Thyroid Dysfunction and Autoantibodies in Early Pregnancy Are Associated with Increased Risk of Gestational Diabetes and Adverse Birth Outcomes

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Context: Maternal thyroid dysfunction, especially in early pregnancy, may lead to pregnancy complications and adverse birth outcomes. Few population-based prospective studies have evaluated these effects and results are discrepant.

Objective: We examined the association of thyroid function and autoimmunity in early pregnancy with adverse pregnancy and birth outcomes.

Setting and Participants: The study used data from the prospective mother-child cohort "Rhea" study in Crete, Greece. A total of 1170 women with singleton pregnancies participated in this analysis. Maternal serum samples in the first trimester of pregnancy were tested for thyroid hormones (TSH, free T_4, and free T_3) and thyroid antibodies (thyroid peroxidase antibody and thyroglobulin antibody). Multivariable log-Poisson regression models were used adjusting for confounders.

Main Outcome Measures: Outcomes included gestational diabetes, gestational hypertension/preeclampsia, cesarean section, preterm delivery, low birth weight, and small-for-gestational-age neonates.

Results: The combination of high TSH and thyroid autoimmunity in early pregnancy was associated with a 4-fold increased risk for gestational diabetes [relative risk (RR) 4.3, 95% confidence interval (CI) 2.1–8.9)] and a 3-fold increased risk for low birth weight neonates (RR 3.1, 95% CI 1.2–8.0) after adjustment for several confounders. Women positive for thyroid antibodies without elevated TSH levels in early pregnancy were at high risk for spontaneous preterm delivery (RR 1.7, 95% CI 1.1–2.8), whereas the combined effect of high TSH and positive thyroid antibodies did not show an association with preterm birth.

Conclusions: High TSH levels and thyroid autoimmunity in early pregnancy may detrimentally affect pregnancy and birth outcomes. (***J Clin Endocrinol Metab*** **97: 4464–4472, 2012)**

Data from human and animal studies suggest that pregnancy alters normal thyroid function (1). Indicative changes include increased concentrations of thyroid hormone-binding globulin, thyroid hormones, and thyroglobulin; enhanced iodine clearance by the kidneys; and a mild thyrotropic effect of rising human chorionic gonadotropin on TSH secretion (2). The prevalence of hypothyroidism in women of reproductive age varies between 2

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Abbreviations: BMI, Body mass index; CI, confidence interval; free T3, free T_3; free T4, free T_4; GDM, gestational diabetes mellitus; RR, relative risk; SGA, small for gestational age; TG-Ab, thyroglobulin antibodies; TPO-Ab, thyroid peroxidase antibodies.

and 4% and is due largely to thyroid autoimmunity (3), whereas thyroid autoantibodies are found in approximately one in 10 women of child-bearing age (4). More importantly, a considerable proportion of pregnant women with thyroid disorders remain undiagnosed or treated insufficiently (5, 6). Maternal thyroid disorders, especially in early pregnancy, may lead to adverse obstetric complications and affect the health status of the offspring (7–18). Severe maternal hypothyroidism has been associated with increased risk of pregnancy-induced hypertension (7), anemia (7), postpartum hemorrhage (7), spontaneous abortion (7), fetal death (8), and possibly of low birth weight offspring (9). Similar data regarding subclinical hypothyroidism are less conclusive (10).

At the other end of the spectrum, uncontrolled hyperthyroidism due to Grave's disease has been associated with preeclampsia (11), stillbirths (11), miscarriages (11), preterm delivery (12), congestive heart failure (11), and intrauterine growth restriction or low-birth-weight offspring (13). Subclinical hyperthyroidism during pregnancy has not been associated with any adverse pregnancy or perinatal outcomes (14). The presence of thyroid autoantibodies has been associated with an increased risk of miscarriage in various populations of pregnant women (15), placental abruption (16), preterm rupture of membranes (17), perinatal mortality (18), and preterm birth (15). Although there is a general consensus regarding the negative impact of thyroid dysfunction during gestation, differences between existing studies in terms of study design, availability of extensive laboratory data, and ethnic differences in TSH concentration hinder the generalization of the results. In addition, there are no studies evaluating the combined effect of thyroid dysfunction and autoimmune status with pregnancy and birth outcomes in large-scale populations.

The objective of the present study was to assess the prevalence of thyroid dysfunction and autoimmunity in Greek pregnant women and to examine their relation with adverse pregnancy and neonatal outcomes in a large-scale, population-based cohort of an iodine-sufficient area of the Mediterranean: Crete, Greece.

Materials and Methods

Study design and population

The mother-child cohort in Crete (Rhea study)

The Rhea project is a mother-child study that prospectively examines a population-based cohort of pregnant women and their children at the prefecture of Heraklion, Crete, Greece (19). Pregnant women (Greek and immigrant) who became pregnant within a 12-month period, starting in February 2007, were contacted and asked to participate in the study. The first contact was made before 15 wk gestation at the time of the first major ultrasound examination and participants were invited to provide blood and urine samples and to participate in a face-to-face interview. The inclusion criteria for study participants were as follows: residents in the study area; women aged older than 16 yr; first visit: hospitals or private clinics at the time of the major ultra sound examination 10–13 wk of gestation; and no communication limitations. Women were contacted again at various times during pregnancy: at birth, at 8–10 wk after delivery and for the child's follow-up at the sixth and 18th months, and at 4 yr of age. Face-to face completed questionnaires together with self-administered questionnaires and medical records were used to obtain information on dietary, environmental, and psychosocial exposures during pregnancy and early childhood. The study was approved by the Ethical Committee of the University Hospital of Heraklion (Crete, Greece), and all mothers provided written informed consent after complete description of the study.

Detailed characteristics of the study population have been described elsewhere (19). Participants not providing blood samples (n = 271), or providing serum samples after the 18th wk of gestation (n = 80) as well as women with incomplete information on outcome variables (n = 40) were excluded from the analysis. Additionally, we have excluded multiple pregnancies (n = 40) and abortions or fetal deaths (n = 52). Thus, a cohort of 1170 women (73% of the total study population) was available for the present analysis.

Thyroid function during pregnancy

Maternal serum samples were collected at the first prenatal visit in 10 ml vacutainer tubes, were centrifuged, and were then stored in aliquots at −80 C until assayed. Thyroid function was assessed in early pregnancy (mean gestational age 14.1 wk; SD 3.6) by quantitative analysis of serum TSH, free T_4 (free T4), free T_3 (free T3), thyroid peroxidase (TPO-Ab), and thyroglobulin antibodies (TG-Ab) [Immulite 2000 immunoassay system (Siemens Healthcare Diagnostics, Los Angeles, CA)]. The intra- and interassay coefficients of variation were less than 12.5% and less than 12.5% for TSH (levels: 0.016–39 mIU/ml), less than 7.8% and less than 7.1% for free T4 (levels: 6.56–62.03 pmol/liter), less than 9.1% and less than 10% for free T3 (levels: 3.84–19.96 pmol/liter), less than 4.9% and less than 5.8% for TG-Ab and less than 7.4% and 7.2% for TPO-Ab.

The concentrations of serum thyroid hormones were categorized as low, normal, and high after the application of population-trimester-specific reference intervals (20). The reference intervals of serum TSH levels for the first trimester were 0.05–2.53 μIU/ml, free T3, 2.37–8.02 pmol/liter; and free T4, 12.23–19.69 pmol/liter. For the second trimester, respective reference intervals were as follows: 0.18–2.73 μIU/ml for TSH, 2.73–8.13 pmol/liter for free T3, and 11.20–18.66 pmol/liter for free T4. Women were categorized into trimesters of pregnancy according to gestational age at the time of sampling. Mothers with TSH serum concentrations within the reference range were considered to have normal TSH status (n = 1062; 90.8%), whereas mothers with TSH concentrations higher or lower than the trimester-specific reference range were considered to have high (n = 79, 6.8%) or low (n = 29, 2.5%) TSH, respectively. Accordingly, 1110 women (95.4%) had normal, 18 (1.5%) low, and 36 (3.1%) high free T3 levels. For free T4, the respective numbers were as follows: 1060 (90.8%) normal, 19 (1.6%) low, and 89 (7.6%) high levels. TPO-Ab and TG-Ab were considered ele-

vated if levels were 35 IU/ml or greater (n = 153, 13%) and greater than 40 IU/ml (n = 83, 7%), respectively. Mothers with thyroid autoimmunity were compared with antibody-negative mothers.

Obstetric and fetal outcomes

Gestational diabetes mellitus (GDM)

Women were screened for GDM at 24–28 wk and were classified as having GDM at the index pregnancy if two or more of the four plasma glucose values obtained during the 100-g, 3-h oral glucose tolerance test were abnormal according to criteria proposed by Carpenter and Coustan (21): fasting of 95 mg/dl or greater, 1 h of 80 mg/dl or greater, 2 h of 155 mg/dl or greater, and 3 h of 140 mg/dl or greater.

Gestational hypertension/preeclampsia

Information on gestational hypertension/preeclampsia was obtained through computer-assisted interviews and medical records.

Cesarean section

Data on cesarean section was obtained from the hospital medical records.

Gestational age

Gestational age was based on the interval between last menstrual period and date of delivery of the baby for 84% of the subjects. When the menstrual estimate of gestational age was inconsistent by 7 or more days within the ultrasound measurement taken in the first trimester of pregnancy (n = 187, 16%), a quadratic regression formula describing the relationship between crown-rump length and gestational age was used instead (22).

Preterm birth

Preterm birth was defined at less than 37 wk of gestation. A spontaneous preterm delivery was defined as a vaginal birth or an urgent cesarean section, whereas a medically indicated preterm delivery was defined as a programmed cesarean section or an induced vaginal birth.

Low birth weight

Low birth weight was defined as birth weight below 2500 g [birth weight range 1040–2490, mean (SD): 2203 (269.4)].

Small for gestational age neonates (SGA)

SGA neonates were defined as live-born infants below the 10th percentile of birth weight for gestational age in a referent population (23). In the current analysis, Spanish growth curves were used to calculate SGA neonates because Greek growth curves are not available (24).

Potential confounders

Potential confounders included characteristics that have an established or possible association with thyroid function, preterm birth, fetal growth, and pregnancy complications, including maternal age; maternal origin (Greek/other); marital status (married/other); parity (primiparous/multiparous); maternal education (low level: ≤6 yr of school; medium level: ≤12 yr of school; high level: university or technical college degree); prepregnancy body mass index (BMI) (kilograms per square meter); weight gain during pregnancy (kilograms); physical activity before and during pregnancy (yes/no); smoking during pregnancy (yes/no); alcohol intake during pregnancy (grams per day); working during pregnancy (yes/no); sleep duration during pregnancy (≥8 h/6–7 h/≤5 h); medication for thyroid disease (yes/no); family history of thyroid disease (yes/no); and infant gender (male, female).

Statistical analysis

Statistical analysis was performed using the statistical package SPSS, version 18 (SPSS Inc., Chicago, IL). The primary outcome variables of interest were pregnancy complications (gestational diabetes and gestational hypertension/preeclampsia), type of delivery (vaginal/cesarean), preterm birth, low birth weight, and SGA neonates. Univariate associations between dependent and independent variables were studied using Pearson's χ^2 test for categorical variables (with Fisher exact test for groups with less than five subjects expected in a cell) and Student *t*/ANOVA tests for continuous ones. In cases of nonnormally distributed variables (tested by the Shaphiro-Wilk's normality test), nonparametric Mann-Whitney or Kruskal-Wallis tests were used instead.

Multivariable log-Poisson regression models were performed to examine the association between thyroid function in early pregnancy and the outcomes of interest after adjusting for confounders. Potential confounders related with the outcomes and/or the exposure of interest in the bivariate models with a $P < 0.05$ were included in the multivariable models, except of maternal age and maternal education that were included *a priori* in all analyses. Relative risks (RRs) and 95% confidence intervals (95% CI) were computed to estimate the degree of association. To account for the possibility of residual confounding, the remaining demographic, lifestyle, and pregnancy characteristics that were available in this data set were then sequentially forced into the parsimonious models to ensure that the estimates associated with thyroid function remained unchanged. All association testing was conducted assuming a $P = 0.05$ significance level and a two-sided alternative hypothesis.

Results

Demographic characteristics of mothers according to TSH values and thyroid autoimmunity status are presented in Table 1. A total of 364 women reported family history of thyroid illness, and 154 were treated with T_4 or antithyroid medication. Women with high TSH and positive thyroid autoantibodies in early pregnancy were more likely to be older, multiparous, married, under medication for thyroid illness, and less likely to work during pregnancy compared with women with normal TSH and negative thyroid antibodies.

Table 2 presents multivariate associations of TSH levels and thyroid autoimmunity in early pregnancy with pregnancy complications. The prevalence of gestational diabetes, gestational hypertension/preeclampsia, and cesar-

TABLE 1. Sociodemographic and clinical characteristics by thyroid function in early pregnancy, Rhea birth cohort, Crete, 2007–2009

	Combination of thyroid autoimmunity and high TSH values[a]			
	Normal TSH Abs (−) (n = 914)	**Normal TSH Abs (+) (n = 148)**	**High TSH Abs (−) (n = 47)**	**High TSH Abs (+) (n = 32)**
Maternal age (yr), mean (SD)	**29.2 (5.1)**	**30.4 (5.0)**	**29.3 (5.0)**	**30.1 (5.5)**
Greek origin, n (%)	820 (91.0)	133 (90.5)	46 (97.9)	30 (93.8)
Married, n (%)	**765 (87.9)**	**125 (86.2)**	**33 (73.3)**	**29 (90.6)**
Maternal education, n (%)				
Low	190 (21.9)	25 (17.4)	6 (13.0)	10 (31.3)
Medium	435 (50.1)	75 (52.1)	22 (47.8)	11 (34.4)
High	244 (28.1)	44 (30.6)	18 (39.1)	11 (34.4)
BMI before pregnancy (kg/m^2), mean (SD)	24.2 (4.8)	24.6 (5.2)	24.3 (5.3)	24.3 (3.7)
Smoking during pregnancy, n (%)	384 (44.2)	58 (40.6)	25 (54.3)	13 (40.6)
Working during pregnancy, n (%)	**422 (48.6)**	**77 (53.8)**	**14 (30.4)**	**13 (40.6)**
Primiparous, n (%)	**331 (38.7)**	**62 (42.8)**	**28 (62.2)**	**11 (35.5)**
Vaginal delivery, n (%)	470 (52.3)	67 (45.6)	15 (32.6)	15 (46.9)
Medication for thyroid disease, n (%)	**71 (8.2)**	**50 (34.5)**	**12 (26.1)**	**17 (54.8)**
Family history of thyroid disease, n (%)	272 (32.5)	54 (40.3)	21 (45.7)	10 (31.3)

Abs, Antibodies. *Bold* indicates significant differences ($P < 0.05$) of ANOVA for continuous variables and χ^2 analysis for categorical variables. Numbers may not correspond to the total due to missing numbers.

[a] TSH reference limits: 0.05–2.53 μIU/ml for the first trimester and 0.18–2.73 μIU/ml for the second trimester; Abs(+) are TPO-Abs 35 IU/ml or greater and/or TG-Abs greater than 40 IU/ml.

ean section was 8.8% (n = 88), 4.8% (n = 47), and 49.6% (n = 571), respectively. The combination of high TSH and thyroid autoimmunity was associated with a 4-fold increased risk for gestational diabetes (RR 4.3, 95% CI 2.1–8.9), whereas the solitary presence of autoantibodies in euthyroid women did not show such an association (RR 1.4, 95% CI 0.7–2.4). All women with high TSH levels and thyroid autoimmunity who developed gestational diabetes (n = 6) had TPO-Ab levels greater than 100 IU/ml, whereas only one had TSH levels greater than 4 μIU/ml. High TSH values without the presence of thyroid autoimmunity were associated with a 10% increased risk of overall cesarean sections (adjusted RR 1.1, 95% CI 1.0–1.2). No association was found between thyroid hormones and/or thyroid autoimmunity in early pregnancy and gestational hypertension/preeclampsia.

TABLE 2. Associations of thyroid autoimmunity and high TSH values in early pregnancy with gestational diabetes, gestational hypertension/preeclampsia and caesarean delivery (n = 1170), Rhea Birth Cohort, Crete, 2007–2009

	Gestational diabetes (n = 88)		**Gestational hypertension/ preeclampsia (n = 47)**		**Cesarean section**					
					All (n = 571)		**Urgent (n = 194)**		**Programmed (n = 316)**	
	n	**RR (95% CI)[a]**	**n**	**RR (95% CI)[b]**	**n**	**RR (95% CI)[a]**	**n**	**RR (95% CI)[a]**	**n**	**RR (95% CI)[a]**
Clinical entities[c]										
Normal TSH/Abs (−) (n = 914)	60	Reference	38	Reference	440	Reference	149	Reference	247	Reference
Normal TSH/Abs (+) (n = 148)	15	1.4 (0.7, 2.4)	6	0.6 (0.2, 1.7)	82	1.0 (1.0, 1.1)	32	**1.3 (1.0, 1.9)**	42	1.0 (0.8, 1.3)
High TSH/Abs (−) (n = 47)	7	1.4 (0.5, 3.8)	3	1.1 (0.3, 4.1)	32	**1.1 (1.0, 1.2)**	9	1.3 (0.7, 2.4)	16	**1.4 (1.0, 2.1)**
High TSH/Abs (+) (n = 32)	6	**4.3 (2.1, 8.9)**	0	NA	17	1.0 (0.9, 1.2)	4	0.9 (0.4, 2.0)	11	1.1 (0.6, 1.8)

All models are adjusted for maternal age, maternal education, and prepregnancy BMI. Abs, Antibodies. *Bold* indicates statistically significant difference ($P < 0.05$).

[a] Also adjusted for parity, family history of thyroid disease, and smoking during pregnancy.

[b] Also adjusted for smoking during pregnancy.

[c] TSH reference limits: 0.05–2.53 μIU/ml for the first trimester and 0.18–2.73 μIU/ml for the second trimester; Abs(+): TPO-Ab, 35 IU/ml or greater and/or TG-Ab greater than 40 IU/ml.

TABLE 3. Associations of thyroid autoimmunity and high TSH values in early pregnancy with preterm birth, low birth weight, and SGA neonates (n = 1170), Rhea birth cohort, Crete, 2007–2009

	Preterm birth						Low birth weight				SGA			
	All (n = 133)		Spontaneous (n = 99)		Medically indicated (n = 34)		All (n = 60)		Preterm excluded (n = 25)		All (n = 56)		Preterm excluded (n = 48)	
	n	RR (95% CI)[a]	n	RR (95% CI)[a]	n	RR (95% CI)[a]	n	RR (95% CI)[b]	n	RR (95% CI)[b]	n	RR (95% CI)[c]	n	RR (95% CI)[c]
Clinical entities[d]														
Normal TSH/Abs (−) (n = 914)	103	Reference	75	Reference	28	Reference	44	Reference	16	Reference	44	Reference	38	Reference
Normal TSH/Abs (+) (n = 148)	24	1.3 (0.8, 2.1)	22	**1.7 (1.1, 2.8)**	2	NA	7	0.9 (0.5, 1.9)	2	0.4 (0.1, 2.7)	7	0.9 (0.3, 2.2)	6	1.1 (0.4, 2.7)
High TSH/Abs (−) (n = 47)	3	0.7 (0.2, 2.1)	1	0.3 (0.1, 2.4)	2	1.6 (0.4, 7.0)	4	**2.6 (1.1, 5.9)**	3	**2.7 (1.0, 7.3)**	3	1.5 (0.5, 4.4)	3	1.1 (0.3, 4.1)
High TSH/Abs (+) (n = 32)	3	0.8 (0.2, 3.0)	1	0.5 (0.9, 3.4)	2	1.6 (0.2, 10.9)	5	**3.1 (1.2, 8.0)**	4	**3.7 (1.4, 9.7)**	2	0.7 (0.1, 4.9)	1	0.9 (0.1, 5.5)

All models are adjusted for maternal age, maternal education, and parity. NA, Not applicable; Abs, antibodies. *Bold* indicates statistically significant difference ($P < 0.05$).

[a] Also adjusted for prepregnancy BMI and sleeping duration.

[b] Also adjusted for smoking during pregnancy, physical activity before pregnancy, and gestational age.

[c] Also adjusted for smoking during pregnancy and physical activity before pregnancy.

[d] TSH reference limits: 0.05–2.53 μIU/ml for the first trimester and 0.18–2.73 μIU/ml for the second trimester; Abs(+): TPO-Ab 35 IU/ml or greater and/or TG-Ab greater than 40 IU/ml.

The prevalence of preterm birth, low birth weight, and SGA neonates was 11.6% (n = 133), 5.4% (n = 60), and 5.1% (n = 56), respectively. Table 3 presents multivariate associations of TSH levels and thyroid autoimmunity in early pregnancy with birth outcomes. Thyroid antibodies *per se* increased the risk of spontaneous preterm birth by 70% (RR 1.7, 95% CI 1.1–2.8), whereas the combined effect of the high TSH and positive thyroid antibodies did not show an association with preterm birth. The combination of high TSH and thyroid autoimmunity was associated with a 3-fold increased risk for low-birth-weight neonates (RR 3.1, 95% CI 1.2–8.0). High TSH values without thyroid autoimmunity were also associated with increased risk for low-birth-weight neonates (RR 2.6, 95% CI 1.1–5.9), whereas the sole presence of thyroid antibodies was not associated with fetal growth restriction. To evaluate the possibility of introducing confounding by preterm birth or comorbid disorders, additional analyses were performed excluding all preterm births, and the results were very similar to the original analysis (Table 3).

In an additional analysis, we studied separately the associations of TSH, free T3, and free T4 hormones as well as thyroid antibodies in early pregnancy with pregnancy complications and birth outcomes: a per-unit increase in TSH value was associated with an increased risk for gestational diabetes (RR 1.1, 95% CI 1.0–1.2), programmed cesarean section (RR 1.1, 95% CI 1.1–1.2), a medically indicated preterm birth (RR 1.2, 95% CI 1.1–1.3), low birth weight (RR 1.2, 95% CI 1.2–1.3) and SGA neonates (RR 1.1, 95% CI 1.0–1.3). No significant associations were found for women with low TSH values in early pregnancy (RR gestational diabetes: 1.4, 95% CI 0.4–5.5; RR preterm birth: 0.9, 95% CI 0.4–1.7; RR low birth weight: 1.1, 95% CI 0.3–4.7). The presence of TPO-Ab but not TG-Ab was associated with a higher risk of gestational diabetes (RR 1.8, 95% CI 1.1–3.0), whereas TG-Ab conferred an increased risk for spontaneous preterm delivery (RR 2.1, 95% CI 1.2–3.6). Free T3 and free T4 were treated as continuous and as categorical variables and were not associated with any of these outcomes, except from the association of low free T4 with an increased risk for low birth weight neonates (RR 6.1, 95% CI 2.5–14.6).

To distinguish between overt and subclinical hypothyroidism as responsible for the observed findings, we checked the status of free T4 hormone in women with high TSH. Almost all women had normal free T4 values [74 had normal values and five had slightly increased values (<90th percentile)], indicating that subclinical hypothyroidism is the responsible clinical entity for all aforementioned maternal and fetal effects.

Finally, to elucidate whether medication for thyroid disease modified the observed results, we performed a sensitivity analysis in which we excluded all women who were under medication for thyroid illness (n = 154, TSH range 0.01–7.04 μIU/ml). Results did not differ substantially from those derived from the main analysis (Supplemental Tables 1 and 2, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

Discussion

The present study provides evidence that the combination of high TSH and thyroid autoimmunity in early pregnancy

increases the risk of adverse birth outcomes such as gestational diabetes and low-birth-weight neonates, whereas thyroid autoimmunity increases the risk of spontaneous preterm delivery. This is the first study measuring thyroid hormones and autoantibodies in early pregnancy in Crete, Greece. The presence of high TSH in pregnant women was almost twice more common in our cohort compared with results from studies in other countries (8, 25), and future studies are needed to investigate underlying causes for this high prevalence. Thyroid autoimmunity occurred in 15.6% of the pregnant women in our cohort, with a preponderance of TPO-Abs against TG-Abs, in agreement with other studies (26, 27).

We found a 4-fold increased risk of gestational diabetes in women with high TSH levels and thyroid autoimmunity in early pregnancy. This is the first study to our knowledge that evaluated the combined effect of high TSH and thyroid autoimmunity in early pregnancy and estimated such a high risk for gestational diabetes. Women with thyroid autoimmunity frequently progress to hypothyroidism during pregnancy, despite a euthyroid status before pregnancy, because the maternal thyroid requirements increase but cannot be met (3). Progressive hypothyroidism therefore often develops or worsens as gestation progresses (28). Previous observational studies evaluated the effect of hypothyroidism on gestational diabetes; most failed to show such an association (29–31), whereas a recent study found that the risk for gestational diabetes increases with TSH levels (32). Männistö *et al.* (33) showed an association between overt hypothyroidism during pregnancy and subsequent diabetes morbidity later in life, whereas other studies have linked diabetes (both type 1 and 2) to thyroid dysfunction and autoimmunity in adults (34, 35). In addition, a study based on Swedish Health Registries showed that women who were on thyroid supplementation during pregnancy had an increased rate of diabetes (pre-existing or gestational) compared with women who did not report the use of thyroid hormones in pregnancy (36). The frequent presence of thyroid autoantibodies in several nonthyroidal autoimmune diseases supports a hypothesis of an underlying enhanced global autoimmune state and immune dysfunction being relevant to these clinical outcomes (15, 37). Maratou and colleagues (38) found decreased rates of insulin-stimulated glucose transport inside cells of hypothyroid patients in an effort to explain the insulin resistant phenotype commonly observed in hypothyroid patients. Further studies with repeated measurements of specific biomarkers related to autoimmunity (glutamic acid decarboxylase, islet cell, or insulin antibodies) during pregnancy could investigate whether the association between high TSH and thyroid autoimmunity with gestational diabetes is the consequence of a widespread autoimmune disorder (39).

Women with thyroid autoimmunity also had an increased risk of spontaneous preterm delivery. Thyroid antibodies could be associated with a subtle decrease of thyroid function or may reflect a generalized activation of the immune system and specifically a deregulated activity of the immune system at the fetal-maternal interface (15, 40). Because pregnancy represents an inflammatory process with a shift in the regulation of cytokine networks within the local placental-decidual environment, a deregulation of the local inflammatory processes can be associated with miscarriage and premature delivery (40–42). Supporting our results, a recent meta-analysis showed that maternal thyroid autoimmunity increased 2-fold the risk of preterm delivery in women with biochemically normal thyroid function (15).

Women with high TSH levels were found to carry a 2-fold increased risk of having a low-birth-weight neonate, whereas the combination of high TSH and thyroid autoimmunity increase 3-fold the risk of low-birth-weight neonates. Due to small numbers in this group, we cannot entirely exclude the possibility of by-chance findings, and for the same reason, we were not able to distinguish between different categories of low-birth-weight neonates (<1000, 1000–1500, 1500–2500 g). These results are consistent with other birth cohort studies that evaluated the effect of hypothyroidism on preterm birth and intrauterine growth restriction (9, 25). There are several potential mechanisms linking maternal overt or subclinical hypothyroidism with fetal growth restriction. Thyroid hormones are essential for infant growth and maturation of many target tissues, including the brain, bone, and skeleton, through the actions of GH and IGF-I (43). More specifically, insufficient T_4 supply to the fetus may negatively affect the development of the pituitary-thyroid axis of the newborn (44) and interfere with normal vascular responsiveness and cardiovascular homeostasis *in utero* (45). The present analysis showed a 10% increased risk of overall cesarean sections in women with high TSH values in early pregnancy. This increase could be attributed to coexisting pregnancy complications (*i.e.* gestational diabetes) or fetal distress and fetal growth restriction as supported by our results.

Previous studies have reported that thyroid supplementation in pregnancy was associated with higher rates of gestational diabetes, gestational hypertension/preeclampsia, and cesarean delivery (36). Data are less consistent regarding thyroid supplementation and birth outcomes (44, 46). In our study, a sensitivity analysis removing women who were on thyroid substitution, and thus could have lower thyroidal reserve during pregnancy, showed

that the rates of adverse pregnancy and birth outcomes did not change significantly.

Strengths of the present study include the population-based prospective design, the large numbers of available biological samples and infants with anthropometric measurements at birth as well as the high participation rate (82%). We had the possibility to use our population-specific-trimester reference ranges for thyroid hormones (20) that are in accordance with the proposed trimester-specific reference ranges for TSH by the American Thyroid Association guidelines (47). Because studies have been carried out in different parts of the world, ethnic differences in TSH concentration and regional differences in iodine levels interfere with reference range calculations; consequently, no previously established reference ranges can be reliably applied in our cohort. These trimester-specific, population-based reference ranges allowed for correct interpretation of thyroid hormone values and accurate classification of thyroid disorders. Additionally, because preterm birth is a heterogeneous rather than a homogeneous entity, we had the possibility to distinguish between spontaneous and medically indicated preterm delivery. The exclusion of twins as well as the adjustment for several variables reduced the likelihood of confounding. We did not observe any substantial differences between the crude and the adjusted models. Thus, it is unlikely that overadjustment affected our findings. The study population included women from the follow-up of a birth cohort, providing the opportunity to account for the effect of exposures during pregnancy prospectively within the cohort. A selection bias could be theoretically generated by the possibility that we included only women receiving an early ultrasound. However, all pregnant women in Greece have to attend several compulsory prenatal visits, one of which takes place between 12 and 15 wk of gestation, which is the time of our enrollment phase. Moreover, the study sample in the Rhea cohort included pregnant women who visited both public hospitals and private maternity clinics, so the study population is representative of the entire population of pregnant women in the Heraklion prefecture.

There are several limitations in the present study that deserve acknowledgment. Pregnant and lactating women may require additional iodine intake to eliminate iodine deficiency disorders. Unfortunately, we did not have available data on possible dietary iodine supplementation, nor did we measure urinary iodine excretion to test for individual iodine status. However, median urinary iodine excretion (the best parameter to evaluate the adequacy of iodine nutrition in a population) during the last 2 decades in Greece has been estimated to be greater than 200 μg/g creatinine (48), which is well within normal limits. These findings indicate that, at present, Greece may be considered as an iodine-sufficient country. In addition, we had no information regarding potential use of substances that may have an effect on thyroid function (*i.e.* drugs like amiodarone or corticosteroids, kelp supplements, perchlorate exposure, iodine containing contrast media, *etc.*). Due to the small numbers, we were not able to investigate the effect of abnormal thyroid function and/or autoimmunity in early pregnancy on the risk of miscarriages/abortions. In the present study, we have used Spanish growth curves to calculate SGA neonates because validated Greek intrauterine growth curves are not available. Finally, although we incorporated extensive information on potential social and environmental factors that are associated with fetal growth, there may be other unidentified factors linked both with pregnancy complications, fetal growth, and thyroid function during pregnancy that could explain this association.

In summary, our results suggest that women with thyroid autoimmunity were more likely to experience a spontaneous preterm delivery, whereas the combination of high TSH and positive thyroid antibodies was associated with a 4-fold increased risk of gestational diabetes and a 3-fold increased risk of low-birth-weight neonates. Future longitudinal studies are needed to better dissect the underlying processes of thyroid physiology during gestation together with efficient preventive strategies to preclude the adverse impacts of thyroid disease in both the mother and child.

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