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Clinical Research Article

Controlled Antenatal Thyroid Screening Study III: Effects of Gestational Thyroid Status on Adolescent Brain Morphology

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Abstract

Context: Children born to mothers with gestational hypothyroidism or hyperthyroidism may have increased risk of adverse neurodevelopmental outcomes. However, the effects of maternal thyroid status on offspring brain development are unclear.

Objective: This work aimed to establish whether adolescent brain morphology is affected by suboptimal gestational thyroid function (SGTF).

Methods: The Controlled Antenatal Thyroid Screening (CATS) study randomly assigned mothers with SGTF to levothyroxine or no supplementation from approximately 12 weeks' gestation. At age 9, children born to mothers who were overtreated with levothyroxine had a higher risk of conduct and hyperactivity traits. For the current CATS III study, children underwent neuroimaging studies, including T1-weighted structural magnetic resonance imaging (MRI). A total of 85 children aged 11 to 16 years had usable T1-weighted MRI data (exposed to untreated SGTF [n = 21], normal GTF [n = 24], or treated SGTF [optimally treated (n = 21), overtreated (n = 20)]). The primary outcome was to examine the association of SGTF and its treatment with global brain volumes. Secondary and exploratory outcomes were to investigate the association of maternal thyrotropin (TSH) and free thyroxine (FT4) levels with global and subregional brain volumes. Results were adjusted for age, sex, and pubertal scores.

Results: There were no significant differences in global brain volumetric measures between groups, including total gray matter volume ($P = .373$). Weak positive correlations were found between maternal TSH, but not FT4, levels and several brain volumes, but these did not survive testing for multiple comparisons.

Conclusion: We found no evidence that SGTF was associated with differences in adolescent brain morphology, and no effect of levothyroxine supplementation.

Key Words: thyroid, pregnancy, attention deficit hyperactivity disorder, magnetic resonance imaging

Abbreviations: ADHD, attention deficit hyperactivity disorder; CATS, Controlled Antenatal Thyroid Screening; DT, diffusion tensor; FSIQ, full-scale intelligence quotient; FT4, free thyroxine; GTF, gestational thyroid function; IQR, interquartile range; MRI, magnetic resonance imaging; SGTF, suboptimal gestational thyroid function; TSH, thyrotropin.

Human fetal brain development is critically dependent on maternal thyroid hormone availability throughout pregnancy. Maternal thyroid hormone is the sole source of fetal thyroid hormone until mid-gestation but remains critically important during the remainder of pregnancy while the fetal thyroid gland develops full maturity. Severe, untreated maternal hypothyroidism is associated with permanent and significant cognitive and motor deficits in their offspring (1). A number of mechanisms may be in operation (2). Rodent models have shown effects on disrupted neuronal proliferation, migration and differentiation, neurite outgrowth, myelination, and synaptogenesis (3, 4), even if reductions in maternal thyroid hormone supply are only transient and moderate (5, 6). Furthermore, many of these effects on brain developmental trajectories, such as neuronal morphology and migration, are reversible only when thyroid hormone supplementation is commenced soon after the induction of experimental hypothyroidism (3, 5, 7). These neuroanatomical alterations may account for the behavioral and cognitive effects described in animal models of developmental hypothyroidism and thyroid hormone resistance (2, 8-12).

These observations suggest that suboptimal maternal thyroid function in pregnancy may have adverse effects on offspring neurodevelopment. Indeed, observational studies have reported associations between gestational thyroid dysfunction and a variety of cognitive and neurobehavioral disorders, including attention deficit hyperactivity disorder

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(ADHD) (13-15). Brain morphology may also be affected by gestational thyroid hormone concentration: In the Generation R cohort, an inverse U-shaped association was noted between maternal thyroid function and gray matter (cortical and subcortical) volume (16, 17), with effects particularly apparent in early gestation (17). However, the specific mechanisms underpinning these alterations, and the effects of thyroid hormone supplementation on neurodevelopmental trajectories, are not known.

The CATS (Controlled Antenatal Thyroid Screening) study was a large (>22 000 pregnant women in the United Kingdom and Italy) randomized controlled trial that studied the effect of thyroid hormone replacement on offspring IQ and behavior, initially at age 3 years (18) and more recently in 452 CATS II children at age 9 years (18, 19). Treatment dose was relatively high (150 µg), so that one-third of treated women developed free thyroxine (FT4) levels greater than the 97.5th percentile of the entire CATS cohort when measured at 20 and/or 30 weeks' gestation. Application of 3 validated instruments to assess child neurodevelopment (strengths and difficulties questionnaire [SDQ], child ADHD questionnaire, social communication questionnaire [SCQ]) (20-22) showed that scores above clinically relevant thresholds for conduct and hyperactivity were significantly more prevalent in children exposed to overtreatment or overt hypothyroidism (23). We therefore hypothesized that abnormal thyroid hormone bioavailability during neonatal development could alter brain maturation with a potential effect on brain morphology. Our main objective, applying advanced neuroimaging to the CATS cohort, sought to investigate this.

Materials and Methods

Study Design and Participants

The present study is a follow-up of the CATS studies, CATS I (18) and CATS II (23-25). The original CATS study recruited approximately 22 000 women in the United Kingdom and Italy between June 2002 and May 2006 (18). Women were screened at a median of 12 weeks, 3 days' gestation and randomly assigned to either screen or control groups; both provided serum samples at recruitment, with the screen group having their thyroid function tested immediately, and the controls after their child was born. Women with an FT4 concentration less than the 2.5th percentile and/or thyrotropin (TSH) greater than the 97.5th percentile were classified as having suboptimal gestational thyroid function (SGTF); percentiles were calculated from the CATS cohort. Women in the screen group with SGTF were treated with levothyroxine (starting dosage 150 µg) for the remainder of their pregnancies. Dosages were adjusted where necessary, aiming to maintain a serum TSH of 0.1 to 1.0 mIU/L, following measurement of TSH and FT4 at 6 weeks posttreatment initiation and at 30 weeks' gestation. Women diagnosed with SGTF after delivery were advised to visit their general practitioner for further management.

The primary outcome of CATS I was the children's IQ at age 3 years, among 390 (303 in the UK) treated (screen) and 404 (306 in the UK) untreated (control) mothers. Assessors were blinded to treatment group. A further 20 789 (15 593 in the UK) women made up the normal GTF group. The CATS II study revisited more than 400 of the CATS children from the UK cohort for testing of IQ, neurodevelopment, anthropometric, and cardiometabolic status at age 9 years (23-25). IQ was measured in the CATS II study using the Wechsler Intelligence Scale for Children, Fourth Edition UK (WISC-IV), which generated a full-scale IQ (FSIQ) (23). In CATS II, 95.2% of participants self-reported as White, 2.1% as Asian or Afro-Caribbean, and 2.7% had no ethnicity recorded. For the present study, mothers who had previously provided consent to be contacted about future research studies were recruited from the CATS II database. We sought to recruit mother-child pairs from each of the 4 study groups (normal gestational thyroid function [normal GTF]: untreated SGTF; optimally treated SGTF [FT4 and TSH within reference range at 20 and 30 weeks' gestation]; overtreated SGTF [FT4 > 17.7 pmol/L at either 20 or 30 weeks' gestation as defined by the top 2.5th percentile in the entire CATS UK cohort at consent into the trial]) (23). To avoid selective recruitment and the potential for bias, we initially sought to identify all potentially eligible individuals and randomly selected those to be approached to take part, adjusting for any imbalance between groups with respect to age and sex as recruitment progressed. Individuals were recruited between November 2016 and February 2020. Full research governance approval was received from Cardiff University (reference SPON 1502-16) and Wales Research Ethics Committee 1 (16/WA/0237). Written and informed consent was obtained from the parents, and assent from the children.

Eligibility and Investigational Measurements

Adolescents were eligible for inclusion if they were aged 10 to 16 years and if they lived sufficiently close to enable travel to and from the Cardiff University Brain Research Imaging Centre (CUBRIC) within 1 day. Participants with contraindications to magnetic resonance imaging (MRI), including nonremovable metal dental braces or piercings, a pacemaker, or other implanted devices, were excluded from participation. After informed consent, a safety screening questionnaire was completed before participants entered an initial "mock" MRI scanner to allow for acclimatization. Since pubertal status was a potentially important confounder of brain maturation, the children completed a validated pubertal self-assessment questionnaire based on the Tanner classification (26). This gives a combined score from a 2-part pictorial questionnaire, rated 1 to 5 for each relevant body area; higher scores correlate with more advanced pubertal development.

Imaging

Images were acquired on a Siemens MAGNETOM Prisma 3T scanner (Siemens Healthcare) using a 32-channel receive-only radiofrequency head coil, and a 3-dimensional inversion recovery prepared fast spoiled gradient recalled sequence (repetition time = 2300 ms, echo time = 3.06 ms, inversion time = 850 ms, flip angle = 9°, pixel bandwidth = 230 kHz, matrix 288×256, isotropic resolution 1 mm^3).

Image Processing and Quality Assurance

T1-weighted images were processed and analyzed using FreeSurfer (27) automated segmentation software (Free Surfer, version 6.0; https://surfer.nmr.mgh.harvard.edu/). A representation of the cortical surface was constructed and automated segmentation using the Desikan Killiany atlas applied to produce global and regional measures (area and volume) of cortical and subcortical areas (27). To account for possible movement artifacts and processing error, image quality assessment was performed using 2 approaches: 1) after images were

processed, 2 authors (L.B. and A.S.) independently visually assessed the cortical surface images, with all deemed useable; and 2) the ENIGMA Cortical Quality Control Protocol 2.0 (April 2017) (28) (http://enigma.ini.usc.edu/) was used to objectively assess the quality of FreeSurfer outputs, with all rated as "good."

Statistical Analysis

Statistical analyses were performed using R statistical software, version 4.3.1 (29). Analysis scripts for this project are available on the Open Science Framework (OSF; DOI 10.17605/OSF.IO/FQ4XK).

Age, sex, and pubertal status have all been shown to affect brain maturation during adolescence (30, 31). Consequently, analyses were adjusted for child sex, child age at time of MRI scan, and self-rated pubertal score. For completeness, we report results both with and without adjustment of covariates.

Data were inspected to establish whether they met assumptions for parametric testing, using the Shapiro-Wilk test for normality and Bartlett test for homogeneity of variances; where assumptions were violated, nonparametric tests were used. Missing data were treated in one of two ways: 1) where a variable was treated as a covariate (eg, total Tanner [pubertal] score), missing data were replaced with the median score for the rest of the cohort (the ages of participants with missing Tanner scores were close to the median age of the cohort, hence replacing missing puberty score data with the median of the whole cohort was deemed appropriate); 2) where a variable was a variable of interest (dependent or independent variable), participants with missing data were excluded from the analysis for that test only.

Outliers were identified using the interquartile range (IQR) criterion; observations $> IQ_{.75} + 1.5 \times IQR$ or $< IQ_{.25} - 1.5 \times IQR$ were deemed outliers. Where outliers were detected, we present results excluding these observations to allow for the assumption of normally distributed residuals to be met for multiple regression. Results were not significantly different with outliers included or excluded. Results, including all data, are available from the authors on request.

Primary Analyses: Effects of Suboptimal Gestational Thyroid Function and Treatment on Global Brain Structure

The effect of group (normal GTF; untreated SGTF; optimally treated SGTF; and overtreated SGTF) on global measures of brain volume (including total gray matter volume, cortex volume, cerebral white matter volume, subcortical gray matter volume, and estimated total intracranial volume) was assessed using a one-way analysis of variance where data met assumptions of normality and heteroscedasticity, or the Kruskal-Wallis test where these assumptions were violated (ie, for subcortical gray matter data). To account for potential confounders of sex, age, and pubertal status, we employed multiple regression.

Secondary Analyses: Effects of Maternal Thyroid-Stimulating Hormone and Free Thyroxine on Global Brain Structure

The effects of baseline TSH and FT4 levels (measured at entry into CATS I; 12 weeks' gestation) on global measures of brain structure were assessed using full and partial (accounting for sex, age, and pubertal status) correlations. Since TSH and FT4 violated assumptions of normality, nonparametric correlation tests were used. Kendall tau was employed for its increased rigidity, beneficial in small samples and when dealing with tied observations in the data.

For those in the treated and overtreated SGTF groups, we also explored full and partial correlations between FT4/TSH levels at 20 and 30 weeks' gestation and the same global measures of brain volume.

Values of P less than .05 were considered statistically significant for all primary and secondary analyses. Given the nonindependence of total, cortical, and subcortical brain volumes, we did not perform corrections for multiple comparisons on these results; this should be considered when interpreting P values close to the α threshold of .05.

Exploratory Analyses: Effects of Maternal Thyroid-Stimulating Hormone and Free Thyroxine on Regional Brain Structure

In exploratory analyses, we investigated the effects of maternal TSH and FT4 levels at baseline on subregional cortical and subcortical gray matter volumes using full and partial (accounting for sex, age, and pubertal status) correlations. As this analysis was exploratory, no outliers were removed from the data set. Bonferroni correction was used to correct for the many multiple comparisons associated with analyzing each subregion of the brain independently.

Sample Size

The structural T1-weighted data presented here were acquired as part of a longer scanning protocol that included diffusion tensor magnetic resonance imaging (DT-MRI) and quantitative magnetization transfer protocols. The sample size for the study was calculated based on the planned DT-MRI analysis and is not immediately transferable to the present study. Instead, we performed a sensitivity analysis to establish the minimum effect size (f) we would have 80% power to detect, given our sample size of 20 or more participants per group (for our primary analysis). Our sample size provided 80% power to detect medium to large effect sizes ($f \geq .38$) in an analysis of variance comparing 4 groups.

Results

Demographic Characteristics

Figure 1 summarizes the number of participants at each stage of the CATS studies. A total of 86 adolescents underwent MRI scanning as part of the CATS III study. One participant was excluded after failing to acclimatize to the mock scanner, leaving 85 participants whose image data were available for analysis (normal GTF = 24, untreated SGTF = 21, optimally treated SGTF = 20, overtreated SGTF = 20), all of whom passed quality assessment for their T1-weighted images. Mirroring the CATS II population, 95.3% of participants self-reported as White, with 1 participant self-reporting as Asian, 1 as Afro-Caribbean, and 2 with no ethnicity recorded.

Demographic characteristics are presented in Table 1. Thyroid function test results at time of consent into CATS are presented per group, as well as additional test results at later time points for the optimally treated and overtreated SGTF groups. As expected, median maternal TSH concentrations were higher, and median maternal FT4 concentrations lower, in the 3 SGTF groups compared to the normal GTF group (23). Child age, sex, IQ at age 9 years, and pubertal status were not significantly

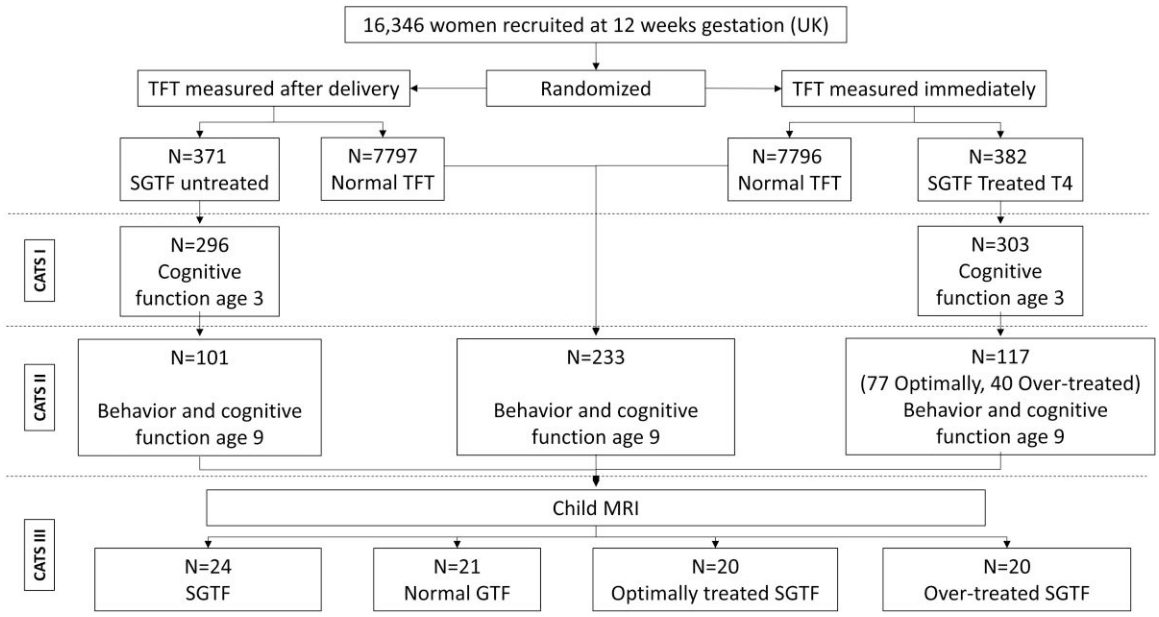

Figure 1. Flow of participants through the CATS studies. Illustrates the initial recruitment in South Wales, UK, for CATS I, when mothers were randomly assigned to screening and treatment for SGTF or not. In CATS I child IQ was assessed at age 3 years; the follow-up study, CATS II, in which child IQ and behavior was assessed at age 9 years; and CATS III, when children underwent magnetic resonance imaging (MRI) scans. CATS, controlled antenatal thyroid screening study; GTF, gestational thyroid function; SGTF, suboptimal gestational thyroid function; TFT, thyroid function tests.

different between groups. Thyroid function results for each group were similar to those in the CATS II study.

Of the 20 women who were overtreated with levothyroxine (FT4 concentration >17.7 pmol/L at 20 or 30 weeks' gestation), 5 had sustained overtreatment throughout the second and third trimesters. The median (IQR) FT4 concentrations at 20 weeks and 30 weeks for the overtreated group were 18.9 pmol/L (17.9-19.6 pmol/L) and 17.3 pmol/L (16.6-18.5 pmol/L), respectively, while median (IQR) TSH values at these times were 0.11 mIU/L (0.02-0.33 mIU/L) and 0.16 mIU/L (0.02-0.33 mIU/L), respectively. In the optimally treated SGTF group, the median (IQR) FT4 concentrations at 20 and 30 weeks' gestation were 15.6 pmol/L (13.8-15.8 pmol/L) and 17.3 pmol/L (16.6-18.5 pmol/L) respectively, with median (IQR) TSH values of 0.36 mIU/L (0.17-1.20 mIU/L) and 0.27 mIU/L (0.12-0.65 mIU/L).

Primary Analyses: Effects of Suboptimal Gestational Thyroid Function and Treatment on Global Brain Structure

Global brain tissue volumes are presented in Fig. 2. There were no significant differences between the 4 groups in total gray matter volume ($F(3,81) = 1.055$; $P = 0.373$), cortical volume ($F(3,81) = 1.084$; $P = .361$), cerebral white matter volume ($F(3,81) = 0.402$; $P = .752$), subcortical gray matter volume ($H(3) = 1.63$; $P = .652$), or total intracranial volume ($F(3,81) = 0.752$; $P = .525$). Accounting for potential confounders (sex, age, and pubertal status) using multiple regression did not reveal any differences in global tissue volume between groups (data not shown).

Secondary Analyses: Effects of Maternal Thyroid-Stimulating Hormone and Free Thyroxine on Global Brain Structure

Baseline levels of TSH (measured at 12 weeks' gestation) were significantly positively correlated with global measures of brain volume (Table 2); however, this effect was not robust to the inclusion of age, sex, and pubertal status in the model (see partial correlation estimates in Table 2). No statistically significant effects of baseline FT4 on global measures of brain volume were observed.

Among those whose mothers were randomly assigned to receive treatment with levothyroxine (optimally treated and overtreated SGTF), no effects of 20 or 30 weeks' gestation levels of maternal TSH or FT4 on global measures of brain volume were observed (32). Only total gray matter volume was shown to correlate with TSH at 30 weeks' gestation in those who were optimally treated, but this did not survive correction for multiple comparisons.

One participant had overt hypothyroidism, with a TSH of 38 mIU/L, almost 4 times the next highest TSH result. This participant was excluded from analysis, along with any participant whose brain volumetric measurements met criteria for outlier-based exclusion (see "Materials and Methods"). Results were similar when all data were included (data not shown).

Although groups were well matched for sex, it was a statistically significant variant in partial correlation calculations. In a previous observational population cohort study, an association between maternal thyroid function and ADHD traits was found in girls, but not in boys (15). Therefore, we performed an exploratory analysis to examine if any interaction was found between treatment group and sex. There was no significant effect of sex on the effect of treatment group on any global brain volume ($P > .05$).

Exploratory Analyses: Effects of Maternal Thyroid-Stimulating Hormone and Free Thyroxine on Regional Brain Structure

Exploratory analyses revealed positive correlations between TSH but not FT4 levels at 12 weeks' gestation (baseline) and regional brain volume across several regions, even after accounting for age, sex, and pubertal status (32). However,

Table 1. Baseline characteristics of the CATS III study cohort

Characteristic	Normal GTF N = 24	Untreated SGTF N = 21	Optimally treated SGTF N = 20	Overtreated SGTF N = 20	Test statistic; *P*
TSH at 12 wk[c] (study entry; mIU/L)	0.96 (0.49-1.40)	3.65[a] (0.83-5.20)	3.71[b] (1.63-4.53)	4.35[b] (3.87-5.04)	H = 32.0; *P* < .001
TSH at 20 wk (mIU/L)	–	–	0.37 (0.17-1.20)	0.11 (0.02-0.42)	W = 277.5; *P* = .037
TSH at 30 wk[d] (mIU/L)	–	–	0.28 (0.12-0.65)	0.16 (0.02-0.33)	W = 228; *P* = .161
FT4 at 12 wk[c] (study entry; pmol/L)	14.1 (13.7-14.6)	10.8[b] (10.7-12.8)	10.7[a] (10.0-13.6)	12.5 (10.6-12.8)	H = 19.7; *P* < .001
FT4 at 20 wk (pmol/L)	–	–	15.6 (14.6-16.3)	18.9 (17.9-19.6)	W = 37.5; *P* < .001
FT4 at 30 wk[d] (pmol/L)	–	–	14.6 (13.8-15.8)	17.3 (16.6-18.5)	W = 46.5; *P* < .001
Male children, %	41.7	52.4	50.0	50.0	H = 0.602; *P* = .896
Age of children, y	13.4 (12.4-15.0)	13.9 (13.1-14.8)	14.6 (12.9-15.5)	12.9 (12.5-13.7)	H = 7.52; *P* = .057
Pubertal score[e]	6.0 (5.8-8.0)	8.0 (5.0-8.0)	7.0 (6.8-8.0)	6.5 (5.8-7.0)	H = 5.81; *P* = .121
FSIQ at age 9, mean (SD)	104.8 (9.69)	105.6 (11.40)	105.6 (11.15)	107.5 (10.31)	F = 0.251, *P* = .86
CATS II cohort					
TSH at 12 wk[c] (study entry; mlU/L)	1.2 (0.7-1.8)	3.6 (1.1-4.5)	4.1 (1.9-5.1)		
FT4 at 12 wk[c] (study entry; pmol/L)	14.1 (1.7)	11.7 (1.9)	12.0 (1.9)		

Values are medians (first and third quartile) unless stated otherwise.
Abbreviations: CATS III, Controlled Antenatal Thyroid Screening III; FSIQ, full-scale intelligence quotient; FT4, free thyroxine; GTF, gestational thyroid function; SGTF, suboptimal gestational thyroid function; TSH, thyrotropin.
[a]*P* less than .01 vs normal GTF; [b]*P* less than .001 vs normal GTF (post hoc pairwise Wilcoxon test).
[c]Missing thyroid function tests, n = 1.
[d]Missing thyroid function tests, n = 2.
[e]Four participants missing total Tanner scores. Data shown here are excluding these participants.

none survived correction for multiple comparisons using the Bonferroni method ($\alpha = .00008$), so results should be interpreted with care.

Discussion

In this prospective follow-up analysis of adolescents from the CATS study, we found no differences in brain morphology among adolescents born to mothers with normal or SGTF, nor an effect of treatment with T4 (whether optimally or overreplaced). We found weak positive associations between maternal TSH at 12 weeks' gestation and total gray matter, cortical, and cerebral white matter volumes, which became insignificant after adjustment for age, sex, and pubertal status. In exploratory analysis, several brain region volumes were correlated with TSH at 12 weeks' gestation, but none survived correction for multiple comparisons. Our findings suggest that any effects of excess maternal thyroid function after 12 weeks' gestation on child neurodevelopment (23) are not accompanied by altered brain morphology in adolescents, at least not at a macrostructural level.

In contrast to animal model data, human studies of the effects of altered gestational thyroid function on offspring neurodevelopment are more limited and largely observational in nature (33). Some studies have observed an association between mild-to-moderate maternal thyroid dysfunction and several child developmental outcomes, including intelligence, reaction time, verbal development, and motor function, particularly when thyroid status is altered in early pregnancy (34-42). These associations have since been confirmed in several meta-analyses (43, 44), although interpretation is difficult due to heterogeneity in study design, including differences in ethnicity, iodine status, definitions of hypothyroidism, timing of sampling in pregnancy, and timing of assessment of the childhood neurodevelopmental outcomes. In contrast, our analysis of the original CATS study children at age 3 years (18) and reanalysis at age 9 years (24) found no effect of maternal SGTF on IQ, nor an effect on cognition of thyroid hormone replacement. Casey et al (45), in a further randomized controlled trial of T4 supplementation in maternal SGTF, similarly found no benefit on IQ or other neurodevelopmental indices in early childhood. In contrast to IQ, however, we did find an effect of maternal T4 supplementation on child behavior (conduct and hyperactivity) in the CATS cohort at age 9 years, an observation that was confined to overt hypothyroidism and the subgroup of mothers who had been overreplaced with T4 during pregnancy (23). These findings are consistent with observational studies and a meta-analysis that confirmed an association between maternal thyroid dysfunction (both hypothyroidism and hyperthyroidism) and childhood ADHD (13, 14, 46). However, the mechanisms in operation in humans are unknown.

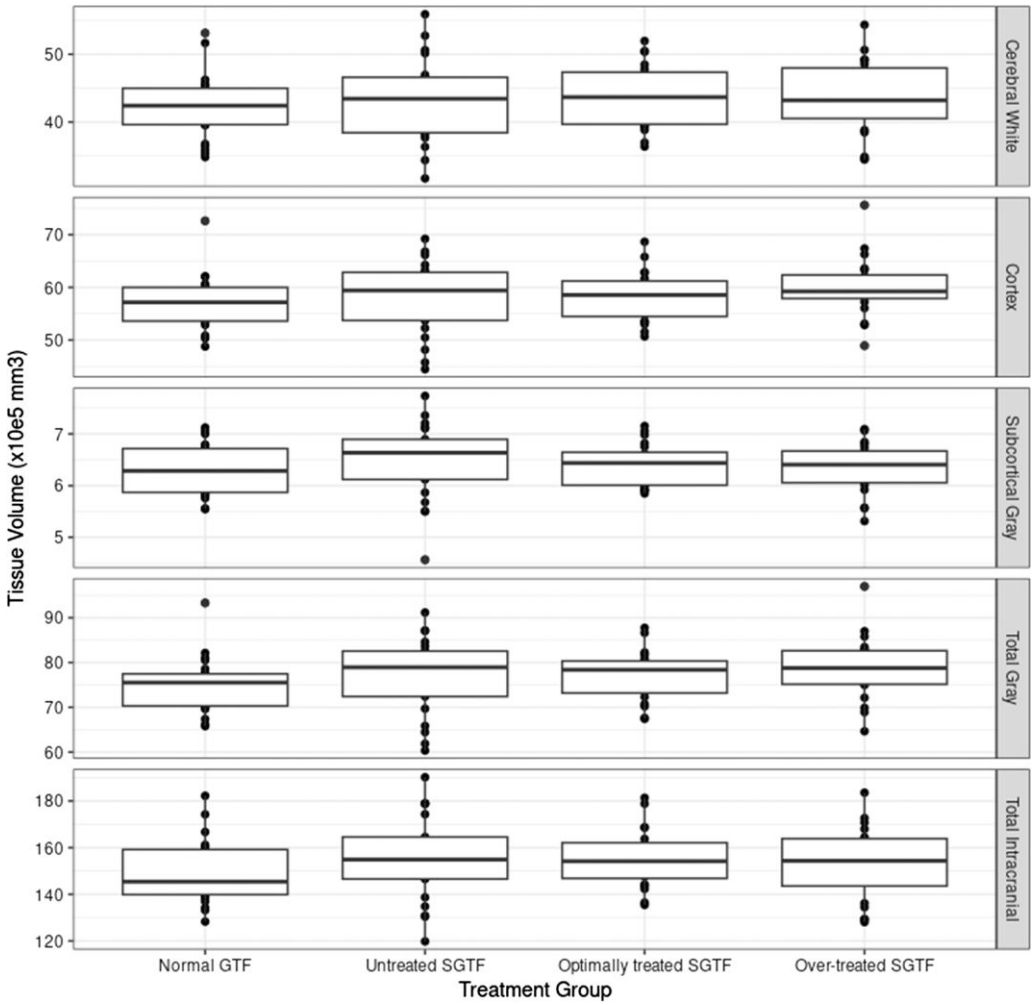

Figure 2. Global brain tissue volumes within each treatment group. Box plots represent medians and interquartile ranges. GTF, gestational thyroid function; SGTF, suboptimal gestational thyroid function.

Recent advances in human neuroimaging have enabled an examination of adolescent brain maturation in unprecedented detail. We were able to extend our study of childhood behavior to a detailed analysis of brain morphology. While other studies have examined the associations between childhood brain morphology and maternal thyroid status (16, 17, 37, 47-49), to our knowledge, ours is the largest study of adolescent brain morphology undertaken predominantly in offspring born to mothers with SGTF, and the first to consider the effects of maternal T4 supplementation on neurodevelopmental trajectories.

In their subanalysis of 652 8-year-old children from the Generation R cohort who underwent MRI assessment, Ghassabian et al (37) found no association between maternal hypothyroxinemia in early gestation and brain volume, cortical thickness, or surface area despite a 4.3 point reduction in IQ. However, the definition of maternal hypothyroxinemia was broad, defined as the lowest 5% rather than 2.5% of the cohort's FT4 results, a total of 27 cases. Korevaar et al (16) later demonstrated an inverse U-shaped association between maternal FT4 concentrations and child IQ, child gray matter volume, and cortical volume in this cohort, a relationship that persisted even after exclusion of mothers with overt thyroid disease. At age 10 years, maternal TSH exhibited an inverse U-shaped association with child total gray matter volume and cortical volume in this population, a relationship that was most evident in early gestation (17).

We found only a weak positive association between maternal TSH at 12 weeks' gestation and child gray matter volume, despite adopting a similar methodological approach (3T MRI and automated segmentation by FreeSurfer image analysis). Potential explanations for these differences may lie in our older adolescent population needing adjustment for the potential confounding influences of puberty and its effects on gray matter maturation (50) and the later sampling time of maternal TSH. Jansen et al (17) found that the inverse U-shaped association of maternal TSH with gray matter volume in their study was evident only in early gestation, disappearing entirely after 14 weeks. A more significant effect of maternal thyroid status on child brain volume may therefore have been missed in our study due to the slightly later thyroid sampling. Our statistical approach also differed: By design, most participants in our study were from mothers with SGTF and TSH values that were therefore skewed. Furthermore, there was no trend in our raw data that suggested that a nonlinear analysis was more appropriate than a linear model, hence we did not undertake cubic spline analysis to examine for nonlinear associations.

Table 2. Effects of baseline (12 weeks' gestation) maternal thyrotropin and free thyroxine levels on global brain volumes

	Full correlation estimate (Kendall tau)	Full correlation *P*	Partial correlation estimate (Kendall tau)	Partial correlation *P*
Baseline TSH (N = 84)				
Total gray matter volume	0.191	.011	0.148	.054
Cortical volume	0.183	.015	0.140	.068
Cerebral white matter volume	0.178	.017	0.137	.073
Subcortical gray matter volume	0.150	.046	0.102	.183
Total intracranial volume	0.172	.021	0.131	.085
Baseline FT4 (N = 84)				
Total gray matter volume	0.013	.862	−0.044	.560
Cortical volume	0.024	.753	−0.034	.652
Cerebral white matter volume	0.039	.607	−0.004	.962
Subcortical gray matter volume	0.020	.789	−0.022	.776
Total intracranial volume	0.036	.634	−0.002	.983

Partial correlation models accounted for sex, age, and pubertal status at the time of imaging. No corrections for multiple comparisons were used for this analysis.
Abbreviations: FT4, free thyroxine; TSH, thyrotropin.

We did not find any evidence of an effect of maternal supplementation with levothyroxine on offspring brain macrostructure, even in overtreated mothers in whom offspring conduct and hyperactivity scores above clinically relevant thresholds were more prevalent at age 9 years (23). This may reflect a lack of power due to the smaller sample size or suggest that any neurobehavioral differences are not accompanied by changes in brain macrostructure. Alternatively, it is possible that any effects of maternal thyroid function on neurodevelopment are transient: ADHD is often considered as a disorder of delayed brain maturation (51) associated with amelioration of the phenotype with age (52).

In a previous study of more than 18 000 image-derived phenotypes in the United Kingdom, we found that a recorded diagnosis of hypothyroidism in adults was associated with a reduction in cerebellar and pallidum gray matter volume (53). Recognizing that an effect of maternal thyroid status might thus be apparent only at a regional brain level, we undertook additional analyses to examine for associations with subcortical volumes. As with the more global measures, weak positive correlations of baseline maternal TSH with several brain regions were identified, although these are presented uncorrected for possible type 1 error and are considered purely exploratory in nature.

Our study has a number of strengths, including the increased signal-to-noise ratio offered by 3T MRI, and brain imaging analysis of children whose mothers had received levothyroxine supplementation in pregnancy within a randomized clinical trial design. We were also careful to adjust for the potential confounding influences of puberty as well as age and sex on brain maturation. However, our study also has several limitations, including the lack of repeat neurobehavioral assessment and an evaluation of pubertal status by self-assessment rather than clinician rating. The self-assessment of pubertal status approach is nevertheless valid. Participants were also scanned only once, hence we are unable to offer insight into any potential effect of maternal thyroid status on the trajectory of brain development, and were predominantly White, which may limit the generalizability of our findings to other populations. Finally, we recognize that the study may have been underpowered to demonstrate an effect on brain macrostructure, since our sample size calculations were based on microstructural indices.

In conclusion, we found no evidence of a significant effect of treating mild maternal thyroid dysfunction on adolescent brain morphology in this follow-up of participants from the CATS study. Secondary analysis found maternal TSH in the first trimester to have a weak correlation with several gray matter regional volumes, but none survived correction for multiple testing. Further studies, including an assessment of brain microstructure, are needed to investigate the mechanisms by which gestational thyroid function affects human brain development.

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Disclosures

The authors declare no conflict of interest.

Data Availability

The data that support the findings of this study are available from the corresponding author on reasonable request.

Analysis scripts for this project are available on the Open Science Framework (OSF; DOI 10.17605/OSF.IO/FQ4XK).

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