The role of triiodothyronine (T3) and T3/free thyroxine (fT4) on glucose metabolism during pregnancy: the Ma’anshan Birth Cohort Study

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Abstract

Objectives

Compared with other thyroid markers, fewer studies explored the associations between triiodothyronine (T3) and T3/free thyroxine (fT4) and glucose abnormality during pregnancy. Thus, we aimed to: (1) Examine the associations of T3 and T3/fT4 with glucose metabolism indicators; and (2) Evaluate, in the first trimester, the performance of the two markers as predictors of gestational diabetes mellitus (GDM) risk.

Methods

Longitudinal data from 2723 individuals, consisting of three repeated measurements of T3 and fT4, from the Man’anshan birth cohort study (MABC), China, were analyzed using a time-specific generalized estimating equation (GEE). The receiver operating characteristic curve (ROC) - area under the curve (AUC) and Hosmer-Lemeshow goodness of fit test were used to assess the discrimination and calibration of prediction models.

Results

T3 and T3/fT4 presented stable associations with the level of fasting glucose, glucose at 1h/2h across pregnancy. T3 and T3/fT4 in both the first and second trimesters were positively associated with the risk of GDM, with the larger magnitude of association observed in the second trimester (Odds ratio (OR) = 2.50, 95%CI = 1.95, 3.21 for T3; OR = 1.09, 95%CI = 1.07, 1.12 for T3/fT4). T3 ((AUC) = 0.726, 95%CI = 0.698, 0.754) and T3/fT4 (AUC = 0.724, 95%CI = 0.696, 0.753) in the first trimester could improve the performance of the predicting model, however, the overall performance is not good.

Conclusion

Significant and stable associations of T3, T3/fT4 and glucose metabolism indicators were
documented. Both T3 and T3/fT4 improve the performance of the GDM predictive model.

**Keywords:** longitudinal studies; triiodothyronine; triiodothyronine/free thyroxine; glucose; pregnancy
Introduction

The thyroid gland experiences great stress during pregnancy, increasing about 10-40% in size, with the production of thyroxine (T4) and triiodothyronine (T3) surging by 50% (1). As a result, thyroid dysfunction such as hypothyroidism, hyperthyroidism and autoantibody positivity complicate up to 17% of all pregnancies (1). It is well accepted that thyroid function abnormalities have a deleterious impact on pregnancy (2, 3).

Hyperglycemia during pregnancy, typically diagnosed as gestational diabetes mellitus (GDM) is a highly prevalent complication during pregnancy and affects around 15-20% of pregnancies (4) and the world has been witnessing a rapid increase in GDM incidence (5). Its negative health consequences for women and their offspring occur both during pregnancy (5) and beyond (6).

Thyroid hormones are involved in glucose metabolism and homeostasis through multiple ways (7) (8) (9, 10), and the associations between thyroid dysfunction and glucose abnormality have been well established. However, previous studies mainly focused on thyroid-stimulating hormone (TSH), fT4 and T4. T3 has long been overlooked, even though T3 plays a significant role in glucose regulation (11). In recent years, T3 has gained popularity in the scientific literature due to it being the active metabolic form of thyroid hormone that can induce endogenous glycemic activity (12). Most peripheral T3 is produced in-part by the conversion of T4 by two deiodinase enzymes, type 1 and type 2 iodothyronine deiodinases (D1 and D2, respectively) (13), with the rest produced directly by the thyroid gland. Therefore, the ratio of T3 (or fT3) to T4 (or fT4) is attracting attention as well but requires extensive further exploration.

As such, based on repeated measurements from a prospective cohort study, we aimed to: (1) Examine the associations of T3 and T3/fT4 with glucose metabolism indicators during pregnancy; (2)
Evaluate the performance of the two markers, in the first trimester, as predictors of the risk for GDM.

Material and Methods

Study population

The Ma’an shan birth cohort study (MABC) is a population-based prospective study conducted in Ma’an shan city of Anhui Province in China. Between May 2013 and September 2014, the MABC recruited 3,474 pregnant women who were attending their first prenatal health visit. The participants were then followed until labor for a maximum of four visits (i.e., the first, second, and third trimesters and delivery). Of these participants, we first excluded those who lacked information on GDM status and were diagnosed with diabetes before conception, yielding a sample of 3,308 women. In addition, we excluded participants without thyroid function data in any trimesters, yielding a final sample of 2,723 women. The present study was approved by the ethics committee of Anhui Medical University. Written informed consent was obtained from all pregnant women.

GDM diagnosis

At 24-28 weeks of gestation, women underwent a GDM screening that consisted of a “one-step” standardized 75 g oral glucose tolerance test (OGTT). Venous blood samples were collected at 0, 1, and 2 h after a glucose load. A positive GDM diagnosis was made when any of the following criteria were met: fasting plasma glucose ≥ 5.1 mmol/L, plasma glucose at 1 h ≥ 10 mmol/L, or plasma glucose at 2 h ≥ 8.5 mmol/L (14). These data, along with fasting glucose in each trimester, were extracted from medical records, and plasma glucose was measured using the glucose oxidase method.

Thyroid hormone measurement

Blood samples were collected at the first, second, and third health visits. Fasting blood was drawn in the morning before 10:00 AM, and serum specimens were isolated and stored at -80°C for further
examination. Serum samples were assayed for levels of fT4, T3, thyrotropin (TSH), antithyroperoxidase autoantibody (TPOAb) and antithyroglobulin autoantibody (TgAb) with electrochemiluminescence using the Cobas e411 automated immunoassay platform (Roche Diagnostics GmbH, Mannheim, Germany). All samples were tested blindly and each batch included two quality control samples. The coefficients of variation for these thyroid profile assays were both less than 10%. The detection limits of fT4, T3, TSH, TPOAb and TgAb were 0.300 pmol/L, 0.300 nmol/L, 0.005 μIU/mL, 5.0 IU/mL and 10.0 IU/mL, respectively. TPOAb and TgAb were considered positive if concentrations were greater than 34.0 IU/mL and 115.0 IU/mL, respectively.

Covariates

Extensive data were collected using a structured self-report questionnaire that was administered by trained interviewers at each health visit. Smoking in early pregnancy was defined as either ongoing smoking or former smoking that ceased after the woman became aware of her pregnancy status. Alcohol consumption was categorized as never, occasional, or regular. Education level was categorized into five groups: 1) Primary school or below; 2) Middle school; 3) High school; 4) Junior college; and 5) Undergraduate or above. Monthly income levels (Chinese Yuan) were divided into four groups: 1) < 1000; 2) 1000-2500; 3) 2500-4000; and 4) ≥ 4000. Seasons for the Northern Hemisphere were defined as spring (i.e., February to April), summer (i.e., May to July), fall (i.e., August to October) and winter (i.e., November to January).

Statistical analysis

After log transformation, values outside +/- 4 standard deviation (SD) of the mean were regarded as either laboratory error or undiagnosed disease and were treated as outliers. The outliers were omitted as following, in each trimester: Eight outliers for T3, 17 for fT4 and 12 for TSH in the first trimester,
One for T3, 7 for fT4 and 13 for TSH in the second trimester; Five for T3, 5 for fT4 and 10 for TSH in the third trimester.

The Mann-Whitney U-test was used to compare median concentrations of the different thyroid markers between the GDM and non-GDM groups. Fasting glucose differences between different trimesters were compared using paired samples T-test. A generalized estimating equation (GEE) was used to examine the associations of demographic or lifestyle factors with thyroid markers. The multiple informant model with a nonstandard version of GEE was used to examine trimester-specific associations of thyroid markers with fasting plasma glucose, plasma glucose at 1 h/ 2 h, and the risk of GDM. For fasting plasma glucose, data from three trimesters were analyzed, while for glucose at 1h/2h and the risk of GDM, only data from the first and second trimesters were included in the model.

Stratified analyses of the associations between thyroid markers and the risk of GDM were conducted according to pre-pregnancy BMI, parity, smoking status, TPOAb and TgAb status using logistic regression. The multiplicative interaction term was evaluated by a likelihood ratio test. Sensitivity analyses of the association between T3, fT4 and T3/fT4 and the risk of GDM were conducted according to the item with which the diagnosis of GDM was made (i.e., high fasting glucose versus high postprandial glucose versus both).

To further specify the clinical significance of T3 and T3/fT4, we built multivariate predictive models along with routine variables (i.e., maternal age, pre-pregnancy BMI, history of family diabetes, gestational seasons, fasting plasma glucose), and compared each of their performances in predicting GDM. Sensitivity, negative predictive value (NPV) and positive predictive value (PPV) were calculated under a fixed specificity of 90.0% and a fixed false positive rate of 10% for different models. The receiver operating characteristic curve (ROC)-area under curve (AUC) was drawn, and the
Z statistic was used to compare the discrimination of different models. Hosmer-Lemeshow goodness of fit test were conducted to assess the calibration of predictive models.

Analyses were conducted using Stata 13.0, R 3.5.1. and MedCalc 18.11.3. Statistical significance was set at \( P < 0.05 \). All tests were 2-sided.

**Results**

Table 1 presents the characteristics of the 2723 participants in our study. A total of 336 (12.3%) patients with GDM were documented. There was detectable difference of GDM incidence between individuals with (n = 336; 12.3%) and without thyroid function data (n = 585; 15.9%) (\( P \) for \( \chi^2 = 0.02 \)). The average age was 26.7 years. Fasting glucose level decreases with increasing gestational age (\( P \) for the difference between 1st and 2nd trimester < 0.001; \( P \) for the difference between 2nd and 3rd trimester < 0.001).

Table 2 presents the concentrations of different thyroid hormones in different gestational age groups as a whole and according to GDM status. T3 concentration increases gradually with increasing gestational age, from 2.35 nmol/L in the first trimester to 2.69 nmol/L in the third trimester. fT4 drops sharply from the first to the second trimester from 16.7 pmol/L to 11.9 pmol/L and increases slightly afterward to 13.3 pmol/L. T3/fT4 increases substantially from 139 in the first trimester to 214 in the second trimester, while decreases a little bit to 202 in the third trimester. TSH increases significantly from 1.58 μIU/mL in the first trimester to 2.42 μIU/mL in the second trimester, while decreases slightly to 2.21 μIU/mL in the third trimester. There are differences in T3 concentrations between the GDM and non-GDM groups across all three periods, as was similar for T3/fT4 concentrations. For fT4 and TSH, the difference was only detected in the second trimester.

Fig. 1 shows the consistent percentage of individuals whose concentrations of T3, fT4 and T3/fT4 were above 75% or below 25% across the three trimesters. For T3, the top 25% individuals in the first
trimester became 11.9% in the second trimester and 6.3% in the third trimester. Likewise, the change for fT4 progressed from 25% in the first trimester, to 12.2% in the second trimester, and 7% in the third trimester. T3/fT4 changed from 25% in the first trimester, to 12.3% in the second trimester and 10.5% in the third trimester.

Table S1 shows the associations between different thyroid markers and related factors. Independent of the adjusted factors, all three thyroid markers were significantly associated with pre-pregnancy BMI, gestational weeks, smoking, and sampling seasonality. As for other factors, age was significantly related to T3 and fT4 concentrations; drinking was significantly associated with T3 and T3/fT4 ratio; parity was significantly associated with fT4 concentrations; gestational age and smoking were significantly associated with TSH concentrations.

Table 3 presents trimester-specific associations between thyroid markers and glucose metabolism indicators by GEE. The effect sizes for T3, fT4, T3/fT4 and TSH are indicated in per nmol/L, per pmol/L, per 10 Unit and per μIU/mL, respectively. Regarding fasting glucose and glucose at 1h/2h, T3 in all periods were significantly associated with all three indicators, except fasting glucose in the first trimester, with the largest magnitude of association observed in the second trimester (β = 0.16, 95%CI = 0.11, 0.20 for fasting glucose; β = 0.41, 95%CI = 0.28, 0.55 for glucose at 1h; β = 0.21, 95%CI = 0.11, 0.31 for glucose at 2h). fT4 in all periods were significantly associated with all the three indicators, except fasting glucose in the third trimester, with the largest magnitude of association observed in the second trimester (β = -0.02, 95%CI = -0.03, -0.01 for fasting glucose; β = -0.14, 95%CI = -0.18, -0.10 for glucose at 1h; β = -0.08, 95%CI = -0.11, -0.05 for glucose at 2h). T3/fT4 in all periods were significantly associated with all the three indicators, with the largest magnitude of association observed in the second trimester (β = -0.02, 95%CI = -0.03, -0.01 for fasting glucose; β =
-0.14, 95%CI = -0.18, -0.10 for glucose at 1h; $\beta = -0.08$, 95%CI = -0.11, -0.05 for glucose at 2h; TSH in both first and second trimester were significantly associated with glucose at 1h/2h. In the first trimester, only T3 (OR = 1.67, 95%CI = 1.33, 2.10) and T3/fT4 (OR =1.08, 95%CI = 1.04, 1.12) were significantly associated with the risk of GDM, while in the second trimester, T3 (OR = 2.50, 95%CI = 1.95, 3.21), fT4 (OR = 0.89, 95%CI = 0.82, 0.96), T3/fT4 (OR = 1.09, 95%CI = 1.07, 1.12) and TSH (OR = 0.91, 95%CI =0.83, 0.99) in the second trimester were all significantly associated with the risk of GDM.

Stratified analyses of associations between three thyroid markers and the risk of GDM are presented in Table S2. Sensitivity analyses of the associations of three thyroid markers and the risk of GDM according to the item with which the GDM is diagnosed are shown in Table S3.

Because T3 and T3/fT4 in the first trimester were both significantly associated with the risk of GDM, we further assessed their performances in predicting GDM. As shown Table 4 and in Fig. 2, adding T3 and T3/fT4 in the models respectively, the performance of model 3 (AUC = 0.726, 95%CI = 0.698, 0.754) and model 4 (AUC = 0.724, 95%CI = 0.696, 0.753) improved and showed comparable medium degree of discriminative ability, and the AUC of the models ranked as model 3 (0.726) > model 4 (0.724) > model 2 (0.710) > model 1 (0.703). As shown in Table S4, the comparison between model 3 and model 1 ($P = 0.004$); model 3 and model 2 ($P = 0.02$); model 4 and model 1 ($P = 0.01$); model 4 and model 2 ($P = 0.04$) reached statistical significance. The Hosmer-Lemeshow goodness of fit test indicated that the calibration capability of the four models was good ($P$ value > 0.05). However, the calibration diagram indicated that the calibration capability of model 4 (Fig.3b) was better than model 3 (Fig. 2a).The best multivariate predictive model of GDM including T3 (or T3/fT4), maternal age, pre-pregnancy BMI, fasting plasma glucose in the first trimester, sampling gestational week, the
season of conception and family history of diabetes.

Discussion

Our current study demonstrated trimester-specific associations between T3, fT4 and T3/fT4 with glucose metabolism indicators, with the largest magnitude of association observed in the second trimester for all three thyroid markers, and the most stable associations for T3/fT4 across pregnancy. Along with routine factors, T3 and T3/fT4 in the first trimester could improve the performance of the GDM predicting model and exhibited a comparable degree of discrimination ability and good calibration.

Our study echoed, and built upon, a recent nested case-control study from the US which indicated that both fT3 and fT3/fT4 were significantly positively associated with the risk of GDM (15). T3 is the primary active hormone involved in glucose metabolism (16) (12), yet it has long been neglected regarding its association with GDM. Given low fT4 hormonal activity, it is unlikely to influence plasma glucose directly. As a result, two studies (17, 18) reporting the associations between fT4 concentrations and the risk of GDM proposed that deiodinase activity might be the underlying factor. Only a handful of longitudinal studies (15, 19, 20) explored the associations of T3, fT3, or deiodinase activity (often indicated as fT3/fT4 or T3/fT4) with the risk of GDM. Neither a study from the United Arab Emirates with 301 participants (19) nor a study from Greece with 1170 participants (20) indicated that early pregnancy fT3 concentrations were associated with the risk of GDM. To our knowledge, the remaining studies were all cross-sectional in nature. Other than dichotomous GDM status (21, 22), some also focused on the glucose metabolic profile (23-25), and all reported significant associations between fT3/fT4 or T3/fT4 and glucose metabolism-related indicators.

In addition to stimulating endogenous glucose production (12), T3 could be involved in glucose
metabolism in various ways. For example, T3 can positively regulate mitochondrial functioning and induce mitochondria biogenesis (26, 27), dysfunction of the latter emerged as a new insight into the etiology of diabetes and glucose abnormality (11). In addition, T3 can exert an important effect on the control of energy expenditure. For instance, under the effect of T3, skeletal muscle energy metabolism is switched into oxidative mitochondrial metabolism mediated by PGC-1α (28), which plays an indispensable role in glucose and fatty acid metabolism (29). Furthermore, T3 can increase glucose uptake independent of insulin in skeletal muscle cells, such as through rapidly increasing GLUT4 expression (30), and the latter plays an important role in whole-body glucose homeostasis.

Collectively, our data strongly support the authentic roles of T3 in the etiology of GDM.

T3 and T3/fT4 in the first trimester could improve the performance of the GDM predicting model, however, even along with routine factors, judging from the NPV and PPV of the predictive model, the performance of the model is not good. A recent review (31) indicated that universal versus risk-based screening for thyroid problems did not affect the risk of GDM, and the evidence originally derived from a trial (32) that included 4516 women. All women from the trial in the universal screening group and high-risk women in the case finding group had their sera immediately tested for TSH, fT4, and TPOAb to identify thyroid dysfunction, however, subsequent medication interventions did not benefit those women in preventing GDM. Our study doesn’t conflict with this trial, because we emphasized the importance of T3 and T3/fT4, although the extent to which the screening of T3 and T3/fT4 in the first trimester could benefit women, regarding GDM prevention, is still uncertain and requires the collection of more data. Although OGTT becomes a routine test, given its inconvenience, not every woman may opt to undergo this test. While thyroid function TSH and T4 testing is being routinely adopted in early pregnancy around China, our study justifies adding T3 into this routine, which would help identify high
risk individuals for GDM. Recently, a large sample-sized study (33) from China tempted to build the GDM predicting model. The study team selected 17 variables from 73 extracted variables, of which thyroid function (i.e., TT3 and TT4) were included. Interestingly, the results indicated the TT3 and TT4 levels had better predictive power than FT3 and FT4.

Another interesting finding was that the consistency of fasting glucose throughout pregnancy is better than thyroid markers. Previous studies on thyroid function mainly focused on early pregnancy and lacked longitudinal data to illustrate the natural course of gestational thyroid function. Our study showed that individuals with T3, fT4, and T3/fT4 levels among the highest or lowest 25% of the populations, in the first trimester, persisted in 6.3%, 7.0%, and 10.5% of the population in the third trimester is in line with a recent study (34) which indicated that early pregnancy thyroid disease only persists until the third trimester in 8.4–24.8% of cases when left untreated. So, the necessity of further thyroid function tests during pregnancy should not only be decided by previous measures.

Our study has several strengths. GDM diagnoses are often made during the second trimester (24–28 gestational weeks), which presents a limited time for an intervention. Our study innovatively proposed including thyroid functioning in the GDM predicting model, which could help identify high-risk individuals at an early stage. In addition, taking advantage of the MABC longitudinal data with repeated measurements, we were able to assess the persistence of the thyroid markers across three trimesters, which could aid clinicians in making decisions regarding thyroid function testing during pregnancy. Some limitations also need to be noted. First, iodine status at the individual level was not determined in our study, which could have influenced our results to some extent. However, according to 2019 statistics from the Centers for Disease Control and Prevention (CDC), people in Ma’anshan city are generally exposed to appropriate iodine conditions, and the median urinary iodine
concentration of women is 174.05 ug/L. Second, the level of fT3 was not evaluated in our study, which limits comparisons of its importance with that of T3. Third, since we don’t have data on thyroxine binding globulin (TGB), this could affect the interpretation of our results.

Our current study documented significant and stable associations between T3, T3/fT4 and glucose metabolism indicators and both T3 and T3/fT4 could improve the performance of the GDM predicting model.

Conflicts of interest: None.

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Statement of Authorship

Beibei Zhu: Formal analysis and Writing original draft; Yan Han: Resources; Fen Deng: Methodology; Shuangqin Yan and Kun Huang: Project administration; Jiahu Hao and Peng Zhu: Writing Review & Editing; Fangbiao Tao and Beibei Zhu: Funding acquisition. Fangbiao Tao: Conceptualization and Supervision;
Figure Legends

Fig.1. Consistency of the three thyroid markers across three trimesters of pregnancy.

The first bar indicates the highest or lowest 25%. The following two bars indicate the percentage of individuals who are above 75% or below 25% in the first trimester, persisting through the second and third trimesters.

Fig.2. Comparison of multivariate predictive models.

Fig.3. Calibration chart of multivariate predictive models including thyroid hormones.

a. Model including T3; b. Model including T3/fT4.
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| Variables                  | All subject | GDM       | Non-GDM   |
|---------------------------|-------------|-----------|-----------|
| N                         | 2723        | 336 (12.3)| 2387 (87.7)|
| Age                       | 26.7 (3.6)  | 28.0 (4.3)| 26.5 (3.4)|
| Body Mass Index           | 20.8 (2.8)  | 22.2 (3.1)| 20.6 (2.7)|
| Gestational age           |             |           |           |
| First trimester           | 10.0 (2.1)  | 9.9 (2.0) | 10.0 (2.1)|
| Second trimester          | 25.6 (1.0)  | 25.6 (0.9)| 25.6 (1.0)|
| Third trimester           | 34.0 (1.0)  | 34.0 (1.1)| 34.0 (1.0)|
| Multiparous               | 290 (10.7)  | 47 (14.0)| 243 (10.2)|
| Ethnic                    |             |           |           |
| Han                       | 2682 (98.5) | 329 (97.9)| 2353 (98.6)|
| Hui                       | 23 (0.8)    | 4 (1.2)   | 19 (0.8)  |
| Other                     | 18 (0.7)    | 3 (0.9)   | 15 (0.6)  |
| Residence                 |             |           |           |
| Urban                     | 2134 (78.4) | 272 (81.0)| 1862 (78.0)|
| Suburb                    | 316 (11.6)  | 42 (12.5)| 274 (11.5)|
| Town                      | 48 (1.7)    | 7 (2.1)   | 41 (1.7)  |
| Rural                     | 226 (8.3)   | 15 (4.5)  | 210 (8.8) |
| Education                 |             |           |           |
| Primary school or below   | 33 (1.2)    | 4 (1.2)   | 29 (1.2)  |
| Middle school             | 500 (18.4)  | 76 (22.6)| 425 (17.8)|
| High school               | 606 (22.3)  | 69 (20.5)| 537 (22.5)|
| Junior college            | 851 (31.3)  | 101 (30.1)| 749 (31.4)|
| Undergraduate or above    | 733 (26.9)  | 86 (25.6)| 647 (27.1)|
| Family income per capital (Chinese Yuan) | | | |
| <1000                     | 50 (1.8)    | 8 (2.4)   | 42 (1.8)  |
| 1000,2500                 | 669 (24.6)  | 104 (31.0)| 566 (23.7)|
| 2500,4000                 | 1167 (42.9)| 142 (42.3)| 1042 (43.7)|
| >4000                     | 837 (30.7)  | 82 (24.4)| 755 (31.6)|
| Smoking                   |             |           |           |
| No                        | 2614 (96.0) | 323 (96.1)| 2291 (96.0)|
| Yes                       | 109 (4.0)   | 13 (3.9)  | 96 (4.0)  |
| Drinking                  |             |           |           |
| Never                     | 2505 (92.0) | 309 (92.0)| 2196 (92.0)|
| Occasionally              | 214 (7.9)   | 26 (7.7)  | 188 (7.9) |
| Regularly                 | 4 (0.1)     | 1 (0.3)   | 3 (0.1)   |
| Fast glucose              |             |           |           |
| First trimester           | 4.7 (0.4)   | 4.8 (0.6)| 4.7 (0.5) |
| Second trimester          | 4.3 (0.5)   | 5.0 (0.7)| 4.2 (0.5) |
| Third trimester           | 4.2 (0.6)   | 4.7 (0.9)| 4.2 (0.6)|

Data presented as mean (SD), or n (%), as applicable.
| Variables | Combined          | Non-GDM           | GDM               | P     |
|-----------|-------------------|-------------------|-------------------|-------|
|           |                   |                   |                   |       |
| **1st trimester** |                   |                   |                   |       |
| T3        | 2.35 (1.54, 3.53) | 2.33 (2.01, 2.68) | 2.50 (2.14, 2.84) | <0.001|
| ftT4      | 16.7 (12.6, 23.5) | 16.7 (15.4, 18.4) | 16.7 (15.1, 18.0) | 0.09  |
| T3/ftT4   | 139 (86, 221)     | 137 (117, 162)    | 148 (125, 175)    | <0.001|
| TSH       | 1.58 (0.02, 5.59) | 1.58 (0.87, 2.54) | 1.58 (0.95, 2.39) | 0.87  |
| **2nd trimester** |                   |                   |                   |       |
| T3        | 2.56 (1.76, 3.56) | 2.53 (2.26, 2.84) | 2.78 (2.46, 3.15) | <0.001|
| ftT4      | 11.9 (9.1, 15.3)  | 12.0 (11.0, 13.0) | 11.5 (10.5, 12.5) | <0.001|
| T3/ftT4   | 214 (142, 328)    | 211 (184, 243)    | 240 (209, 276)    | <0.001|
| TSH       | 2.42 (0.70, 5.89) | 2.46 (1.74, 3.35) | 2.13 (1.56, 3.02) | <0.001|
| **3rd trimester** |                   |                   |                   |       |
| T3        | 2.69 (1.68, 3.75) | 2.68 (2.30, 3.05) | 2.82 (2.46, 3.20) | <0.001|
| ftT4      | 13.3 (9.5, 17.2)  | 13.3 (11.9, 14.8) | 13.2 (11.8, 14.4) | <0.24 |
| T3/ftT4   | 202 (128, 303)    | 199 (172, 233)    | 214 (185, 248)    | <0.001|
| TSH       | 2.21 (0.51, 5.57) | 2.21 (1.55, 3.08) | 2.19 (1.51, 2.98) | 0.21  |

Combined samples are presented as median (2.5th and 97.5th percentile)

GDM and non-GDM groups are presented as median (25th and 75th percentile)

Unit of T3, ftT4 and TSH nmol/L, pmol/L and μIU/mL.

Boldface indicates statistically significant.
Table 3. Trimester-specific associations between three thyroid markers and glucose metabolism indicators by GEE.

| Variables | Fasting glucose | Glucose at 1h | Glucose at 2h | GDM |
|-----------|-----------------|---------------|---------------|-----|
|           | β (95%CI)       | P             | β (95%CI)     | P   | β (95%CI)     | P   | OR (95%CI)    | P   |
| T3        |                 |               |               |     |               |     |               |     |
| 1st trimester | -0.03 (-0.07, 0.06) | 0.10         | 0.22 (0.10, 0.35) | < 0.001 | 0.07 (-0.02, 0.17) | 0.15 | 1.67 (1.33, 2.10) | < 0.001 |
| 2nd trimester | 0.16 (0.11, 0.20) | < 0.001      | 0.41 (0.28, 0.55) | < 0.001 | 0.21 (0.11, 0.31) | < 0.001 | 2.50 (1.95, 3.21) | < 0.001 |
| 3rd trimester | 0.13 (0.10, 0.16) | < 0.001      |                 |     |               |     |               |     |
| fT4       |                 |               |               |     |               |     |               |     |
| 1st trimester | 0.01 (0.006, 0.02) | < 0.001      | -0.03 (-0.05, -0.01) | 0.001 | -0.03 (-0.05, -0.01) | 0.001 | 1.00 (0.95, 1.04) | 0.84  |
| 2nd trimester | -0.02 (-0.03, -0.01) | 0.003        | -0.14 (-0.18, -0.10) | < 0.001 | -0.08 (-0.11, -0.05) | < 0.001 | 0.89 (0.82, 0.96) | < 0.001 |
| 3rd trimester | -0.005 (-0.01, 0.004) | 0.29         |                 |     |               |     |               |     |
| T3/fT4    |                 |               |               |     |               |     |               |     |
| 1st trimester | -0.01 (-0.02, -0.01) | 0.01         | 0.04 (0.02, 0.06) | < 0.001 | 0.02 (0.01, 0.04) | < 0.001 | 1.08 (1.04, 1.12) | < 0.001 |
| 2nd trimester | 0.02 (0.01, 0.02) | < 0.001      | 0.05 (0.04, 0.06) | < 0.001 | 0.03 (0.02, 0.04) | < 0.001 | 1.09 (1.07, 1.12) | < 0.001 |
| 3rd trimester | 0.01 (0.01, 0.02) | < 0.001      |                 |     |               |     |               |     |
| TSH       |                 |               |               |     |               |     |               |     |
| 1st trimester | -0.01 (-0.01, 0.00) | 0.21         | 0.04 (0.01, 0.07) | 0.01  | 0.05 (0.03, 0.08) | < 0.001 | 1.03 (0.99, 1.08) | 0.13  |
| 2nd trimester | -0.01 (-0.02, 0.01) | 0.33         | -0.09 (-0.13, -0.05) | < 0.01  | -0.04 (-0.07, -0.01) | < 0.001 | 0.91 (0.83, 0.99) | 0.03  |
| 3rd trimester | -0.01 (-0.02, 0.01) | 0.16         |                 |     |               |     |               |     |

Abbreviations: OR, odds ratio; CI, confidence interval; β and OR was adjusted for age, pre-pregnancy BMI, sampling seasonality, family diabetes history, parity, smoking in early pregnancy, drinking status, gestational weeks, education, income, and residence. β and OR indicated the effect size of per nmol/L, per pmol/L, per 10 Unit and per μIU/mL for T3, fT4, T3/fT4 and TSH, respectively. Boldface indicates statistically significant.
Table 4. Performance of multivariate predictive model of GDM including thyroid hormones and routine variables at a fixed false positive rate of 10%.

| Models | AUC (95%CI) | Sensitivity | Specificity | NPV   | PPV   | Cut-off | $P^d$ |
|--------|-------------|-------------|-------------|-------|-------|---------|------|
| Model 1 | 0.703 (0.686,0.720) | 26.5% | 90.0% | 89.7% | 27.1% | 0.208 | 0.060 |
| Model 2 | 0.710 (0.693,0.729) | 29.2% | 90.0% | 90.0% | 29.1% | 0.211 | 0.477 |
| Model 3 | 0.726 (0.709,0.743) | 30.8% | 90.0% | 90.2% | 30.1% | 0.215 | 0.072 |
| Model 4 | 0.724 (0.707,0.741) | 32.4% | 90.0% | 90.4% | 31.3% | 0.215 | 0.685 |

Abbreviations: AUC, area under curve; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

$P$ value of Hosmer-Lemeshow good of fit test.

Model 1 including maternal age, pre-pregnancy BMI, history of family diabetes, and season of conception;
Model 2 based on model 1, plus fasting plasma glucose and sampling gestational week;
Model 3 based on model 2, plus T3;
Model 4 based on model 2, plus T3/fT4.
Fig. 1. Consistency of the three thyroid markers across three trimesters of pregnancy. The first bar indicates the highest or lowest 25%. The following two bars indicate the percentage of individuals who are above 75% or below 25% in the first trimester, persisting through the second and third trimesters.
Fig. 2. Comparison of multivariate predictive models. Model 1 including maternal age, pre-pregnancy BMI, history of family diabetes, and season of conception; Model 2 based on model 1, plus fasting plasma glucose and sampling gestational week; Model 3 based on model 2, plus T3; Model 4 based on model 2, plus T3/FT4.
Fig. 3. Calibration chart of multivariate predictive models including thyroid hormones. a. Model including T3; b. Model including T3/FT4.
Table S1. Associations between thyroid markers and related factors by generalized estimating equation (GEE).

| Variables                  | $\beta_1$ (95% CI) | $\beta_2$ (95% CI) | $\beta_3$ (95% CI) | $\beta_4$ (95% CI) |
|----------------------------|---------------------|--------------------|--------------------|---------------------|
| Age                        | -0.007 (-0.012, -0.003) | -0.06 (-0.08, -0.03) | 0.18 (-0.26, 0.63) | 0.02 (-0.01, 0.04) |
| Body Mass Index            | 0.03 (0.02, 0.03)    | -0.08 (-0.10, -0.06) | 3.14 (2.67, 3.63)  | 0.01 (-0.02, 0.03)  |
| Gestational Week           | 0.01 (0.01, 0.01)    | -0.17 (-0.18, -0.17) | 2.94 (2.87, 3.01)  | 0.01 (0.00, 0.02)  |
| Seasonality                |                     |                    |                    |                    |
| Spring                     | Ref                 | Ref                | Ref                |                    |
| Summer                     | 0.04 (0.01, 0.07)    | 0.40 (0.25, 0.56)   | -2.46 (-5.09, 0.17) | -0.06 (-0.19, 0.06) |
| Fall                       | 0.02 (-0.01, 0.05)  | -0.13 (-0.28, 0.01) | 4.57 (1.73, 7.41)  | 0.02 (-0.07, 0.12)  |
| Winter                     | 0.04 (0.01, 0.07)    | 0.29 (0.14, 0.44)   | -1.33 (-4.02, 1.36) | -0.03 (-0.14, 0.08) |
| Education                  |                     |                    |                    |                    |
| Primary school or below    | Ref                 | Ref                | Ref                |                    |
| Middle school              | -0.05 (-0.19, 0.10) | 0.20 (-0.37, 0.78)  | -7.89 (-21.52, 5.75) | 0.35 (-0.15, 0.85)  |
| High school                | -0.06 (-0.20, 0.09) | 0.24 (-0.33, 0.81)  | -9.25 (-22.80, 4.30) | 0.24 (-0.22, 0.71)  |
| Junior college             | -0.07 (-0.21, 0.08) | 0.11 (-0.45, 0.69)  | -8.02 (-21.54, 5.50) | 0.13 (-0.32, 0.59)  |
| Undergraduate or above     | -0.10 (-0.26, 0.04) | 0.14 (-0.44, 0.71)  | -11.48 (-25.11, 2.16) | 0.06 (-0.40, 0.52)  |
| Income                     |                     |                    |                    |                    |
| <1000                      | Ref                 | Ref                | Ref                |                    |
| 1000, 2500                 | 0.07 (-0.05, 0.18)  | 0.21 (-0.24, 0.66)  | 3.39 (-6.67, 13.66) | 0.03 (-0.49, 0.55)  |
| 2500, 4000                 | 0.04 (-0.07, 0.16)  | 0.10 (-0.34, 0.55)  | 2.74 (-7.37, 12.86) | -0.19 (-0.67, 0.30)  |
| >4000                      | 0.05 (-0.07, 0.16)  | 0.06 (-0.39, 0.51)  | 3.94 (-6.26, 14.14) | -0.13 (-0.62, 0.37)  |
| Residence                  |                     |                    |                    |                    |
| Urban                      | Ref                 | Ref                | Ref                |                    |
| Suburb                     | 0.04 (-0.02, 0.09)  | 0.06 (-0.13, 0.26)  | 1.27 (-3.31, 5.65)  | -0.08 (-0.26, 0.10)  |
| Town                       | 0.01 (-0.10, 0.13)  | -0.20 (-0.64, 0.24) | 4.23 (-5.29, 13.76) | -0.19 (-0.67, 0.30)  |
| Rural                      | 0.02 (-0.04, 0.08)  | 0.11 (-0.14, 0.37)  | 0.20 (-4.90, 5.30)  | -0.13 (-0.62, 0.37)  |
| Smoking                    |                     |                    |                    |                    |
| No                         | Ref                 | Ref                | Ref                |                    |
| Yes                        | 0.08 (0.00, 0.16)    | -0.33 (-0.68, -0.03) | 11.82 (4.62, 19.02) | 0.11 (-0.24, 0.46)  |
| Drinking                   |                     |                    |                    |                    |
| Never                      | Ref                 | Ref                | Ref                |                    |
| Occasionally              | 0.06 (0.003, 0.12)   | -0.04 (-0.26, 0.18) | 5.37 (0.32, 10.42)  | -0.04 (-0.25, 0.18)  |
| Regularly                  | 0.46 (0.07, 0.84)    | -1.03 (-2.29, 0.21) | 48.81 (30.02, 67.59) | 1.47 (-0.67, 3.62)  |
| Parity                     |                     |                    |                    |                    |
| Nulliparous                | Ref                 | Ref                | Ref                |                    |
| Multiparous                | 0.01 (-0.04, 0.07)   | 0.27 (0.05, 0.50)   | -4.35 (-9.09, 0.39) | -0.18 (-0.42, 0.06)  |

Abbreviations: CI, confidence interval
$\beta$ adjusted for all the variables listed except the variable itself.
$\beta_1$ is for T3; $\beta_2$ is for fT4; $\beta_3$ is for T3/fT4; $\beta_4$ is for TSH.
Table S2. Stratified analyses of associations between three thyroid markers and the risk of GDM.

| Trimester | Group | OR (95% CI) | P    | OR (95% CI) | P    | OR (95% CI) | P    |
|-----------|-------|-------------|------|-------------|------|-------------|------|
| 1st trimester | | | | | | | |
| BMI       |       | 0.87        | 0.01 | 1.09 (1.04, 1.13) | 0.04 |
| <24       |       | 1.72 (1.32, 2.23) | 0.99 (0.94, 1.04) | 1.05 (0.97, 1.14) | 0.04 |
| ≥24       |       | 1.67 (0.93, 3.00) | 1.13 (1.00, 1.28) | 0.90 (0.87, 1.14) | 0.12 |
| Parity    |       | 0.05        | 0.90 | 1.09 (1.05, 1.14) | 0.12 |
| No        |       | 1.84 (1.43, 2.38) | 0.99 (0.94, 1.04) | 1.02 (0.93, 1.12) | 0.43 |
| Yes       |       | 0.81 (0.41, 1.58) | 0.99 (0.87, 1.14) | 0.99 (0.87, 1.14) | 0.38 |
| Smoking   |       | 0.90        | 0.13 | 1.08 (1.04, 1.12) | 0.93 |
| No        |       | 1.66 (1.30, 2.11) | 1.00 (0.95, 1.05) | 1.29 (1.05, 1.59) | 0.12 |
| Yes       |       | 4.19 (0.88, 19.85) | 0.65 (0.40, 1.05) | |
| TPOAb     |       | 0.88        | 0.11 | 1.09 (1.05, 1.13) | 0.12 |
| Negative  |       | 1.89 (1.45, 2.47) | 1.00 (0.95, 1.05) | 1.07 (0.98, 1.17) | 0.38 |
| Positive  |       | 1.07 (0.59, 1.94) | 0.96 (0.85, 1.09) | 0.96 (0.85, 1.09) | 0.66 |
| TGAb      |       | 0.47        | 0.23 | 1.08 (1.04, 1.12) | 0.93 |
| Negative  |       | 1.70 (1.31, 2.20) | 1.00 (0.95, 1.05) | 1.12 (1.02, 1.25) | 0.38 |
| Positive  |       | 1.86 (0.96, 3.60) | 0.94 (0.81, 1.10) | |
| 2nd trimester | | | | | | | |
| BMI       |       | 0.90        | 0.23 | 1.10 (1.07, 1.13) | 0.44 |
| <24       |       | 2.51 (1.88, 3.36) | 0.87 (0.79, 0.95) | 1.08 (1.02, 1.14) | 0.38 |
| ≥24       |       | 1.95 (1.10, 3.45) | 0.98 (0.81, 1.19) | 0.91 (0.81, 1.19) | 0.96 |
| Parity    |       | 0.40        | 0.91 | 1.09 (1.07, 1.12) | 0.96 |
| No        |       | 2.57 (1.96, 3.37) | 0.88 (0.81, 0.96) | 1.10 (1.02, 1.19) | 0.38 |
| Yes       |       | 1.96 (0.93, 4.12) | 0.90 (0.70, 1.15) | 1.16 (0.99, 1.36) | 0.71 |
| Smoking   |       | 0.31        | 0.38 | 1.09 (1.07, 1.12) | 0.71 |
| No        |       | 2.41 (1.86, 3.12) | 0.88 (0.81, 0.95) | 1.10 (1.07, 1.12) | 0.71 |
| Yes       |       | 9.21 (1.12, 75.68) | 1.00 (0.61, 1.63) | |
| TPOAb     |       | 0.79        | 0.29 | 1.10 (1.07, 1.13) | 0.66 |
| Negative  |       | 2.61 (2.00, 3.42) | 0.89 (0.82, 0.97) | 1.02 (0.93, 1.12) | 0.38 |
| Positive  |       | 1.22 (0.44, 3.34) | 0.80 (0.59, 1.09) | |
| TGAb      |       | 0.92        | 0.25 | 1.10 (1.07, 1.12) | 0.20 |
| Negative  |       | 2.52 (1.95, 3.26) | 0.89 (0.82, 0.96) | 1.29 (0.97, 1.73) | 0.20 |
| Positive  |       | 2.62 (0.22, 31.30) | 0.62 (0.28, 1.37) | |

Abbreviations: OR, odds ratio; CI, confidence interval; P indicates the P value of interaction.
Table S3. Sensitivity analyses of the associations of three thyroid markers and the risk of GDM according to the grounds GDM is diagnosed.

| Trimester | Group                  | $OR_1$ (95%CI) | $OR_2$ (95%CI) | $OR_3$ (95%CI) |
|-----------|------------------------|----------------|----------------|----------------|
| 1st trimester | High fasting glucose   | 1.76 (1.25, 2.47) | 0.96 (0.89, 1.04) | 1.10 (1.05, 1.16) |
|           | High postprandial glucose | 1.66 (1.18, 2.33) | 1.03 (0.97, 1.10) | 1.08 (1.02, 1.14) |
|           | Both                   | 1.38 (0.80, 2.35) | 0.96 (0.86, 1.09) | 1.05 (0.97, 1.15) |
| 2nd trimester | High fasting glucose   | 2.46 (1.72, 3.54) | 0.89 (0.79, 1.00) | 1.10 (1.06, 1.14) |
|           | High postprandial glucose | 2.40 (1.64, 3.49) | 0.87 (0.77, 0.99) | 1.09 (1.05, 1.13) |
|           | Both                   | 2.57 (1.47, 4.51) | 0.89 (0.74, 1.07) | 1.10 (1.05, 1.17) |

Abbreviations: OR, odds ratio; CI, confidence interval; $OR_1$ is for T3; $OR_2$ is for T3/fT4; $OR_3$ is for TSH.
Table S4. Comparison of area under curve (AUC) among different models.

| Models                      | Model 1 | Model 2          | Model 3          | Model 4          |
|-----------------------------|---------|------------------|------------------|------------------|
| Model 1                     | 1       |                  |                  |                  |
| Model 2                     | 1.25 (0.211) | 1                |                  |                  |
| Model 3                     | 2.88 (0.004) | 2.59 (0.010) | 1                |                  |
| Model 4                     | 2.34 (0.019) | 2.04 (0.041) | 0.40 (0.690) | 1                |

Model 1 including maternal age, pre-pregnancy BMI, history of family diabetes, and season of conception;
Model 2 based on model 1, plus fasting plasma glucose and sampling gestational week;
Model 3 based on model 2, plus T3;
Model 4 based on model 2, plus T3/ft4.