a randomly selected subsample of respondents (n = 568), the effect of human chorionic gonadotropin (hCG), assessed at 12 weeks' gestation, was determined on possible different trajectories.

Materials and Methods

We undertook a prospective study of the course of thyroid function in a large cohort of pregnant women in whom TSH, fT4, and TPO-Ab levels were determined. We applied LCGA with trimester-repeated assessments of thyroid function. The primary outcome was identification of trajectories of thyroid function that integrate changes in TSH and fT4 during the three trimesters. Secondary outcomes were to evaluate the role of TPO-Abs and determine the occurrence of thyroid dysfunction. This study is reported in line with the STROBE guidelines (10).

Subjects

During a period of 2 years (2002 to 2004), 1702 women who booked antenatal visits at 12 weeks' gestation were followed up in five community midwife practices in the iodine-sufficient vicinity of Eindhoven, Netherlands (11). Only Dutch Caucasian women (n = 1507) were invited to participate, and 1198 women (79.5%) consented. Exclusion criteria similar to those of the NHANES survey (such as previous thyroid disease, thyroid medication) were noted in 49 women (12); 1149 women were eligible and were followed up at 24 and 36 weeks' gestation.

Complete data were missing for 49 women, and data analysis was performed in the remaining sample of 1100 (Table 1). At 36 weeks' gestation, thyroid data were lacking from 40 women because they had already given birth. To maximize statistical power, thyroid parameters (fT4, TSH, and TPO-Ab status) in these 40 women were estimated with the multiple imputation algorithm. The variables age, parity, parental history of thyroid dysfunction, smoking, pre-pregnancy body mass index, history of miscarriage, and thyroid parameters at 12 and 24 weeks' gestation (fT4, TSH, and TPO-Ab status) were used for the imputation modeling, and pooled estimates were reported (13).

The study was approved by the Medical Ethical Committee of Máxima Medical Centre in Eindhoven/Veldhoven, and all women gave written informed consent.

Assessments

Serum TSH level was measured using a solid-phase, two-site chemiluminescent enzyme immunometric assay (IMMULITE Third Generation TSH Assay; Diagnostic Products Corporation, Los Angeles, CA). Interassay coefficients of variation were 5.0% and 4.4% at concentrations of 0.22 and 2.9 mIU/L, respectively. Trimester-specific TSH reference ranges were defined using 2.5th and 97.5th cutoffs in TPO-Ab–negative women. The serum fT4 concentration was measured with a solid-phase immunometric assay (IMMULITE Free T4 Assay; Diagnostic Products Corporation, Los Angeles, CA). Interassay coefficients of variation were 6.7% and 4.4% at concentrations of 11.6 and 31.5 pmol/L, respectively. Trimester-specific fT4 reference ranges were defined using 2.5th and 97.5th cutoffs in TPO-Ab–negative women.

Serum TPO-Ab levels were determined using the IMMULITE Anti–TPO-Ab Kit (Immulite TPO-Ab Assay; Diagnostic Products Corporation). Interassay coefficients of variation were 9% and 9.5% for concentrations of 40 and 526 IU/mL, respectively. The anti-TPO assay is standardized in relation to the International Reference Preparation for anti-TPO MRC 66/387.

Table 1. Baseline Characteristics of a Sample of 1100 Women at 12 Wks' Gestation

	Mean (SD)	N (%)
Age	30.5 (3.6)	
Lifestyle habits		
Currently smoking		143 (13)
Alcohol use in pregnancy		123 (10)
BMI, kg/m^2	25.6 (4.7)	
Obstetric features		
Parity		
Primiparity		513 (47)
Multiparity		587 (53)
1		464 (79)
2		106 (18)
>2		17 (3)
Spontaneous miscarriage earlier in life		178 (16)
Obstetric complications		
PTB (<37 wk)		55 (5)
GHT		61 (5.5)
Small for gestational age (SGA)[a]		77 (7)
Thyroid function		
Mean TSH (SD), mIU/L	1.32 (0.81)	
Median logTSH (range), mIU/L	0.041 (−2.09 to 0.90)	
Mean fT4 (SD), pmol/L	16.1 (3.5)	
Thyroid function parameters		
Parental history of thyroid dysfunction		87 (7.9)
TPO-Ab >35		95 (8.6)

Abbreviations: BMI, body mass index; PTB, preterm birth.

[a]SGA: birth weight <10th percentile adjusted for term at birth, parity, and sex.

A TPO-Ab titer >35 IU/mL at 12 weeks' gestation was defined as TPO-Ab positive, and this cutoff identified 95 TPO-Ab–positive women (8.6%; Table 1) and 1005 TPO-Ab–negative women.

hCG was assessed at 12 weeks' gestation in a random subsample of 568 participants by the Immulite technique (IMMULITE HCG; Diagnostic Products Corporation, Los Angeles, CA). The coefficient of variation was 5.8% at a concentration of 370 IU/L.

Statistical analysis

Descriptive statistics were performed in SPSS version 24 (IBM). To study longitudinal trajectories of gestational thyroid function, we performed LCGA, a longitudinal mixture modeling technique that can identify homogeneous classes within a larger heterogeneous longitudinal data sample (14). We used MPlus version 7.4 (Muthén & Muthén, Los Angeles, CA) (15). To model thyroid function, we included TSH and fT4 levels at all trimesters. Because TSH values were positively skewed with an abundance of scores close to zero, we modeled trajectories of gestational thyroid function using the maximum likelihood parameter estimates option in MPlus (16). This option is robust to non-normality and prefers overtransforming variables to approximate a normal distribution (17). The starting point was a one-class model, after which models were fitted to increasing numbers of classes. To determine the optimal number of classes, we applied the following fit indices: Bayesian information criterion (BIC), Lo-Mendell-Rubin likelihood ratio test (LMR-LRT), and bootstrapped likelihood ratio test (BLRT) (18). Better-fitting models have lower BIC values, and significant LMR-LRT and BLRT values indicate that a model with an additional class is a better fit to the data. The BLRT is superior to the LMR-LRT for identification of the correct number of classes (18). Apart from these fit indices, we also considered entropy, with entropy values closer to 1 indicating clearer delineation of classes (19). We further accounted for parsimony and interpretability of the models and average posterior probabilities of their classes. We also took into account whether additional classes included >1% of the total sample.

Obstetric complications

We also investigated whether different trajectories were associated with poor obstetric outcomes. These included preterm birth (PTB), GHT (pregnancy-induced hypertension/preeclampsia), and poor fetal growth.

Gestational age was calculated from the date of the last menstrual cycle and from an ultrasonography scan during what was presumed to be the 12th week of pregnancy. When a discrepancy of >7 days was seen between these two measurements, a second ultrasonography scan was performed within 2 weeks to reassess gestational age. PTB was defined as a gestational age <37 weeks at birth. GHT was defined as development of systolic blood pressure (BP) ≥140 mm Hg and/or diastolic BP ≥90 mm Hg after 20 weeks' gestation (at least two BP readings) in previously normotensive women (+ preeclampsia in case of additional presence of proteinuria).The Netherlands Perinatal Registry was used to define small-for-gestational age (SGA) neonates. In this registration system, >95% of all hospital and home births in The Netherlands are registered. The definition of SGA was based on population-based birth weight percentiles using the lowest 10th percentile cutoff (20). Although the cutoff for the definition of SGA and intrauterine growth restriction is arbitrary, in most large studies the lowest 10th percentile is used (21). To define SGA appropriately, birth weight is corrected for gestational age, sex of the baby, and parity.

Differences between possible classes were compared using the χ^2 test for categorical variables and the t test/ANOVA for continuous variables, with Bonferroni correction for multiple testing. Nonparametric testing of variables with considerable departure from normal distribution was performed using the Mann-Whitney U and Kruskal-Wallis tests.

Results

Baseline characteristics of the sample of 1100 pregnant women at 12 weeks' gestation are presented in Table 1. Ninety-five women (8.6%) were considered to be TPO-Ab positive (TPO-Ab >35 kU/L). The reference range of normal thyroid function (between the 2.5th and 97.5th percentiles) in 1005 TPO-Ab–negative women was between 0.15 and 2.9 IU/L for TSH and between 11.9 and 20.8 pmol/L for fT4 at 12 weeks' gestation. At 24 weeks' gestation, these values were 0.4 to 2.7 IU/L and 10.2 to 17.8 pmol/L, respectively, and at 36 weeks' gestation, 0.42 to 3.2 IU/L and 9.5 to 17.0 pmol/L, respectively. Trimester-specific overt thyroid dysfunction was defined as TSH and fT4 levels outside these reference ranges, whereas subclinical thyroid dysfunction referred to

women with a trimester-specific TSH level outside the reference range but a normal fT4 concentration.

In total, 55 women (5%) delivered preterm. Of these 55 PTB women, six were TPO-Ab positive (11%), four (7.3%) had subclinical hypothyroidism, and four (7.3%) had subclinical hyperthyroidism at some time during gestation. Similar figures were found in the remaining 1045 women who delivered at term: 8.5% were TPO-Ab positive, 69 (6.6%) had subclinical hypothyroidism, and 48 (4.6%) had subclinical hyperthyroidism. GHT occurred in 61 women (5.5%) and was significantly more frequent in nulliparous women (45 of 513; 8.8%) than in multiparous women [16 of 587; 2.7%; χ^2 (1): 19; $P < 0.001$]. There were no differences in TPO-Ab–positive status, subclinical hypothyroidism, or subclinical hyperthyroidism between women with GHT and women with normotension (data not shown). The 77 women who gave birth to SGA neonates had similar levels of TPO-Ab positive and subclinical hyperthyroidism (data not shown), but they developed subclinical hypothyroidism more frequently during gestation (10, 13%) than did the remaining 1023 women (63, 6.1%): [χ^2 (1): 5.3; $P = 0.02$].

The mean hCG level in the subsample of 568 women was 57.714 IU/L (SD: 29.380), with a median of 52.733 IU/L and a range from 7043 to 196.211 IU/L. The 90th and 95th percentiles of hCG were 100.691 and 111.317 IU/L. This subsample showed no statistically different baseline characteristics (age, parity, lifestyle habits) compared with the total sample (data not shown). The mean (SD) fT4 level, mean (SD) TSH level, and median (range) logTSH of this subsample were 16.3 (2.5) pmol/L, 1.28 (0.81) mIU/L, and 0.043 mIU/L (range: −0.200 to 0.84 mIU/L), and 50 women (8.8%) were TPO-Ab positive. These numbers were statistically no different from those of the total sample (Table 1).

Trajectories of gestational thyroid function

On the basis of the BIC, LMR-LRT, and BLRT statistics, the three-class model was chosen. This three-class model had adequate class sizes and entropy and high average posterior probabilities (0.84 to 0.98; Supplemental Table 1). These model estimates were replicated using convergence checks, supporting a three-class model as the optimal solution (18).

The three longitudinal thyroid function trajectories that formed each class are shown in Fig. 1. All share a gradual fT4 decline.

Class 1 (n = 1019; 92.4%) was labeled *Low increasing TSH* and was regarded as the reference group. Individuals had a gradual increase in TSH level toward the end of gestation (TSH level at 36 weeks was 1.3 IU/L). Class 2 (n = 30; 2.8%) was labeled *High increasing TSH* and displayed the highest increase in TSH level toward the end of gestation (TSH level at 36 weeks was 3.3 IU/L); individuals had an intermediate mean fT4 level (15.8 pmol/L) at 12 weeks. Class 3 (n = 51; 4.6%) was labeled *Decreasing TSH* and had the highest TSH level (3.2 IU/L) and the lowest fT4 level (mean, 15.3 pmol/L) at 12 weeks' gestation. These women displayed a pattern of decreasing TSH levels throughout gestation to a mean TSH value of 2.4 IU/L and a mean fT4 value of 13.2 pmol/L at 36 weeks' gestation. Thyroid and other characteristics in relation to the three trajectories of TSH and fT4 are shown in Table 2.

Overt and subclinical hyperthyroidism occurred only in class 1, whereas overt and subclinical hypothyroidism occurred predominantly in class 3. Using trimester-specific reference ranges, in total, there were 73 women (6.6%)

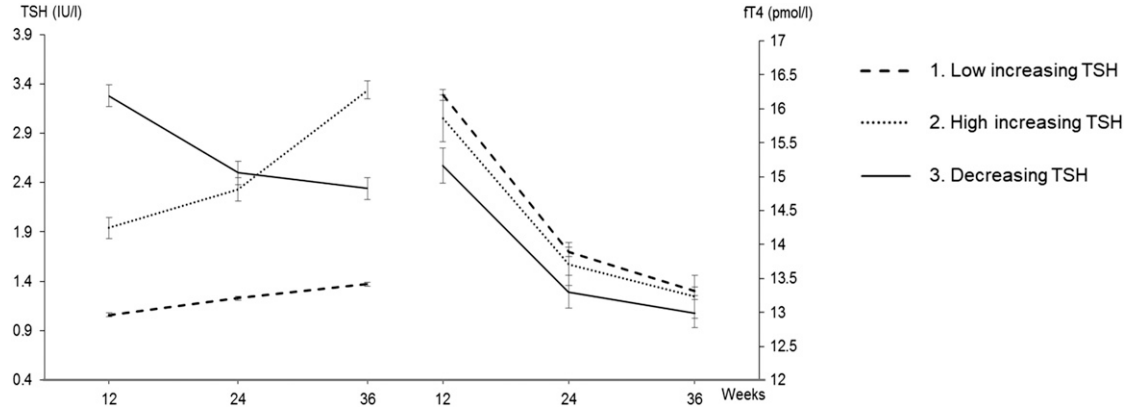

Figure 1. Three trajectories of TSH and fT4 level changes from the first to the third trimester of pregnancy. Note: For ease of interpretation, the figure displays raw means for TSH and fT4 levels instead of estimated means derived from the LCGAs. Estimated means from the LCGAs can be found in Supplemental Table 1. Women in class 1 (n = 1019; 92.4%) are characterized by the lowest initial TSH levels, which showed a gradual increase toward the end of gestation, and the highest fT4 levels at baseline, which gradually decreased further at 36 weeks' gestation. Women in class 2 (n = 30; 2.8%) had intermediate TSH levels at baseline that increased toward the end of gestation. Women in class 3 (n = 51; 4.6%) are characterized by the highest initial TSH levels, which decreased during pregnancy, and the lowest fT4 levels at baseline, which gradually further decreased to the lowest levels at 36 weeks' gestation.

Table 2. Subgroups of Thyroid Dysfunction According to the Three Subclasses (Total N = 1100)

	Class 3 (n = 51)		Class 2 (n = 30)		Class 1 (n = 1019)	
	N (%)	Mn (SD)	N (%)	Mn (SD)	N (%)	Mn (SD)
Thyroid function at 12 wk						
Overt hypothyroidism	3 (5.8)		1 (3.3)		0	
Subclinical hypothyroidism	**32 (62.7)**		**4 (13.3)**		**1 (0.1)**	
Overt hyperthyroidism	0		0		11 (1.1)	
Subclinical hyperthyroidism	0		0		21 (2.1)	
Thyroid function at 24 wk						
Overt hypothyroidism	0		0		1 (0.1)	
Subclinical hypothyroidism	17 (33.3)		12 (40)		1 (0.1)	
Overt hyperthyroidism	0		0		2 (0.2)	
Subclinical hyperthyroidism			0		27 (2.6)	
Thyroid function at 36 wk						
Overt hypothyroidism	1 (1.9)		0		1 (0.1)	
Subclinical hypothyroidism	8 (15.4)		18 (56.3)		0	
Overt hyperthyroidism	0		0		5 (0.5)	
Subclinical hyperthyroidism	0		0		22 (2.1)	
Thyroid-related parameters at 12 wk						
Multiparity	**26 (51)**		**9 (30)**		**552 (54)**	
1	18 (69)		8 (89)		438 (79)	
2	7 (27)		1 (11)		98 (18)	
<2	1 (4)		0		16 (3)	
Spontaneous miscarriage	9 (17.6)		4 (13.3)		209 (20.5)	
Age, y		31.0 (3.6)		30.3 (3.9)		30.5 (3.5)
Smoking	5 (9.8)		4 (13.3)		120 (11.8)	
Alcohol intake	6 (11)		3 (10)		135 (13)	
Pre-pregnancy BMI, kg/m^2		26.1 (4.9)		24.7 (3.6)		25.5 (4.5)
Parental history of thyroid dysfunction	**11 (21.6)**		**2 (6.7)**		**74 (7.3)**	
TPO-Ab						
Median TSH values, mIU/L						
At 12 wk	3.20		2.05		1.00	
At 24 wk	2.30		2.45		1.20	
At 36 wk	2.30		3.45		1.40	
Median fT4 values, pmol/L						
At 12 wk	14.8		15.85		16.0	
At 24 wk	13.1		14.0		13.7	
At 36 wk	13.0		13.5		13.0	
Women with TPO-Ab >35						
At 12 wk	**25 (49)**		**4 (13.3)**		**66 (6.4)**	
At 24 wk	**22 (43)**		**3 (10)**		**53 (5.2)**	
At 36 wk	19 **(37)**		**1 (3.3)**		**46 (4.5)**	
TPO-Ab titers						
At 12 wk						
Median	**25**		**9**		**9**	
Minimum	9		9		9	
Maximum	3600		140		1900	
At 24 wk						
Median	**16**		**9**		**9**	
Minimum	9		9		9	
Maximum	2400		67		920	
At 36 wk						
Median	**15**		**9**		**9**	
Minimum	9		9		9	
Maximum	1549		53		580	
Obstetric complications						
PTB (<37 wk)	3 (5.9)		2 (6.7)		50 (4.9)	
GHT	2 (3.9)		4 (13.3)		55 (5.4)	
SGA[a]	6 (11.7)		5 (16.6)		66 (6.5)	

Class 3: decreasing TSH; class 2: high increasing TSH; class 1: low-increasing TSH. Reference range thyroid function assessed in 1026 TPO-Ab–negative women: TSH, 0.15 to 2.9 IUL, and fT4, 11.9 to 20.8 pmol/L. Bold characters represent statistically significant differences between groups.

Abbreviation: Mn, mean.

[a]SGA: birth weight <10th percentile at delivery, adjusted for term of gestation, parity, and sex.

with subclinical hypothyroidism at any trimester, of whom 28 women (38%) were TPO-Ab positive, and 52 women (4.7%) with subclinical hyperthyroidism, of whom seven were TPO-Ab positive (13.5%). Subclinical hypothyroidism resolved by 24 and 36 weeks' gestation in 20 (of whom 50 were TPO-Ab positive) of 32 women in class 3 who showed subclinical hypothyroidism at 12 weeks (Table 2). Within class 3, five women had subclinical hypothyroidism in all trimesters (60% TPO-Ab positive). In class 2, the four women with subclinical hypothyroidism at 12 weeks had subclinical hypothyroidism in all trimesters (100% TPO-Ab positive). Of the remaining 14 women in class 2 who developed subclinical hypothyroidism by 36 weeks, four were TPO-Ab positive (28%). A family history of thyroid dysfunction occurred more often in class 3 women [χ^2 (2) = 13; P = 0.003], and TPO-Abs were also most prevalent in class 3. Multiparity occurred less frequently in class 2 than in class 1 [χ^2 (1) = 6.8; P = 0.0093] or class 3 [χ^2 (1) = 3.4; P = 0.066].

PTB was equally distributed in all classes: 4.8% in class 1, 6.3% in class 2, and 5.8% in class 3. In class 1, there were 66 SGA neonates (6.5%); in class 2, five (16.6%); and in class 3, six (11.7%), which was significantly different: χ^2 (2) = 6.5; P = 0.039. Compared with the reference group (class 1), class 2 had an OR of 2.8 (95% CI: 1.13 to 7.3) for SGA. With regard to GHT, LCGA did not identify a trajectory of change in thyroid function that was associated with increased risk: χ^2 (2) = 3.8; P = 0.15.

Table 3 shows similar trajectories in the subsample of 568 women in whom hCG was also assessed, as well as mean (SD) and median (range) of hCG.

The mean hCG concentrations were the highest in class 1 and the lowest in class 2, but they were not significantly different [ANOVA: F(2) = 1.40; P = 0.22]. Similarly, the median hCG level was the highest in class 1 and the lowest in class 2, but they were not significantly different [Kruskal-Wills: χ^2 (2) = 2.5; P = 0.28]. In the reference group (class 1), the maximum hCG level was 196.211 IU/L, whereas this figure was 84.243 IU/L in class 2 (representing the 83th percentile of class 1) and 98.292 IU/L in class 3 (representing the 89th percentile of class 1). The numbers (%) of TPO-Ab–positive women in these subsample trajectories were similar to those (Table 2) of the total sample: 32 (6.1%) in class 1, three (17.6%) in class 2, and 15 (51.7%) in class 3. Also, the numbers of women with overt hyperthyroidism (six; 1%) and subclinical hyperthyroidism (11; 1.9%) were comparable to those of the total sample (Table 2) and occurred only in class 1. Of the six women with overt hyperthyroidism of class 1, none had an hCG concentration >90th percentile, whereas in the 11 women with subclinical hyperthyroidism, five (55%) had an hCG level >90th percentile.

Because 70% of the women in class 2 were nulliparous compared with 49% and 46% in classes 3 and 1, respectively, we repeated LCGA in nulliparous and multiparous women separately. In nulliparous women [Fig. 2(a); n = 513], three classes were again identified; their characteristics were similar to those of the whole group of 1100 women: class 1 (n = 486; 94.7%), *low increasing TSH (*reference group); class 2 (n = 13; 2.6%) *high increasing TSH;* and class 3 (n = 14; 2.7%) *decreasing TSH* (parameter estimates are shown in Supplemental Table 2.)

In multiparous women, however, only two classes were identified [Fig. 2(b); N = 587]: class 1 (n = 553; 94%) *low increasing TSH* (reference group) and class 3 (n = 34; 6%) *decreasing TSH,* with characteristics similar to those of class 3 in the whole cohort and the nulliparous group (parameter estimates are shown in Supplemental Table 3).

Thyroid and other characteristics in relation to longitudinal trajectories of TSH and fT4 in nulliparous and multiparous women are shown in Table 4. The prevalence of TPO-Ab in class 1 subjects did not differ between nulliparous and multiparous women and remained stable throughout pregnancy.

The prevalence of TPO-Abs in class 3 women decreased in nulliparous women by 14% (from 21% at week 12 to 7% at week 36), whereas a significantly greater decrease of 38% (from 41% at week 12 to 2.9% at week 36) was observed in multiparous women

Table 3. hCG Concentrations at 12 Wks' Gestation in a Subset Sample (N = 568) According to Three Trajectories of Thyroid Function (TSH and fT4) Throughout Gestation

	Class 3 (n = 29)	Class 2 (n = 17)	Class 1 (n = 522)
hCG level, IU/L			
Mean (SD)	52.423 (19.418)	48.555 (18.796)	58.306 (30.060)
Median	52.232	48.123	53.448
Minimum	26.408	19.869	7.043
Maximum	98.292	84.243	196.211
Range	72.884	64.374	189.168

Class 3: decreasing TSH; class 2: high increasing TSH; class 1: low increasing TSH.

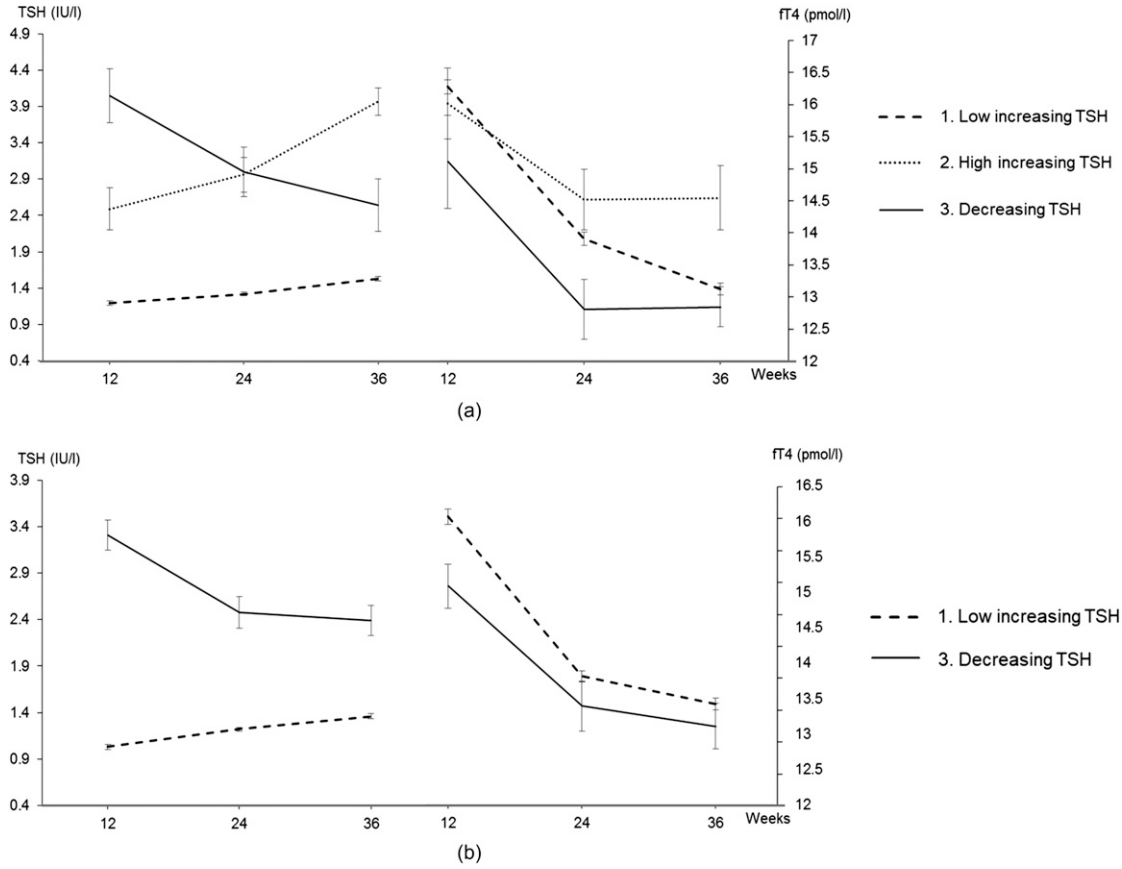

Figure 2. Trajectories of TSH and fT4 level changes from the first to the third trimester of pregnancy separated for (a) nulliparous and (b) multiparous women. Note: For ease of interpretation, the figure displays raw means for TSH and fT4 levels instead of estimated means derived from the LCGAs. Estimated means from the LCGAs can be found in Supplemental Tables 2 and 3.

[χ^2 (1) = 11.8; P = 0.001]. The highest prevalence of subclinical hypothyroidism occurred in class 2 nulliparous women (69%). The number of women reporting a parental history of thyroid dysfunction was also highest in class 2 nulliparous women. Throughout gestation, the highest concentrations of TPO-Abs were present in nulliparous women. The prevalence of TPO-Abs was highest (54%) at week 12 in nulliparous class 2 women and remained high until late gestation (31%). The prevalence of TPO-Abs in nulliparous class 2 women was higher than in class 3 (7%) and class 1 (6.1%) [χ^2 (2) = 12.0; P = 0.006] nulliparous women and in class 3 (2.9%) and class 1 (5.4%) multiparous women at all stages of pregnancy.

The prevalence of GHT among nulliparous women was not significantly different between classes [χ^2 (2) = 2.1; P = 0.36]: 15.4% (2 of 13) in class 2, 8.8% (43 of 486) in class 1, and no cases in class 3. In multiparous women, these figures were significantly different between classes [χ^2 (1) = 5.0; P = 0.025]: 8.8% (3 of 34) in class 3 and 2.4% (13 of 553) in class 1. Of the 55 women with PTB, 36 were nulliparous and 19 were multiparous women [χ^2 (1) = 8.3; P = 0.002]. Table 4 shows that in nulliparous women, the prevalence of PTB was not different between classes 1, 2, and 3 (6.3%, 15.4%, and 21.4%, respectively), but the absolute numbers were rather low [χ^2 (2) = 6.1; P = 0.12]. In multiparous women, all 19 PTBs (3.4%) occurred in class 1 and none in class 3.

Discussion

We performed a prospective study of longitudinal changes in thyroid function in individual women during pregnancy. LCGA enabled women to be grouped into classes according to longitudinal trajectories of thyroid status. Three classes were identified, a distinction that could not have been made if only cross-sectional data analysis was performed. LCGA analysis and classification thus elucidated pathophysiological understanding of the relationship between thyroid status, thyroid autoimmunity, and the progression of pregnancy.

Three longitudinal trajectories of thyroid function

A physiological decrease in fT4 level and a rise in TSH level as pregnancy progressed was seen in >90% of women in class 1, the reference group. Low TSH levels in the first trimester result from the thyroid-stimulating effect of high hCG levels (1). The rise in TSH during

Table 4. Trajectories of Thyroid Dysfunction in Nulliparous and Multiparous Women

	Nulliparous Women (N = 513)		
	Class 3 (n = 14)	**Class 2 (n = 13)**	**Class 1 (n = 486)**
Thyroid function at 12 wk, N (%)			
Overt hypothyroidism	0	3 (23)	0
Subclinical hypothyroidism	**3 (21)**	**9 (69)**	**7 (1.4)**
Overt hyperthyroidism	0	0	2 (0.4)
Subclinical hyperthyroidism	0	0	7 (1.4)
Thyroid-related parameters at 12 weeks, N (%)			
Parental history of thyroid dysfunction, N (%)	0	**3 (23)**	**38 (7.8)**
Women with TPO-Ab >35, N (%)			
At 12 wk	**3 (21)**	**7 (54)**	**35 (7.2)**
At 24 wk	**2 (14)**	**6 (46)**	**34 (6.9)**
At 36 wk	**1 (7)**	**4 (31)**	**30 (6.1)**
Median TSH value, mIU/L			
At 12 wk	3.26	1.95	1.10
At 24 wk	2.30	2.65	1.20
At 36 wk	2.20	3.45	1.40
Median fT4 values, pmol/L			
At 12 wk	14.5	16.05	16.2
At 24 wk	13.2	13.9	13.8
At 36 wk	12.7	13.35	13.2
TPO-Ab titers			
At 12 wk			
Median	**9**	**72**	**9**
Minimum	9	9	9
Maximum	2100	3600	1900
At 24 wk			
Median	**9**	**33**	**9**
Minimum	9	9	9
Maximum	1600	2400	920
At 36 wk			
Median	**9**	**16**	**9**
Minimum	9	9	9
Maximum	1000	1549	350
Obstetric complications, N (%)			
PTB (<37 wk)	3 (21.4)	2 (15.4)	31 (6.3)
GHT	0	2 (15.4)	43 (2.4)

	Multiparous Women (N = 587)		
	Class 3 (n = 34)	**——**	**Class 1 (n = 553)**
Thyroid function at 12 wk, N (%)			
Overt hypothyroidism	1 (7)		0
Subclinical hypothyroidism	**18 (53)**		**0**
Overt hyperthyroidism	0		7 (1.3)
Subclinical hyperthyroidism	0		14 (2.5)
Thyroid-related parameters at 12 wk, N (%)			
Parental history of thyroid dysfunction, N (%)	**6 (18)**		**40 (7)**
Median TSH values, mIU/L			
At 12 wk	3.20		0.96
At 24 wk	2.20		1.12
At 36 wk	2.30		1.30
Median fT4 values, pmol/L			
At 12 wk	14.8		15.8
At 24 wk	13.8		13.7
At 36 wk	12.9		13.3
Women with TPO-Ab >35, N (%)			
At 12 wk	14 **(41)**		**36 (6.5)**
At 24 wk	**2 (5.9)**		**34 (6.1)**
At 36 wk	1 (2.9)		30 (5.4)
TPO-Ab titers			
At 12 wk			
Median	16		9
Minimum	9		9
Maximum	1200		1300
At 24 wk			
Median	10		9
Minimum	9		9
Maximum	830		790
At 36 wk			
Median	9		9
Minimum	9		9
Maximum	470		580
Obstetric complications, N (%)			
PTB (<37 wk)	0		19 (3.4)
GHT	3 (8.8)		13 (2.4)

Class 1: decreasing TSH trajectory; class 2: high increasing TSH trajectory; class 3: low increasing TSH trajectory. Reference range thyroid function assessed in 1026 TPO-Ab–negative women: TSH, 0.15 to 2.9 IU/L, and fT4, 11.9 to 20.8 pmol/L. Bold characters represent statistically significant differences between groups.

late gestation, when the thyroid-stimulating effect of hCG has subsided, likely reflects the capability of the thyroid gland to meet increasing demands for thyroid hormone during pregnancy. Such increased demands result from the fetal need for maternal T4 in the first trimester, increased levels of serum thyroxine-binding globulin, increased renal iodide clearance, and increased degradation of thyroid hormones by placental deiodinase type 3 (1, 7). Nevertheless, even though TSH concentrations increase as pregnancy progresses, they remain at low-normal levels, suggesting that the occurrence of subclinical and overt hyperthyroidism in class 1 women was related to high levels of hCG, especially because the concentration of TPO-Ab decreased in the second and third trimesters. This was partially supported by hCG assessment in a subsample with limited epidemiological power: None of the six women with overt hyperthyroidism had an hCG level >90th percentile, whereas 55% of women with subclinical hyperthyroidism had an hCG level >90th percentile.

The trajectory in class 3 women may reflect the thyroidal response in pregnant women who are prone to develop or already have autoimmune thyroiditis. Thus, 20% of class 3 women had a family history of thyroid disease, and 50% were TPO-Ab positive. Women in class

3 had a higher TSH concentration in the first trimester, reflecting thyroid autoimmunity; however, it is notable that the TSH concentration decreased with advancing gestation in class 3 women compared with class 1 and 2 women in whom the TSH concentration increased in the second and third trimesters. Therefore, it seems that upregulation of the HPT axis to meet the increased thyroid hormone demands of pregnancy was compromised in class 3 women. In support of this interpretation, their fT4 concentrations were lower throughout gestation, consistent with recent findings that the thyroidal response to hCG is restricted in TPO-Ab–positive pregnant women (22).

Nevertheless, the outcome of hCG assessment in a subsample could also suggest another mechanism. Up to 50% of women in class 3 had elevated TPO-Ab titers, which were already present before conception. An elevated TPO-Ab titer is one of the most important determinants of high TSH concentration. Thus, these women also likely had an increased preconception TSH concentration. The β-unit of hCG has a TSH-like effect, so it is possible that because of higher preconception TSH levels in these women, hCG stimulation of the TSH receptor was less pronounced than in "healthy" TPO-Ab–negative women (class 1, reference group).

The prevalence and concentration of TPO-Abs decreased in the second and third trimesters but was less pronounced in class 3 than in classes 1 and 2. Immune tolerance of the partial allograft in pregnancy is largely dependent on $CD4^+$ regulatory T-cells (Tregs), which increase in number and function during gestation and are crucial inhibitors of autoimmunity (23–25). Thus, augmentation of Tregs is viewed as an important mechanism behind the observed decrease of thyroid antibodies and improvement of autoimmune thyroid diseases during gestation (26). The modest decrease in TPO-Abs in class 3 women underlines the increased prevalence of thyroid autoimmunity in these women compared with women in classes 1 and 2. We propose, therefore, that class 3 women might have an impaired increase in Tregs during pregnancy. Indeed, deficient numbers of Tregs have been documented in nonpregnant women with Hashimoto thyroiditis (26). Women in class 2 have an intermediate prevalence of hypothyroidism, a family history of thyroid disease, and TPO-Abs compared with women in classes 1 and 3. Thus, it can be concluded that class 2 comprises women with less-advanced thyroid autoimmunity, in whom Treg mechanisms may continue to prevent worsening of thyroid function in pregnancy. Interestingly, class 2 and 3 women of the subsample showed the lowest hCG levels compared with the reference group (although not significantly lower because of insufficient power). hCG is known to attract Treg cells at the fetal-maternal interface and has the potential to provoke the conversion of non–Treg cells into Treg cells (27). Class 2 and 3 women showed the highest numbers of TPO-Abs with the lowest levels of hCG and the highest numbers of pregnancy complications, such as SGA and GHT. However, the number of women with an hCG subsample analysis was too low to differentiate further for parity and/or fetal sex. GHT predominantly occurs in primiparous women (overrepresented in class 2), and hCG seems to induce T suppressor cells in lymphocytes only from females, not from males (27). Therefore, lower concentrations of hCG in class 2 and 3 women could decrease production of Treg cells overall, resulting in impaired fetal tolerance.

Because SGA, by definition, is corrected for parity, we were able to investigate a possible association between SGA and thyroid function trajectory in the main LCGA with adequate epidemiological power. Women in class 2 had a threefold increased risk of delivering SGA neonates. In a large cohort study from Rotterdam, an association between high maternal fT4 level during early gestation and SGA was identified, with no effect on maternal TSH or TPO-Ab status (28). This conclusion was based on a single assessment of thyroid function, whereas in the current study, LCGA was performed using three sequential measures of thyroid function and indicated that women in class 2, with the highest increase in TSH level throughout gestation and with fT4 levels above the reference group, had the highest incidence of SGA neonates. Also, class 2 women had a trend toward the highest incidence of PTB, further linking high TSH level and modestly low fT4 levels throughout gestation to poor obstetric outcome. However, the LCGA also showed that the highest incidence of GHT occurred in class 3 women with the highest TSH levels and lowest fT4 levels at early gestation. A possible explanation could be that women in class 3 (as shown in the analyses of the subsample) have lower hCG levels during early gestation, resulting in a lower rise in fT4 concentration and a higher TSH concentration. Moreover, by increasing the number of uterine natural killer cells, hCG in general contributes to proper remodeling of the maternal spiral arteries crucial for sufficient fetal nourishment (27). Impaired spiral artery development (because of lower hCG level) results in poor placentation, which is a major risk factor of GHT (7).

Another explanation could be that class 3 women had a threefold higher rate of TPO-Ab–positive results than class 2 women. It was recently suggested that the fT4 stimulating effect of hCG on fT4 production was less prominent in TPO-Ab–positive women (22). High hCG levels in healthy pregnant women will result in higher FT4 levels and thereby indirectly to lower TSH levels. In TPO-Ab–positive women, the thyroid gland may already be compromised to a certain extent, and the response of the thyroid gland to hCG may be compromised as well (lower release of T4 in response to the same amount of hCG). Alternatively,

SGA and GHT may result from differing pathogenetic mechanisms.

Nulliparous vs multiparous women

The LCGA demonstrated an absence of class 2 trajectory in multiparous women, whereas the characteristics of classes 1 and 3 in multiparous women were not different from the characteristics of the whole cohort of 1100 women. In nulliparous women, all three classes of thyroid function trajectory were observed with the highest prevalence of overt and subclinical hypothyroidism, TPO-Ab, and family history of thyroid disease in class 2 women. In the whole cohort of 1100 women, this was the case in class 3 women. These findings suggest differences in immune regulation between nulliparous and multiparous women. During a first pregnancy, the pregnancy-related immune stimulation induces type 2 cytokines and downregulates type 1 cytokines. Thus, in normal pregnancy, there is a skew toward type 2 immunity (29, 30). This type of immune stimulation also activates a subset of memory T-cells that has the potential for long-term survival. In the case of reexposure to antigens in a second pregnancy (from the same father), memory T-cells undergo rapid expansion and mediate more robust T-effector functions (29, 30). Thus, multiparous women more readily respond to a second fetal allograft with another type 2 immune reaction, or from the opposite perspective, the skewed type 2 immune response is less pronounced in nulliparous women.

The finding that GHT occurred more frequently in nulliparous women than in multiparous women is consistent with previous reports and likely results from differences in immune stimulation at conception (30). Furthermore, class 3 multiparous women with the highest prevalence of elevated TPO-Abs had a fourfold higher incidence of GHT than reference group 1. In class 3 nulliparous women, in whom the prevalence of elevated TPO-Abs dropped from 21% at 12 weeks to 7% at 36 weeks, it is notable that no cases of GHT were reported; in contrast, in class 2 women with the highest levels of TPO-Abs at 12 and 36 weeks, 15.4% developed GHT. These differences in the prevalence of GHT between the individual classes of nulliparous and multiparous women correlate with the concentration of TPO-Abs and also reemphasize the differences in type 2 immune responses that occur during pregnancy in nulliparous and multiparous individuals (31).

Strengths and limitations

The major strengths of this study are (1) its prospective nature, (2) the application of LCGA to thyroid status (TSH + fT4) assessed in each trimester of individual women to allow identification of individual trajectories of changes in thyroid function during pregnancy, and (3) use of a large cohort representative of Caucasian pregnant women living in an iodine-sufficient region. Although classification into three longitudinal trajectories met all the criteria for adequate LCGA (including the subanalyses), classes 2 and 3 were rather small in absolute numbers of pregnant women. Furthermore, we assessed hCG in only a subset of the total sample. We found substantial differences in hCG levels and patterns among the different trajectories, but they were not significantly different because of low power. Therefore, we were unable to stratify further for parity (GHT is predominantly found in primiparous women) or fetal sex (hCG-related induction of T suppressor cells occurs only in lymphocytes from females) (27). Incorporating hCG levels in LCGA would, however, require a much larger sample of ~2000 to 3000 women and could further explain differences in thyroid function patterns and obstetric complications in different longitudinal trajectories. Iron status was also not assessed, and it has been demonstrated that low iron status is associated with a higher prevalence of elevated TPO-Ab titers (and hence a higher rate of increased TSH levels) (32, 33). Thus, it is possible that differences in iron status could account for differences in TPO-Abs and hence TSH patterns between the three identified classes of women. Finally, thyroglobulin antibodies were not determined, so their relationships with different classes of women and possible effects on pregnancy outcome could not be investigated (34). There is an ongoing discussion about the reliability of fT4 assessments during late gestation (2).

Implications

In 5% to 8% of apparently healthy pregnant women, thyroid function during gestation does not follow the classic trajectory of slightly increasing TSH level and decreasing fT4 level (class 1), but instead follows trajectory 3 (decreasing TSH level) or trajectory 2 (pronounced increase in TSH level) associated with thyroid autoimmunity and hypothyroidism. It may be that pregnancy outcomes correlate better with the three identified longitudinal trajectories of thyroid function than with thyroid function tests obtained from cross-sectional studies. The same has recently been suggested for correlations with infant neurodevelopment (35). Such possibilities require further investigation, with special focus on the issue of screening of thyroid function.

Although the LCGA according to parity identified differences between nulliparous and multiparous women, the subgroups were small; larger, more detailed studies are required to investigate the possible interacting roles of iodine intake, ethnicity, and iron status on thyroid trajectory, antibody status, and pregnancy outcome. The attenuated immune response in subsequent

pregnancies should also be explored in more detail in relation to thyroid autoimmunity. The adaptive response was recently proposed as the immunological basis for partner-specific protection against complications in subsequent pregnancies compared with the first pregnancy (30). One could postulate that this phenomenon is related to the lower prevalence of GHT in multiparous women and is a possible explanation for the observed association between (subclinical) hypothyroidism and GHT (2). Furthermore, in normal pregnancy, it has become clear that microvesicles of the syncytiotrophoblast enter the peripheral maternal circulation, a process called microchimerism (the presence of small amounts of foreign particles or DNA detectable in a genetically distinct individual) (36). Microchimerism is thought to play a role in recurrent abortion and also to explain differences in pregnancy-related immune response between primiparous and multiparous women (37). In addition, it has been suggested that several pregnancy complications (including GHT, fetal growth restriction, and PTB) show a sex-specific pattern. In a recent study of the Generation R project, it was demonstrated that male sex was associated with poor maternal vascular adaptation to pregnancy (38). A very recent meta-analysis including >3 million participants showed that male sex was associated with increased risk of GHT in a non-Asian population (39). Thus, future research looking at differences in pregnancy complications between thyroid function trajectories adjusted to parity should take sex into account.

Finally, identification of the longitudinal thyroid function trajectory that a particular pregnant woman follows can be recognized only at the end of pregnancy. The future challenge will be to identify risk factors during early pregnancy that predict to which trajectory (with its specific adverse outcomes) women belong. A possible candidate could be pulsed Doppler ultrasonography of the umbilical and uterine arteries to assess flow velocity forms as a reflection of placental vascular resistance. Thyroid hormone receptors are expressed in the trophoblast cells, and thyroid hormone is important for appropriate placentation (40). Higher vascular resistance has been related to GHT, SGA, and PTB. A recent study of the Generation R project showed that fT4 in the higher range—but not TSH—was associated with higher vascular resistance (41). The authors suggested that this discrepancy was hCG related. Another approach could be an LCGA in which possible trajectories of combined fT4, TSH, and hCG functions are compared using pulsed Doppler ultrasonography. A limitation of this method is that it is reliable only from 20 weeks' gestation, making it less applicable to detect women at risk for longitudinal thyroid trajectories associated with poor obstetric outcome at early gestation (10 to 12 weeks).

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