An Inverse Relationship Between Weight and Free Thyroxine During Early Gestation Among Women Treated for Hypothyroidism

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Background: Following treatment sufficient to normalize thyrotropin (TSH), nonpregnant hypothyroid adults display higher free thyroxine (FT₄) concentrations than a reference population. Our aim is to determine whether FT₄ concentrations are higher during pregnancy among women treated for hypothyroidism and whether their weight is associated with FT₄ levels. Weight/FT₄ relationships have not previously been reported in treated hypothyroid adults (either pregnant or nonpregnant).

Methods: Thyroid-related measurements were available from over 10,000 women at two early pregnancy time periods from the FaSTER (First and Second Trimester Evaluation of Risk for Fetal aneuploidy) trial (1999–2002). All women were receiving routine prenatal care. Present analyses were restricted to 9267 reference women and 306 treated, hypothyroid women with TSH between the 2nd and 98th reference percentiles. We compared FT₄ values between those groups at 11–14 and 15–18 weeks' gestation, using linear regression to estimate FT₄/maternal weight relationships, after accounting for treatment and other potential covariates.

Results: In comparison to reference women, median FT₄ values and percent of FT₄ values ≥95th reference percentile were significantly higher in treated women at both 11–14 and 15–18 weeks' gestation ($p < 0.001$) overall and after stratification by weight into tertiles. Among both treated and reference women, median FT₄ decreased monotonically with increasing weight, regardless of anti-thyroperoxidase antibody status. Maternal age, maternal weight, and treatment status were important predictors of FT₄ levels ($p < 0.001$, defined by partial r^2 values of 1% or higher). Anti-thyroperoxidase antibody status, TSH values (after logarithmic transformation), and all interaction terms were well below an r^2 of 1%. FT₄ levels were 1.45 pmol/L higher in treated than reference women, independent of other factors. Maternal age and weight reduced FT₄ levels by 0.0694 pmol/L/y and 0.0208 pmol/L/kg, respectively.

Conclusions: FT₄ concentrations are higher among treated hypothyroid pregnant women than among reference women, and higher maternal weight is associated with lower FT₄ levels, regardless of treatment status. This inverse relationship is not associated with higher TSH levels. While no immediate clinical implications are attached to the current observations, increased peripheral deiodinase activity in the presence of higher weight might explain these findings. Further investigation appears worthy of attention.

Introduction

AMONG NONPREGNANT ADULTS WITH HYPOTHYROIDISM, L-thyroxine (LT₄) substitution sufficient to normalize thyrotropin (TSH) levels is associated with free thyroxine (FT₄) levels in serum that are higher, on average, than pre-

dicted from a reference population (1–5). To date, however, FT₄ levels have not been examined from this perspective during pregnancy. Furthermore, the relationships between weight and FT₄ levels have not been explored in treated hypothyroid adults (either pregnant or nonpregnant), even though considerable attention has been given to taking

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weight or body mass index (BMI) into account when calculating LT₄ dosage (6–8). The present study examines FT₄ levels at two intervals during early pregnancy in women being treated for hypothyroidism in comparison to a reference population, both overall and after stratification by weight. Regression analysis is used to account for possible covariates. The reference group is defined by untreated women with TSH values between the 2nd and 98th percentiles, and comparisons are limited to treated hypothyroid women whose TSH measurements also fall within that range.

Materials and Methods

The multicenter FaSTER (First and Second Trimester Evaluation of Risk for Fetal aneuploidy) trial has been previously described (9). Participants were asked to give supplementary consent to allow their residual sample to be used for additional research studies at 5 of the 15 recruitment centers (Montefiore Medical Center, Bronx, NY; Swedish Medical Center, Seattle, WA; LDS Hospital, Salt Lake City, UT; Utah Valley Regional Medical Center, Provo, UT; and McKay Dee Hospital, Ogden, UT). Women with singleton pregnancies who consented were eligible for inclusion in the present study. Women with pregnancies affected by Down syndrome were excluded.

Among 10,218 women documented to have thyroid-related measurements available from samples obtained on two occasions (11–14 weeks' gestation and 15–18 weeks' gestation) and gestational dates established by ultrasound, 156 women were excluded due to missing data regarding known hypothyroidism, leaving 9670 women not known to be hypothyroid, along with 392 hypothyroid women. Reference data are derived from a subset of these 9670 women with TSH values between the 2nd and 98th percentiles (10). TSH percentiles are calculated separately for 11–14 weeks of gestation and 15–18 weeks of gestation. The FT₄ measurements are then compared with those from a subset of the 392 hypothyroid women whose TSH values are also within that reference range (11).

Samples were collected between 1999 and 2002, stored at –80°C, and tested between July 2004 and May 2005 (stored for 3–5 years). Levels of TSH, FT₄, and thyroid antibodies were measured using the Immulite 2000 methodology (Siemens Medical Solutions Diagnostics, Tarrytown, NY). Samples were thawed overnight before assay, and first and second trimester samples from each woman were assayed within 24 hours of each other. Long-term coefficients of variation were 5.3%, 6.9%, and 3.8% at TSH concentrations of 0.53, 4.5, and 21.9 mU/L; 8.1%, 6.5%, and 7.9% at FT₄ concentrations of 0.9, 1.8, and 3.2 ng/dL; 2.5%, 6.6%, and 5% at anti-thyroperoxidase (TPO) antibody concentrations of 30, 39, and 546 IU/mL. Anti-TPO antibody status was defined as positive (≥ 35 IU/mL) or negative.

Postdelivery follow-up was performed by the research coordinator at each site or by telephone interview. A single perinatologist and a pediatric geneticist reviewed maternal and pediatric medical records for the following patient subsets: abnormal first and/or second trimester screening results, adverse obstetric or pediatric events, and 10% of normal subjects randomly selected from the trial database. A purpose-designed computerized tracking system with up to 10 contacts per subject was used to ensure complete outcome

collection. Pregnancy and pediatric outcomes were obtained in more than 98% of study subjects.

Statistical analyses were performed using SAS version 9.4 (Cary, NC) and included *t*-tests, chi-square tests, linear regression, and two-way ANOVA. TSH underwent a logarithmic transformation. Significance was two-tailed at the 0.05 level.

To explore the impact of thyroid treatment and maternal weight on the FT₄ levels, we performed a multivariate stepwise linear regression analysis. The FT₄ measurement was the dependent variable, with maternal weight and treatment category as independent variables. Additional variables considered by the model included any demographic or assay finding that differed significantly between the treatment groups. A significant coefficient for the treatment variable and reference would indicate a systematic difference in FT₄ measurements between the two groups, while the coefficient for maternal weight would indicate a similar linear relationship in the two groups. Interaction terms with the treatment category were included, and the lack of importance of a main effect or interaction term (partial r^2 value $< 1\%$) would indicate that the model is applicable to both treatment groups.

Results

A total of 9267 reference and 306 treated women were found to have TSH values between the 2nd and 98th percentiles of the reference population. Table 1 compares selected features of pregnancies among women being treated for hypothyroidism with reference pregnancies. Gestational age at sampling was the same for the two groups of women in both the late first and early second trimester. Treated women were older and weighed more than reference women ($p < 0.001$), and gestational diabetes occurred more frequently ($p = 0.04$). Median gestational age at delivery and birth weight were not different between the two groups, and percentages of deliveries at < 37 weeks and < 34 weeks of gestation were not significantly higher among treated women.

Table 2 shows median TSH, FT₄ values, percentage of FT₄ values ≥ 95 th percentile, and percentage of women with elevated anti-TPO antibody levels at 11–14 and 15–18 weeks' gestation. All of these measurements were significantly higher in treated women than in reference women ($p < 0.001$).

Table 3 shows median FT₄ values and percentage of FT₄ values ≥ 95 th percentile at 11–14 and 15–18 weeks' gestation according to thyroid treatment status, stratified by tertile of treated women's weight. Numbers of women are shown for each subset. A preponderance of reference women were in the lowest weight tertile, reflecting the higher average weight of treated women (Table 1). While remaining relatively higher among treated women at each weight interval after stratification, median FT₄ concentrations also decreased monotonically with increasing weight in both treated and reference women. Differences were significant in both column effects (treated versus reference women) and row effects (weight-stratified comparisons) by two-way ANOVA ($p < 0.001$). The relationship of lower FT₄ medians with increasing weight was not due to TSH levels. The median TSH in the lowest weight versus the highest weight categories were nearly identical (1.83 vs. 1.87 mU/L among treated women, first trimester; 1.04 vs. 1.03 mU/L among reference women, first trimester; 1.83 vs. 1.54 mU/L treated women,

TABLE 1. WOMEN TREATED FOR HYPOTHYROIDISM VERSUS REFERENCE WOMEN: SELECTED DEMOGRAPHIC AND OUTCOME DATA^a

Characteristic	Treated	Reference	p
No. of women	306	9267	
Age, years ^b	31.0 (5.1)	29.0 (5.5)	<0.001
Weight, kg ^b	69.3 (16.7)	65.0 (14.6)	<0.001
Gestational age at first sampling, weeks	12.6 (0.7)	12.6 (0.7)	0.34
Gestational age at second sampling, weeks	16.0 (0.9)	16.0 (0.9)	0.46
Gestational diabetes, %	4.9	2.9	0.04
Preeclampsia, %	1.0	1.0	0.77
Gestational age at birth, weeks ^b	39.3 (2.3)	39.4 (2.2)	0.35
Birthweight, g ^b	3402 (538)	3402 (514)	0.19
<37 weeks, %	8.5	7.2	0.39
<34 weeks, %	2.3	2.4	0.89

^aLimited to subjects with thyrotropin (TSH) between 2nd and 98th percentiles.^bMedian (SD).

second trimester; and 1.23 vs. 1.16 mU/L referent women, second trimester). Percentages of FT₄ values $\geq$ 95th percentile were significantly higher among treated women than among reference women at all weight intervals ($p < 0.001$), and these percentages decreased monotonically as weight increases.

A multivariate stepwise regression analysis considered treatment status, maternal weight, maternal age, TSH levels, and anti-TPO antibody status as main effects in predicting FT₄ levels. Interaction terms included all pairwise combinations of these variables with treatment status. Only treatment status, maternal weight, and maternal age were included in the final model ($p < 0.001$, $r^2 > 1\%$). Together, these three variables explained 8% of the variability in FT₄ measurements. Coefficients for the effect of treatment (1=treated, 0=referent), age (in years), and weight (in kilograms) were 1.44885 pmol/L (treatment, yes), -0.06937 pmol/L/y, and -0.02084 pmol/L/kg, respectively. The regression constant was 16.501 pmol/L. Over the range of measurements (e.g., maternal age ranging from 20 to 40 years), these coefficients indicated changes in FT₄ measurements on the order of +1.45, -1.4 , and -1.7 pmol/mL, respectively. None of the

other main or interaction terms satisfied the inclusion criteria and were removed from the final model. Several of these were statistically significant due to the large numbers of study subjects but had very low partial r^2 values (e.g., all $< 0.2\%$ for anti-TPO antibodies). The lack of important interactions with treatment indicates that the two groups differed in FT₄ values by a constant amount and that the two included independent variables (maternal age and maternal weight) were associated with similar changes in the two treatment groups.

Discussion

In 1982, Salmon *et al.* (12) reported elevated T₄ levels in association with normal triiodothyronine (T₃) levels among hypothyroid individuals who were clinically euthyroid following treatment with LT₄. A possible explanation for this upward shift in FT₄ concentration was put forth by Fish *et al.* (13) in 1987. They postulated that T₃ was the primary stimulus to TSH suppression via hypothalamic/pituitary feedback and that the T₃ contribution from normal thyroid gland function (18% of total T₃) was either diminished or absent in

TABLE 2. WOMEN TREATED FOR HYPOTHYROIDISM DURING PREGNANCY VERSUS REFERENCE WOMEN: THYROID HORMONE DATA AT TWO TIME INTERVALS EARLY IN GESTATION^a

	Treated	Reference	p
TSH, mU/L ^b			
11–14 weeks' gestation	1.88 (1.11)	1.06 (0.35)	<0.001
15–18 weeks' gestation	1.69 (0.33)	1.23 (0.27)	<0.001
FT ₄ , pmol/L ^c			
11–14 weeks' gestation	15.2 (2.6)	14.2 (2.1)	<0.001
15–18 weeks' gestation	14.1 (2.6)	13.0 (2.0)	<0.001
FT ₄ $\geq$ 95th percentile, %			
11–14 weeks' gestation	17.0	5.0	<0.001
15–18 weeks' gestation	21.7	5.0	<0.001
Elevated anti-TPO antibodies, %			
11–14 weeks' gestation	47.1	8.3	<0.001
15–18 weeks' gestation	45.4	8.1	<0.001

^aSubjects with thyrotropin between 2nd and 98th percentiles at both 11–14 and 15–18 weeks' gestation.^bMedian (log SD).^cMedian (SD).FT₄, free thyroxine; TPO, thyroperoxidase.

TABLE 3. FREE THYROXINE LEVELS IN THE LATE FIRST AND EARLY SECOND TRIMESTER OF PREGNANCY IN WOMEN TREATED FOR HYPOTHYROIDISM AND REFERENT WOMEN, STRATIFIED BY MATERNAL WEIGHT

	Weight, kg	Treated				Reference			
		FT ₄ , pmol/L			FT ₄ ≥95th percentile	FT ₄ , pmol/L			FT ₄ ≥95th percentile
		N	Median	SD		N	Median	SD	
11–14 weeks' gestation ^a	<64.5	102	15.7	2.8	27.5%	4529	14.4	2.0	6.5%
	64.5–77.2	102	15.2	2.5	13.7%	2771	14.1	2.3	4.6%
	>77.2	102	14.4	2.4	9.8%	1967	13.5	1.9	2.0%
15–18 weeks' gestation ^a	<64.5	98	15.4	2.8	36.7%	4586	13.3	1.9	5.6%
	64.5–77.2	98	13.7	2.2	17.3%	2826	13.0	1.8	5.2%
	>77.2	99	13.5	2.4	11.1%	1972	12.5	1.9	3.2%

^aAt both 11–14 and 15–18 weeks' gestation (first and second trimesters, respectively), column effects (treated vs. reference comparisons) and row effects (weight-stratified comparisons) for FT₄ measurements are significantly different ($p < 0.001$, two-way ANOVA). In addition, the percentages of FT₄ values at or above the 95th percentile are significantly different between treated and reference pregnancies at all weight intervals ($p < 0.001$).

FT₄, free thyroxine.

hypothyroid individuals, the remainder being produced by conversion of T₄ to T₃ via deiodinase activity in peripheral organs and tissues. Numerous reports have now documented relatively high FT₄ values in conjunction with normal TSH among treated nonpregnant hypothyroid individuals (1–5), as recently summarized by Biondi and Wartofsky (14).

The present study confirms that FT₄ values are shifted significantly higher (by about 1.45 pmol/L) among pregnant women being treated for hypothyroidism. The percentage of FT₄ values ≥95th percentile is also significantly higher than among reference women. The reference group that formed the basis for our analysis was defined by TSH measurements between the 2nd and 98th percentiles; comparisons were limited to hypothyroid women whose TSH measurements were within those boundaries. As with nonpregnant adults being treated for hypothyroidism, the upward shift in FT₄ values among treated women during early pregnancy in our study might be explained by a diminished contribution of T₃ from the thyroid gland, leading to higher FT₄ levels to achieve a euthyroid state.

In 2013, we reported a reciprocal relationship between FT₄ and maternal weight among euthyroid pregnant women (15). This observation was consistent with an earlier analysis by Mannisto *et al.* (16), who reported an inverse relationship between BMI and FT₄ in antibody-negative pregnant women. While the present analysis includes both antibody-positive and antibody-negative women, TPO status has minimal impact on the FT₄/weight relationships. The analysis by Mannisto *et al.* also showed that BMI and free triiodothyronine (FT₃) were directly related, suggesting the possibility that deiodinase activity might explain BMI-associated FT₃/FT₄ shifts in euthyroid women. Our analysis shows an inverse relationship between maternal weight and FT₄ among pregnant women being treated for hypothyroidism, similar to that observed in reference women; percentages of FT₄ values ≥95th percentile also decrease in stepwise fashion with increasing weight. A recent study by Mehran *et al.* (17) documented a similar association between FT₄ and BMI in nonpregnant euthyroid adults and linked low FT₄ with features of the metabolic syndrome.

In 2013, Ojomo *et al.* (8) examined the efficacy of a standardized, weight-based thyroid hormone replacement

dosage prescribed for 122 patients after thyroidectomy for benign pathology. Among those with a BMI >30 kg/m², 53% were overdosed, while 46% of those with BMI <25 kg/m² were underdosed. Based on their observations, the authors concluded that obese hypothyroid individuals converted more LT₄ to T₃ and proposed lowering the per kilogram LT₄ dosage requirement, as BMI increased (a linear relationship up to 50 kg/m²). This conclusion, while derived by examining LT₄ dosage requirements rather than by FT₄ measurement, is consistent with our present findings.

Strengths of our study include an unbiased convenience sample of pregnant women with hypothyroidism, as part of a much larger cohort of pregnancies, with serum samples collected from the same women at two well-defined, narrow gestational time periods. The FT₄, TSH, and anti-TPO antibody measurements were performed on all of these samples in one laboratory, under standardized conditions. Weaknesses include lack of information about thyroid hormone replacement dosage and composition, although monotherapy with L-thyroxine is the currently recommended treatment during pregnancy, and use of L-triiodothyronine is strongly discouraged (18–20). The original study protocol focused on adverse pregnancy outcomes that might be associated with commonly measured thyroid function tests. As a result, FT₃ measurements were not performed, thereby preventing its assessment.

In conclusion, this study confirms that FT₄ measurements are shifted upward among pregnant women with normal TSH who are being treated for hypothyroidism, consistent with reported findings in nonpregnant, hypothyroid adults. A new finding is our documentation of an inverse relationship between FT₄ levels and weight during early pregnancy among the treated hypothyroid women, a pattern that has previously been noted only among reference women at the same time in pregnancy. Relatively lower FT₄ levels among obese women being treated for hypothyroidism are unlikely to be explained by lower thyroid gland output. A more likely explanation might be that peripheral deiodinase activity is increased in the presence of higher weight. While no immediate clinical implications are attached to the current observations, further investigation of the association between weight and peripheral deiodinase activity appears worthy of attention.

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Author Disclosure Statement

The authors have nothing to disclose.

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