A Longitudinal Study of Thyroid Markers Across Pregnancy and the Risk of Gestational Diabetes

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Context: T3 is the biologically active thyroid hormone involved in glucose metabolism. The free T3 (fT3)/free T4 (fT4) ratio, a marker indicating conversion of fT4 to fT3, is also implicated in glucose homeostasis.

Objective: To examine associations of fT3 and the fT3/fT4 ratio with gestational diabetes mellitus (GDM).

Design: In a case-control study, thyroid markers (fT3, fT4, TSH) were measured and the fT3/fT4 ratio was derived across four visits in pregnancy, including first (gestational weeks 10 to 14) and second (weeks 15 to 26) trimester. Conditional logistic regression adjusting for thyroid autoimmunity status and major GDM risk factors estimated trimester-specific associations of thyroid markers with subsequent GDM risk.

Setting: Twelve US clinical centers.

Participants: One hundred seven GDM cases and 214 non-GDM controls from a multiracial pregnancy cohort of 2802 women.

Main Outcome Measures: GDM diagnosis ascertained from medical records.

Results: Both fT3 and the fT3/fT4 ratio were positively associated with GDM: adjusted OR (95% CI) comparing the highest vs lowest fT3 quartile was 4.25 (1.67, 10.80) at the first trimester and 3.89 (1.50, 10.10) at the second trimester. Similarly, the corresponding risk estimates for the fT3/fT4 ratio were 8.63 (2.87, 26.00) and 13.60 (3.97, 46.30) at the first and second trimester, respectively. Neither TSH nor fT4 was significantly associated with GDM.

Conclusions: Higher fT3 levels, potentially resulting from *de novo* synthesis or increased fT4 to fT3 conversion, may be an indicator of GDM risk starting early in pregnancy. (***J Clin Endocrinol Metab* 103: 2447–2456, 2018**)

Pregnancy has a considerable physiological impact on the thyroid gland and its metabolic function (1). To meet the increased demands during pregnancy, the thyroid gland increases up to 40% in size, accompanied by an upsurge in the production of thyroid hormones T4 and T3 (1). Abnormalities in thyroid function are relatively

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Abbreviations: aOR, adjusted OR; BMI, body mass index; GDM, gestational diabetes mellitus; fT3, free T3; fT4, free T4; NICHD, National Institute of Child Health and Human Development; OGTT, oral glucose tolerance test.

prevalent among pregnant women and have been linked to several obstetric complications, including premature delivery and pregnancy loss, as well as adverse health outcomes in the offspring (1). However, the debate regarding the utility of routine screening and/or treatment of thyroid dysfunction during pregnancy is highly contentious and remains to be resolved.

Given the important role thyroid hormones play in glucose metabolism and homeostasis, thyroid dysfunction has been suggested to play a role in the etiology of gestational diabetes mellitus (GDM), a common metabolic complication in pregnancy (2). However, the existing evidence has been conflicting and longitudinal data are sparse. Whereas a few prospective studies (3–5) report increased incidence of GDM in women who have overt or subclinical hypothyroidism, others (6–8) report no significant differences. Similarly, several (6, 9, 10) but not all (7, 11, 12) prospective studies have found that isolated hypothyroxinemia [normal TSH, low free T4 (fT4)] in pregnancy is associated with increased risk of GDM.

Of the two thyroid hormones T4 and T3, T4 is considered a prohormone, serving as a substrate for the biologically active form T3 (13). The conversion of peripheral T4 to T3, by two deiodinase enzymes, accounts for 80% of all the T3 produced; the rest is produced directly by the thyroid gland (13). T3 is also the primary active hormone involved in glucose metabolism, yet most prior studies have only looked at the associations between fT4 levels and GDM. Recently, two large prospective cohort studies (10, 14) observed an inverse association between fT4 levels and GDM, but speculated that low fT4 levels in GDM women may indicate increased conversion from fT4 to free T3 (fT3) or increased deiodinase activity, which is responsible for this conversion. A commonly used method for estimating the conversion from fT4 to fT3, and potentially serving as a proxy for deiodinase activity, is the fT3/fT4 ratio (15, 16). Although studies examining its association with GDM are lacking, several cross-sectional studies have noted that the fT3/fT4 ratio is associated with higher insulin resistance and glycosylated hemoglobin as well as elevated fasting glucose, fasting insulin, and postload glucose levels (17–19). Thus, a comprehensive analysis examining the subclinical changes in thyroid hormones fT4, fT3, and their ratio with GDM risk may offer novel insights into the pathogenesis of GDM.

In the current study, we prospectively investigated the associations of the fT3/fT4 ratio and related markers of thyroid function (fT3, fT4, TSH) with GDM while accounting for thyroid autoimmunity status. Because thyroid levels can change with the progression of pregnancy, we assessed these associations separately for the first and second trimester. As a secondary objective, we also examined the longitudinal trajectory of the thyroid markers across the entire pregnancy.

Materials and Methods

Study design

This case-control study was nested within the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) Fetal Growth Studies—Singleton Cohort (2009–2013), a multiracial and multicenter pregnancy cohort consisting of 2334 nonobese (20) and 468 obese women. Eligible women, 18 to 40 years of age, were enrolled between 8 and 13 weeks of gestation from 12 US clinical centers and followed throughout pregnancy. Exclusion criteria included pre-existing hypertension, diabetes, renal/autoimmune diseases, psychiatric disorders, cancer, and HIV/AIDS. Additional major exclusion criteria applicable to nonobese women (enrolled for the primary aim of developing US fetal growth standards for four self-identified US racial/ethnic groups) were smoking before pregnancy and history of pregnancy complications (*e.g.*, GDM, severe preeclampsia). Research approval for this study was granted by the institutional review boards of all participating sites, including the NICHD. Women provided written informed consent.

GDM ascertainment

GDM status (n = 107) was ascertained by review of medical records. For GDM diagnosis, we applied the Carpenter and Coustan diagnostic criteria (21) to the oral glucose tolerance test (OGTT) results. The average gestational age at OGTT among GDM cases (n = 95) was 27 weeks (range, 11–36 weeks). Women without OGTT results were classified as GDM if they had an indication of medication-treated GDM on the hospital discharge diagnosis (n = 12). Non-GDM status was confirmed based on 50-g 1-hour glucose challenge test, OGTT, and hospital discharge diagnosis. We matched each case to two non-GDM controls based on age (±2 years), race/ethnicity (non-Hispanic white, African American, Hispanic, Asian/Pacific Islander), and gestational week of blood collection (±2 weeks). Matching factors were selected either because they were well-established risk factors of GDM (age, race/ethnicity) or determinants of blood biomarker levels during pregnancy (gestational age).

Exposure assessment

Blood specimens were collected at four visits during the course of pregnancy, targeted at gestational weeks 8 to 13, 16 to 22, 24 to 29, and 34 to 37; however, the actual ranges were as follows: 10 to 14, 15 to 26, 24 to 31, and 33 to 39 weeks, respectively. Blood specimens collected at the second visit (weeks 15 to 26) were collected after an overnight fast of 8 to 14 hours. All blood specimens were stored at −80°C and thawed immediately before assay.

Utilizing the electrochemiluminescence immunoassay method, concentrations of plasma TSH (mIU/L), fT3 (pmol/L), fT4 (ng/dL), thyroglobulin antibody (IU/mL), and thyroid peroxidase antibody (IU/mL) were measured with Roche reagents (Roche Diagnostics, Indianapolis, IN) on the Roche Cobas e411 analyzer. The fT3/fT4 ratio was derived by dividing plasma concentrations of fT3 (pg/dL) by fT4 (ng/dL). Thyroid markers were measured at all four time points of blood

collection among the GDM cases and one of the two matched controls. The remaining controls only had thyroid markers measured at the two visits prior to GDM diagnosis (*i.e.*, weeks 10 to 14 and 15 to 26). All assays were conducted blinded to case-control status and performed in a central laboratory at the University of Minnesota. The interassay coefficients of variation were ≤5% for the thyroid hormones (fT3, fT4, TSH) and ≤15% for the two thyroid antibodies.

The pregnancy-specific reference range for TSH, as recommended by the 2017 American Thyroid Association guidelines (1), was applied to the study sample. Women with TSH concentrations ≤ 4 mIU/L were considered to have normal TSH levels. Isolated hypothyroxinemia was defined as having normal TSH in conjunction with low fT4 levels (<10th percentile in controls) (22). Overt or subclinical hypothyroidism was defined as having elevated TSH levels (>4 mIU/L) with low or normal fT4 concentration (≤90th percentile in controls). Women with normal TSH status and normal fT4 concentration (10th to 90th percentile in controls) were classified as euthyroid. With respect to thyroid autoimmunity status, women were considered antibody-positive if the thyroid peroxidase antibody levels were >35 IU/mL or the thyroglobulin antibody levels were >40 IU/mL (6).

Covariates

A structured questionnaire administered at enrollment (8 to 13 weeks) collected information on demographics and common risk factors of GDM, including maternal age (years), race/ethnicity (non-Hispanic white, African American, Hispanic, Asian/Pacific Islander), family history of diabetes (yes/no), nulliparity (yes/no), education (less than, equal to, or more than high school), smoking in the 6 months prior to pregnancy (yes/no), and alcohol consumption in the 3 month before pregnancy (yes/no). Prepregnancy body mass index (BMI; <25, 25.0 to 29.9, ≥30.0 kg/m^2) was calculated from self-reported prepregnancy weight and measured height. GDM treatment (diet/lifestyle modification and/or medication) history was extracted from medical records. Gestational age at each blood collection was estimated from the reported date of the last menstrual period, which was confirmed by ultrasound measurement at the time of enrollment. Women also reported any medication use, including medications for thyroid conditions.

Statistical analysis

In descriptive analyses, differences in participant characteristics between cases and controls were assessed by binomial/multinomial logistic regression with generalized estimating equations for categorical variables, and generalized linear mixed effects models for continuous variables including thyroid markers, both accounting for matched case-control pairs.

Conditional logistic regression was used to estimate crude and adjusted ORs (aORs) of GDM for each thyroid marker accounting for the matched case-control pairs. The thyroid markers were analyzed continuously and as quartiles. The quartiles were based on the thyroid marker distributions among the controls. ORs were calculated separately for the two visits prior to GDM diagnosis (*i.e.*, weeks 10 to 14 and 15 to 26). For the multivariable models, a *priori* selected covariates included key demographic factors and conventional risk factors for GDM: education level, parity, family history of diabetes, and prepregnancy BMI (<25, 25.0 to 29.9, ≥30.0 kg/m^2). As maternal age and gestational age at blood collection were only matched between cases and controls within a certain range (±2 years and 2 weeks, respectively), we further adjusted for these two variables to reduce residual confounding and derive conservative estimates. Additionally, because thyroid antibodies may influence both thyroid hormone levels and glucose homeostasis, we also included thyroid autoimmunity status (antibodies positive vs negative) in the models. Of note, we did not include smoking as a covariate, as nonobese women who smoked prior to pregnancy were not eligible for the study and only five obese women in the study reported smoking before pregnancy. Tests of linear trend were performed by using the median value for each quartile as a continuous variable in the conditional logistic regression models. To ensure temporality, we excluded one case at weeks 10 to 14 and five cases at weeks 15 to 26 from our analytical population, as their blood samples were collected after the GDM diagnosis. GDM diagnosis was made at median 15.4 weeks after blood collection at 10 to 14 weeks, and 9.9 weeks after collection at 15 to 26 weeks.

In addition to examining associations with thyroid marker levels, we also estimated ORs of GDM for clinical thyroid conditions including isolated hypothyroxinemia and overt or subclinical hypothyroidism, using women who had euthyroid status as the reference group. In sensitivity analyses, we excluded women who had elevated thyroid antibodies (n = 50 at weeks 10 to 14; n = 37 at weeks 15 to 26) (6), prior history of GDM (n = 6), prior history of preeclampsia (n = 8), smoked before the current pregnancy (n = 5), or had medication-treated GDM (n = 28) (to assess whether the associations still persisted among women with less severe GDM). Of note, none of the women reported use of thyroid medications prior to GDM diagnosis. In models looking at the quartile-specific associations between thyroid markers and GDM, we also repeated the analyses limiting the sample only to women who had euthyroid status. Furthermore, we stratified our analyses by prepregnancy BMI status (BMI < 25.0 vs BMI ≥ 25.0 kg/m^2), race/ethnicity (non-Hispanic white, African American, Hispanic, Asian/Pacific Islander), or family history of diabetes (yes vs no).

As a part of secondary analyses, the median concentration of each thyroid marker was plotted against the four study visits to depict changes in thyroid marker levels over the course of pregnancy. Statistical analyses were conducted using SAS 9.4 (SAS Institute, Cary, NC).

Results

Table 1 shows selected participant characteristics among women with and without GDM. Compared with non-GDM controls, GDM cases were more likely to have a higher prepregnancy BMI and a family history of diabetes. Median fT4 levels were significantly lower among GDM cases, whereas median fT3 and the fT3/fT4 ratio were significantly higher among cases at the two visits in the first (weeks 10 to 14) and second (weeks 15 to 26) trimester before GDM diagnosis (Table 2). TSH levels did not differ significantly between cases and controls at either trimester.

Table 3 shows the associations between thyroid markers in the first and second trimester and GDM status. fT3 and the fT3/fT4 ratio were significantly and

Table 1. Participant Characteristics Among Women Who Had GDM and Their Matched Controls in the NICHD Fetal Growth Studies—Singleton Cohort (2009–2013)

	GDM Cases (n = 107)	Non-GDM Controls (n = 214)	P^a
Age, y	30.5 ± 5.7	30.4 ± 5.4	
Race/ethnicity			
Non-Hispanic white	25 (23.4)	50 (23.4)	
African American	15 (14.0)	30 (14.0)	
Hispanic	41 (38.3)	82 (38.3)	
Asian/Pacific Islander	26 (24.3)	52 (24.3)	
Education			0.18
Less than high school	17 (15.9)	26 (12.1)	
High school graduate or equivalent	15 (14.0)	23 (10.7)	
More than high school	75 (70.1)	165 (77.1)	
Married/living with a partner	92 (86.0)	167 (78.0)	0.12
Nulliparity	48 (44.9)	96 (44.9)	1
Family history of diabetes	40 (37.4)	48 (22.4)	0.003
Smoked before pregnancy[b]	4 (3.7)	1 (0.5)	0.06
Alcoholic beverage consumption before pregnancy	61 (57.0)	137 (64.0)	0.22
Prepregnancy BMI, kg/m^2			<0.001
18.38–24.99	37 (34.6)	123 (57.5)	
25.0–29.99	35 (32.7)	56 (26.2)	
30.0–45.11	35 (32.7)	33 (15.4)	

Data are presented as n (%) for categorical variables and mean (SD) for continuous variables.

[a] *P* values for differences between case and control subjects were obtained by generalized linear mixed effects models for continuous variables and binomial/multinomial logistic regression with generalized estimating equations for binary/multilevel categorical variables, accounting for matched case-control pairs. *P* values are not shown for matching variables (age, race/ethnicity).

[b] Nonobese women who smoked were not eligible for the study.

positively associated with GDM risk at both trimesters: the aOR (95% CI) comparing the highest vs lowest quartile of fT3 was 4.25 (1.67, 10.80) at the first (P_{trend} = 0.001) and 3.89 (1.50, 10.10) at the second (P_{trend} = 0.007) trimester. Similarly, the corresponding risk estimates comparing the highest vs lowest quartile of the fT3/fT4 ratio were 8.63 (2.87, 26.00) and 13.60 (3.97, 46.30) at the first (P_{trend} = 0.001) and second ($P_{trend} < 0.0001$) trimester, respectively. fT4 levels were inversely associated with GDM risk at the second trimester, yet the quartile-specific associations were not significant after adjusting for potential confounders. However, women who were in the top decile of fT4 levels at the second trimester had a significantly decreased risk of GDM compared with those who were in the lowest quartile [aOR (95% CI), 0.17 (0.04, 0.76)]. TSH levels were not associated with GDM risk in either trimester.

Isolated hypothyroxinemia at the second, but not first trimester, was significantly associated with increased GDM risk: the aOR (95% CI) comparing women who had hypothyroxinemia to women who had euthyroid status was 1.56 (0.63, 3.89) in the first and 2.97 (1.07, 8.24) in the second trimester (Table 4). Subclinical or overt hypothyroidism in either first [aOR (95% CI), 2.58 (0.39, 17.01)] or second trimester [aOR (95% CI), 1.78 (0.49, 6.42)] was not related to GDM risk.

The results were similar in sensitivity analyses excluding women who had elevated thyroid antibodies, prior history of GDM or preeclampsia, smoked before pregnancy, or had medication-treated GDM. The associations also persisted when limiting the sample to only women who had euthyroid status (n = 252 at weeks 10 to 14; n = 232 at weeks 15 to 26), or when stratifying the analyses by prepregnancy BMI status (BMI < 25.0 vs BMI ≥ 25.0 kg/m^2), race/ethnicity (non-Hispanic white, African American, Hispanic, Asian/Pacific Islander), or family history of diabetes (yes vs no). In additional exploratory analyses (data not shown), we compared thyroid markers between GDM cases with 2 vs 3+ OGTT measures above the threshold, and we found that the latter group had higher fT3 and an fT3/fT4 ratio at both visits, although the differences were not statistically significant. Additionally, among GDM cases, fT3 and the fT3/fT4 ratio at both visits was found to be significantly and positively correlated with fasting glucose levels from OGTT.

In secondary analyses, we examined the longitudinal changes in the level of thyroid markers during the course of pregnancy (Fig. 1). Both fT3 and fT4 levels declined with the progression of pregnancy. Overall, for most study visits, fT3 and the fT3/fT4 ratio were significantly higher, whereas fT4 was significantly lower among cases, as compared with non-GDM controls. TSH levels appeared to increase sharply from the first to second visit and then level off. The difference between cases and controls was significant only at the last visit (weeks 33 to 39), with higher TSH levels among GDM cases.

Discussion

In this longitudinal study, we provide evidence that thyroid function early in pregnancy may be an indicator for subsequent risk of GDM, a common metabolic complication in pregnancy. To our knowledge, this is the first study to identify fT3 and the fT3/fT4 ratio measured early in pregnancy as independent risk factors of GDM. Increased levels of either marker were associated with a greater GDM risk, even after adjusting for potential confounders such as prepregnancy BMI and family history of diabetes. The fT3/fT4 ratio, a marker indicating the conversion rate from T4 to T3, was most strongly associated with GDM, with women in the

Table 2. Median Plasma Concentrations of Thyroid Markers Among Women With GDM and Their Matched Controls in the NICHD Fetal Growth Studies—Singleton Cohort (2009–2013)

	GDM Cases	Non-GDM Controls	P^a
Weeks 10–14[b]			
fT3, pg/dL	300.00 (276.62, 327.27)	279.87 (263.96, 304.22)	**0.002**
fT4, ng/dL	1.02 (0.94, 1.13)	1.06 (0.98, 1.17)	**0.04**
fT3/fT4 ratio[c]	289.60 (263.13, 322.56)	259.74 (238.94, 288.75)	**0.0001**
TSH, mIU/L	1.25 (0.73, 1.83)	1.28 (0.86,1.82)	0.65
Gestational age at blood collection, wk	13.0 (12.3, 13.6)	13.0 (12.4, 13.6)	
Weeks 15–26[d]			
fT3, pg/dL	292.85 (265.26, 325.00)	275.32 (255.19, 299.67)	**0.0003**
fT4, ng/dL	0.88 (0.81, 1.00)	0.94 (0.86, 1.03)	**0.001**
fT3/fT4 ratio[c]	334.51 (285.16, 375.76)	289.55 (257.69, 332.58)	**<0.0001**
TSH, mIU/L	1.96 (1.26, 2.52)	1.91 (1.26, 2.76)	0.59
Gestational age at blood collection, wk	18.9 (17.3, 21.0)	19.0 (17.7, 21.1)	
Change from weeks 10–14 to 15–26			
fT3, pg/dL	0.14 (0.08, 0.20)	0.12 (0.07, 0.20)	0.69
fT4, ng/dL	8.44 (−9.74, 24.03)	5.19 (−13.00, 24.67)	0.44
fT3/fT4 ratio[c]	−37.87 (−63.27, −20.6)	−30.22 (−47.43, −15.4)	**0.0002**
TSH, mIU/L	−0.54 (−1.09, −0.20)	−0.61 (−1.14, −0.21)	0.67

Data are presented as median (25th and 75th percentile). Boldface indicates statistically significant results.

[a] *P* values for differences between case and controls were obtained by generalized linear mixed effects models for continuous variables accounting for matched case-control pairs. *P* values are not shown for gestational age at blood collection, as it was one of the matching variables.

[b] n = 104 and 214 for cases and controls, respectively.

[c] The fT3/fT4 ratio was obtained by dividing plasma concentration of fT3 (pg/dL) by fT4 level (ng/dL).

[d] n = 94 and 212 for cases and controls, respectively.

highest quartile in the second trimester showing an almost 14-fold increased risk compared with women in the lowest quartile.

Although T3 is the primary, biologically active hormone involved in glucose homeostasis, prior studies examining thyroid biomarkers in relationship to GDM risk have been mostly focused on its precursor hormone, fT4, and their regulatory hormone, TSH. Two prospective studies (11, 23) to date have examined the association between fT3 levels and GDM, and both reported no significant differences in fT3 levels in early pregnancy between women with and without subsequent GDM. Inferences of findings from the two studies (11, 23) were hindered by the relatively small number of GDM cases, which may have limited their statistical power of detecting a significant association. Differences in demographic composition, including race/ethnicity, GDM diagnostic criteria, and population-specific reference intervals for thyroid hormones, may also have contributed to divergent findings. Notably, in our study, we identified the fT3/fT4 ratio, a commonly used proxy of peripheral deiodinase activity (15, 16), as a novel risk factor for GDM. Although the fT3/fT4 ratio has not previously been examined in relationship to GDM risk, our findings are consistent with others (17–19) showing a significant association between the fT3:fT4 ratio and measures of glucose and insulin metabolism (*e.g.*, elevated fasting glucose). Taken together, these findings suggest that higher fT3 levels, which could result from either increased fT4 to fT3 conversion or increased T3 synthesis from the thyroid gland, could be related to the pathophysiology of GDM.

Consistent with previous findings (9, 10), we observed lower fT4 levels among women with GDM, yet the associations were not significant after adjusting for potential confounders, particularly prepregnancy BMI. However, compared with women who had euthyroid status in our study, women who had isolated hypothyroxinemia (normal TSH, low fT4 levels) in the second trimester had an almost threefold greater risk of GDM. Short-term fasting is known to affect TSH and not fT4 levels (24–26), but whether the fasting status in the second trimester influenced this trimester-specific association is not clear in our study. However, other studies have also observed that isolated hypothyroxinemia in the second, but not the first trimester, is related to subsequent GDM risk (6, 9, 14). Neither hypothyroidism nor TSH levels alone were associated with GDM risk in our study, which is consistent with some studies (6–8, 11), but contrasts with others (3–5, 23) that observed an elevated GDM risk among women who had subclinical hypothyroidism or high TSH levels in pregnancy. Differences in population characteristics, study design, and sample size may account for these discrepant findings. Of note, all three studies (6–8) reporting a null association had very few women who developed both GDM and

Table 3. aOR (95% CI) for GDM According to Quartiles of Thyroid Markers at Gestational Weeks 10–14 and 15–26 in the NICHD Fetal Growth Studies—Singleton Cohort (2009–2013)

	Case (n)	Control (n)	Crude Model	Multivariable Model[a]
Gestational weeks 10–14[b]				
fT3, pg/dL				
Quartile 1: 1.18–4.06	13	53	1	1
Quartile 2: 4.07–4.31	18	54	1.31 (0.57, 3.00)	1.32 (0.53, 3.28)
Quartile 3: 4.32–4.68	25	52	2.05 (0.91, 4.60)	1.97 (0.78, 4.96)
Quartile 4: 4.69–7.66	43	53	**4.07 (1.82, 9.11)**	**4.25 (1.67, 10.80)**
Upper decile: 5.02–7.66	29	21	**6.09 (2.47, 15.00)**	**6.08 (2.19, 16.87)**
P for trend			**<0.0001**	**0.001**
Per unit increment			**1.02 (1.01, 1.03)**	**1.02 (1.01, 1.03)**
fT4, ng/dL				
Quartile 1: 0.70–0.98	37	55	1	1
Quartile 2: 0.99–1.06	24	52	0.69 (0.37, 1.31)	0.70 (0.34, 1.45)
Quartile 3: 1.07–1.17	23	59	0.61 (0.32, 1.16)	0.69 (0.33, 1.42)
Quartile 4: 1.18–2.26	19	48	0.58 (0.29, 1.19)	0.63 (0.26, 1.51)
Upper decile: 1.29–2.26	7	19	0.61 (0.22, 1.66)	0.83 (0.27, 2.56)
P for trend			0.11	0.30
Per unit increment			0.23 (0.04, 1.22)	0.54 (0.08, 3.75)
fT3/fT4 ratio[c]				
Quartile 1: 0.83–3.67	9	53	1	1
Quartile 2: 3.68–4.00	12	54	1.43 (0.51, 4.02)	1.12 (0.34, 3.74)
Quartile 3: 4.01–4.44	26	52	**4.03 (1.56, 10.45)**	**5.26 (1.70, 16.20)**
Quartile 4: 4.45–6.85	51	53	**8.03 (3.13, 20.61)**	**8.63 (2.87, 26.00)**
Upper decile: 5.39–6.85	25	22	**10.25 (3.62, 29.01)**	**9.28 (2.76, 31.26)**
P for trend			**<0.0001**	**0.001**
Per unit increment			**1.01 (1.01, 1.02)**	**1.01 (1.01, 1.02)**
TSH, mIU/L				
Quartile 1: 0.06–0.86	29	53	1	1
Quartile 2: 0.87–1.28	22	53	0.77 (0.38, 1.56)	0.71 (0.32, 1.59)
Quartile 3: 1.29–1.82	23	51	0.85 (0.43, 1.69)	0.63 (0.28, 1.44)
Quartile 4: 1.83–30.11	25	52	0.88 (0.46, 1.71)	1.17 (0.54, 2.51)
Upper decile: 2.53–30.11	12	20	1.11 (0.48, 2.57)	1.43 (0.53, 3.84)
P for trend			0.83	0.78
Per unit increment			1.08 (0.94, 1.24)	1.11 (0.93, 1.32)
Gestational weeks 15-26[b]				
fT3, pg/dL				
Quartile 1: 2.95–3.93	14	57	1	1
Quartile 2: 3.94–4.24	19	50	1.78 (0.77, 4.13)	1.25 (0.46, 3.37)
Quartile 3: 4.25–4.61	16	52	1.65 (0.70, 3.89)	1.86 (0.70, 4.92)
Quartile 4: 4.62–6.63	43	53	**3.84 (1.74, 8.48)**	**3.89 (1.50, 10.10)**
Upper decile: 4.93–6.63	27	21	**6.84 (2.65, 17.65)**	**7.30 (2.30, 23.16)**
P for trend			**<0.0001**	**0.007**
Per unit increment			**1.01 (1.01, 1.02)**	**1.02 (1.01, 1.03)**
fT4, ng/dL				
Quartile 1: 0.63–0.86	39	54	1	1
Quartile 2: 0.87–0.94	19	53	**0.46 (0.24, 0.89)**	0.65 (0.31, 1.24)
Quartile 3: 0.95–1.03	22	56	0.52 (0.26, 1.05)	0.57 (0.26, 1.24)
Quartile 4: 1.04–2.53	14	51	**0.31 (0.14, 0.69)**	0.44 (0.17, 1.14)
Upper decile: 1.11–2.53	4	26	**0.14 (0.04, 0.52)**	**0.17 (0.04, 0.76)**
P for trend			**0.005**	**0.047**
Per unit increment			**0.04 (0.00, 0.39)**	**0.07 (0.01, 0.76)**
fT3/fT4 ratio[c]				
Quartile 1: 1.80–3.96	8	53	1	1
Quartile 2: 3.97–4.45	16	53	2.14 (0.84, 5.48)	2.46 (0.78, 7.72)
Quartile 3: 4.46–5.12	21	53	**3.29 (1.31, 8.27)**	**4.37 (1.41, 13.50)**
Quartile 4: 5.13–7.83	47	53	**8.61(3.37, 21.98)**	**13.60 (3.97, 46.30)**
Upper decile: 5.67–7.83	28	22	**9.09 (3.45, 23.98)**	**12.73 (3.71, 43.69)**
P for trend			**<0.0001**	**<0.0001**
Per unit increment			**1.01 (1.01, 1.02)**	**1.01 (1.01, 1.02)**

(*Continued*)

Table 3. aOR (95% CI) for GDM According to Quartiles of Thyroid Markers at Gestational Weeks 10–14 and 15–26 in the NICHD Fetal Growth Studies—Singleton Cohort (2009–2013) (Continued)

	Case (n)	Control (n)	Crude Model	Multivariable Model[a]
TSH, mIU/L				
Quartile 1: 0.21–1.26	24	55	1	1
Quartile 2: 1.27–1.91	21	52	1.09 (0.53, 2.23)	0.95 (0.41, 2.17)
Quartile 3: 1.92–2.76	31	54	1.42 (0.72, 2.81)	1.44 (0.66, 3.11)
Quartile 4: 2.77–8.28	18	53	0.76 (0.37, 1.57)	0.93 (0.39, 2.23)
Upper decile: 3.77–8.28	9	21	0.92 (0.35, 2.46)	1.64 (0.49, 5.44)
P for trend			0.56	0.92
Per unit increment			0.96 (0.78, 1.19)	1.07 (0.83, 1.38)

Boldface indicates statistically significant results.

[a]Adjusted for maternal age (years), gestational age at blood collection (weeks), nulliparity (yes/no), education (less than, equal to, or more than high school), family history of diabetes (yes/no), prepregnancy BMI (<25, 25.0–29.9, ≥30.0 kg/m^2), and thyroid autoimmunity status (antibodies positive vs negative).

[b]Timing of blood sample collection preceded the diagnosis of gestational diabetes in all participants.

[c]The tT3/fT4 ratio was obtained by dividing plasma concentration of fT3 (pg/dL) by fT4 level (ng/dL).

subclinical hypothyroidism. Recently, a meta-analysis of six cohort studies also showed that subclinical hypothyroidism was significantly associated with a 1.35-fold increased risk of GDM as compared with women who had euthyroid status (2). Because our sample only had 20 women who had overt or subclinical hypothyroidism before GDM diagnosis, we cannot rule out the possibility that the observed lack of a significant association could be due to inadequate statistical power.

Our findings are biologically plausible. Thyroid hormones regulate hepatic gluconeogenesis, intestinal absorption of glucose, and uptake of glucose in peripheral tissues (2). Additionally, they modulate messenger RNA and protein expression of glucose transporters, promote pathways that accelerate glycogenolysis, and modify circulating insulin levels and counterregulatory hormones (27, 28). Among the thyroid hormones, T3 is the biologically active hormone responsible for stimulating endogenous glucose production, with several studies noting that fT3 levels are positively associated with insulin secretion and hyperinsulinemia (29, 30). Around 80% of circulating T3 levels are derived from monodeiodination of T4 carried out by peripheral deiodinase activity (13), supporting the notion that the fT3/fT4 ratio could serve as an important marker for glucose homeostasis. Furthermore, a missense variant (Thr92Ala) in the gene encoding type 2 deiodinase, the subtype of deiodinase specific to generating T3 from T4, has been found to be associated with insulin resistance as measured by the hyperinsulinemic–euglycemic clamp (31).

As a secondary objective of our study, we profiled the longitudinal trajectory of the thyroid hormones across the entire course of pregnancy. In our sample, we observed expected changes in the levels of TSH, fT3, and fT4 hormones across pregnancy. Maternal thyroid physiology changes considerably in pregnancy owing to several mechanisms, including transient rise in human

Table 4. Adjusted OR (95% CI) for GDM According to Hypothyroidism or Isolated Hypothyroxinemia at Gestational Weeks 10–14 and 15–26 in the NICHD Fetal Growth Studies—Singleton Cohort (2009–2013)

	Case (n)	Control (n)	Crude Model	Multivariable Model[a]
Gestational weeks 10–14[b]				
Euthyroid[c]	79	171	1	1
Overt/subclinical hypothyroidism[d]	3	3	2.09 (0.41, 10.73)	2.58 (0.39, 17.01)
Isolated hypothyroxinemia[e]	13	15	2.18 (0.96, 4.94)	1.56 (0.63, 3.89)
Gestational weeks 15–26[b]				
Euthyroid[c]	71	163	1	1
Overt/subclinical hypothyroidism[d]	6	14	1.00 (0.36, 2.80)	1.78 (0.49, 6.42)
Isolated hypothyroxinemia[e]	13	11	2.93 (1.19, 7.21)	2.97 (1.07, 8.24)

[a]Adjusted for maternal age (years), gestational age at blood collection (weeks), nulliparity (yes/no), education (less than, equal to, or more than high school), family history of diabetes (yes/no), prepregnancy BMI (<25, 25.0–29.9, ≥30.0 kg/m^2), and thyroid autoimmunity status (antibodies positive vs negative).

[b]Timing of blood sample collection preceded the diagnosis of gestational diabetes in all participants.

[c]Women with normal TSH (≤4 mIU/L) and normal fT4 concentration (between 10th and 90th percentile) were classified as euthyroid.

[d]Overt/subclinical hypothyroidism having elevated TSH levels (>4 mIU/L) with low or normal fT4 concentration (≤90th percentile in controls).

[e]Isolated hypothyroxinemia was defined as having normal TSH (≤4 mIU/L) in conjunction with low fT4 levels (<10th percentile in controls).

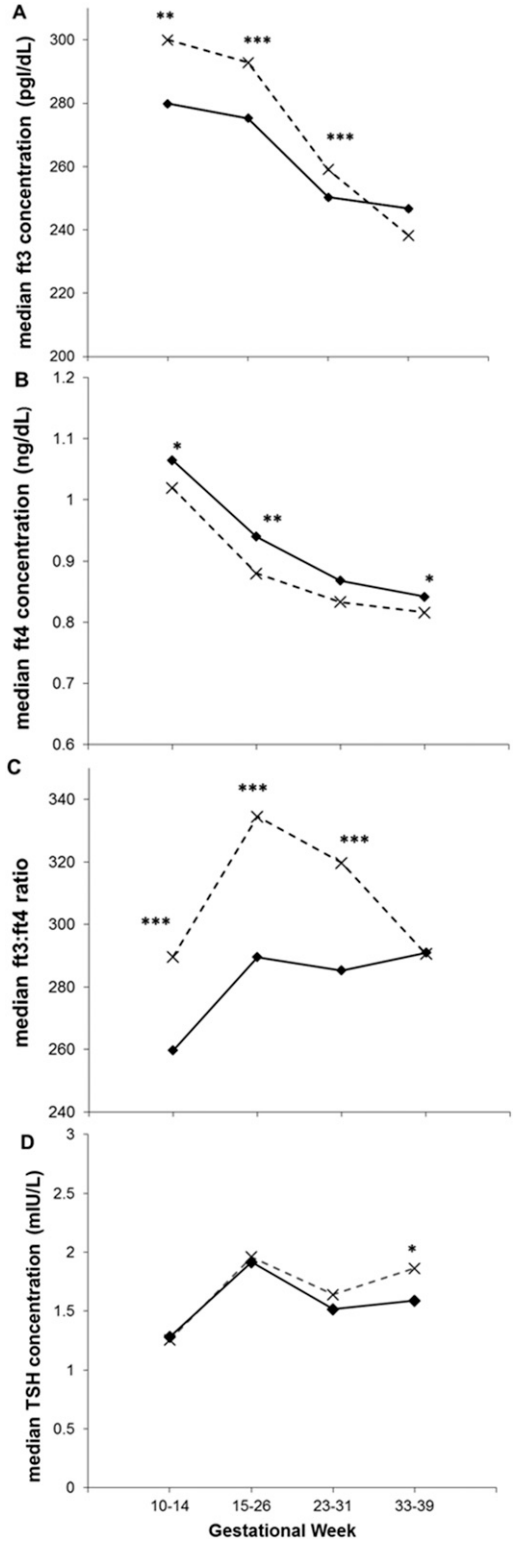

Figure 1. Median plasma concentrations of (A) fT3, (B) fT4, (C) fT3/fT4 ratio, and (D) TSH at each study visit among women with GDM and their matched controls. Solid line indicates non-GDM controls; dashed line indicates GDM cases. Weeks 10 to 14, n = 104 and 214 for cases and controls, respectively; weeks 15 to 26, n = 94 and 212 for cases and controls, respectively; weeks 23 to 31, n = 102 and 106 for cases and controls, respectively; weeks 33 to 39, n = 88 and 101 for cases and controls, respectively. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ for case-control comparisons at each study visit obtained by generalized linear mixed effects models accounting for matched case-control pairs.

chorionic gonadotropin in early pregnancy, increased concentrations of T4 binding proteins, increased thyroid hormone metabolism by the placenta, and greater iodide excretion in the urine (1). Although women were enrolled in our study early in pregnancy between 10 and 14 weeks, we likely missed the human chorionic gonadotropin–induced decrease in TSH levels very early in gestation (1), only capturing the gradual increase in TSH levels thereafter (*i.e.*, second and third trimesters). fT4 and fT3 levels showed a continuous decline with the progression of pregnancy, which was consistent with findings of others (32, 33). Of note, our study was unique in that we longitudinally profiled the thyroid hormone parameters in a relatively large multiracial cohort of pregnant women.

There are several strengths to our study. First, we longitudinally measured several markers of thyroid function across pregnancy, allowing us to prospectively examine the trimester-specific associations between thyroid status and GDM, which is critically important given the changes in thyroid hormones across gestation (32, 33). Second, because thyroid autoimmunity status may influence both thyroid hormone levels and glucose homeostasis, an additional strength of this study was that we measured and accounted for thyroid antibody levels in our primary analyses. Third, our sample had a good representation of four major racial/ethnic groups in the United States, and GDM status in these women was well characterized based on review of medical records. Moreover, our study sample included relatively healthy women without pre-existing thyroid disease or any other chronic conditions. Thyroid medication use in pregnancy was also ascertained and accounted for. One of the limitations of this study was that due to the low frequency of clinical thyroid conditions in our study sample, we could not consider the joint effect of thyroid autoimmunity status and hypothyroidism on GDM risk. Because maternal iodine status is an important determinant of thyroid hormone levels, another limitation was the lack of iodine measurements in our study. However, it is reasonable to assume that our relatively healthy sample of US women would be iodine sufficient. Lastly, trimester-specific ranges for thyroid hormone levels were not available from our laboratory, and, as such, we used reference ranges recommended by the 2017 American Thyroid Association guidelines.

In summary, findings from this longitudinal study suggest that higher fT3 levels, potentially resulting from *de novo* synthesis or increased deiodinase activity, may be involved in the pathophysiology of GDM. At present, the utility of routine screening for thyroid function during pregnancy is controversial. This study adds an important piece of evidence to this debate, as our findings

show that women with thyroid abnormalities in early to middle pregnancy are at an increased risk for GDM and its adverse health sequelae. Our findings, in conjunction with previous evidence of thyroid-related adverse pregnancy outcomes, support the potential benefits of thyroid screening among pregnant women.

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