Downstream Effects of Maternal Hypothyroxinemia in Early Pregnancy: Nonverbal IQ and Brain Morphology in School-Age Children

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Context: Although maternal hypothyroxinemia is suggested to be related to various adverse consequences in a child's neurodevelopment, the underlying neurobiology is largely unknown.

Objective: The objective of the study was to examine the relationship between maternal hypothyroxinemia in early pregnancy and children's nonverbal intelligence quotient (IQ). Furthermore, we explored whether global brain volumes, cortical thickness, and brain surface area differed between children exposed prenatally to hypothyroxinemia and healthy controls.

Design and Setting: The study included a large population-based prospective birth cohort in The Netherlands.

Participants: A total of 3727 mother-child pairs with data on prenatal thyroid function at less than 18 weeks of gestation and nonverbal IQ at 6 years participated in the study. In 652 children, brain imaging was performed at 8 years of age.

Main Measures: Maternal hypothyroxinemia was defined as free T4 in the lowest 5% of the sample, whereas TSH was in the normal range. At 6 years, children's IQ was assessed using a Dutch test battery. Global brain volumetric measures, cortical thickness, and surface area were assessed using high-resolution structural magnetic resonance imaging.

Results: The children of mothers with hypothyroxinemia in early pregnancy scored 4.3 points IQ lower than the children of mothers with normal thyroid status (95% confidence interval −6.68, −1.81; $P = .001$). After adjustment for multiple testing, we did not find any differences in brain volumetric measures, cortical thickness, and surface area between children exposed prenatally to hypothyroxinemia and controls.

Conclusions: Our findings confirm a large adverse effect of maternal hypothyroxinemia on children's nonverbal IQ at school age. However, we found no evidence that maternal hypothyroxinemia is associated with differences in brain morphology in school-age children. **(*J Clin Endocrinol Metab* 99: 2383–2390, 2014)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

Received December 3, 2013. Accepted March 19, 2014.
First Published Online March 31, 2014

Abbreviations: CBCL1½-5, Child Behavior Checklist, preschool version; CI, confidence interval; CRP, C-reactive protein; fT_4, free T_4; IQ, intelligence quotient; MRI, magnetic resonance imaging; SRS, Social Responsiveness Scale; TPO-Ab, thyroid peroxidase antibody.

Maternal thyroid hormones are crucial for normal brain development in early stages of fetal life. Children born to mothers with untreated overt hypothyroidism in pregnancy are at increased risk of cognitive impairment (1). Hypothyroxinemia, characterized by low free T_4 (fT_4) and normal TSH, is also suggested to have adverse consequences for children. Epidemiological studies have shown that the children of hypothyroxinemic mothers are at an increased risk for delayed neurodevelopment in infancy (2), neuropsychological deficits (3), and language delay in toddlerhood (4). The few studies with a longer follow-up have found that children exposed to hypothyroxinemia have slower reaction times in neuropsychological tasks (5), more attention deficit/hyperactivity disorders (6), and greater autistic-like behaviors (7). Yet a recent randomized clinical trial in a large sample of pregnant women showed no effect of early screening and treatment of hypothyroxinemia on children's intelligence quotient (IQ) at age 3 years (8). Thus, the question remains whether low maternal thyroid function, within the normal range, affects children's cognitive development.

Animal studies provide evidence that induced maternal hypothyroxinemia influences important neurodevelopmental processes in offspring such as alternation in neural migration and differentiation in cerebral cortex and hippocampus (9, 10). In addition, maternal hypothyroxinemia in rats causes delays in hippocampal axonal growth (11), limits dendritic growth in cerebellar Purkinje cells (12), and has sex-specific effects on white matter proteins in the cerebellum of the rat progeny (13). To date, few neuroimaging observational studies in humans have investigated structural and functional brain abnormalities in response to maternal clinical hypothyroidism (14) and congenital hypothyroidism (15). These studies have found that the children of hypothyroid mothers had smaller hippocampal volumes (14) and abnormalities in the corpus callosum (16). Children with congenital hypothyroidism also had smaller hippocampal volumes (17), abnormal corpus callosum morphology (18), and atypical hippocampal function (19). Yet prospective neuroimaging studies of children born to hypothyroxinemic mothers are lacking.

Despite existing evidence from animal models and suggestive findings of observational studies regarding the association between maternal hypothyroxinemia and children's cognition, the underlying neurobiology is still largely unknown. In this population-based neuroimaging study, we aimed to investigate whether the children exposed to maternal hypothyroxinemia during early pregnancy had lower nonverbal IQ at 6 years of age. We also tested the relationship between maternal subclinical hypothyroidism (normal fT_4 and high TSH) and a child's IQ. We hypothesized that maternal hypothyroxinemia in early pregnancy had adverse consequences on children's IQ and that any IQ difference would correspond to structural alternations observed in magnetic resonance imaging (MRI) of the brain. Based on findings in animals, which show a decrease in the cortical thickness and impairment in cortical maturation due to maternal hypothyroxinemia (9, 10), we hypothesized that brain measures, and specifically the cortical thickness, hippocampus, and corpus callosum, would be affected in the children of hypothyroxinemic mothers.

Materials and Methods

Participants

This study was embedded in the Generation R Study, a population-based birth cohort in Rotterdam, The Netherlands, which follows up children from fetal life onward (20). The Medical Ethics Committee of the Erasmus Medical Center and the Central Committee of Research Involving Human Subjects approved the study. Written informed consent was obtained from parents and confidentiality was guaranteed.

From 7069 pregnant women enrolled during early pregnancy, thyroid parameters were assessed in 5100 mothers. Because of potential differences in fetal brain development and nutritional intake between multiple and singleton pregnancies, we excluded 107 twins. From the remaining 4993 children, nonverbal IQ was assessed in 3727 children at 6 years of age (75%) [mean age 6.2 y (0.5), range 4.9–9.0 y].

A subgroup of children was also invited to participate in an MRI study. An overview of the neuroimaging component, including participant selection, has been described elsewhere (21). A total of 1070 children [mean age 7.9 (1.0) y, range 6.1–10.7 y] were scanned between September 2009 and August 2013. Of these children, 147 children were excluded based on poor image quality (n = 144) or incidental findings (n = 3). Furthermore, we excluded the children from twin pregnancies (n = 17). This resulted in a sample of 906 children, from which data on maternal thyroid function was available in 652 children.

We compared the 3727 mother-child pairs included in the analyses with the 1266 pairs (25% of 4993) excluded because of missing information on the child's nonverbal IQ. We found that the children who were excluded were more likely to be boys (53.8% vs 49.5%, $P = .01$), to have a non-Western national origin (36.1% vs 30.9%, $P = .002$), and to have mothers with lower education (30.0% vs 20.4%, $P = .001$) when compared with the nonrespondents. The children included were also more likely to be first born (46.1% vs 40.7%, $P = .001$) and to have older mothers (mean difference 1.2 y, $P < .001$) than those excluded. Maternal fT_4 and TSH levels did not differ between two groups.

We also explored whether the imaging subsample (n = 652) was different from the rest of the sample with IQ data. There were no differences in maternal fT_4 and TSH, children's nonverbal IQ, and demographics. However, the children within the imaging subsample had more problem behavior compared to the children without imaging data ($P < .001$). Furthermore, the chil-

dren in the imaging study were more likely to be Dutch compared with the children who were not involved in the imaging study.

Thyroid hormone assessments

Maternal thyroid parameters [fT_4 and TSH and thyroid peroxidase antibodies (TPO-Abs)] were assessed in blood samples obtained during the first prenatal visit [mean 13.5 (2.0) wk, range 5.1–17.9 wk]. Within 24 hours plasma was stored at −80°C and processed in batches over 6 months using chemiluminescence assays (Vitros ECI immunodiagnostic system; Ortho Clinical Diagnostics). Reference values of fT_4 for nonpregnant women in our laboratory were 11–25 pmol/L. Inter- and intraassay coefficients of variation for fT_4 were 4.7%–5.4% and 1.4%–2.7%, respectively. We used the reference values for maternal TSH in pregnancy as recommended (TSH levels between 0.03 and 2.5 mIU/L) (22). An alternative cutoff value of 3.0 mIU/L for TSH was used to explore whether the associations were independent of cutoff choice. Inter- assay and intraassay coefficients of variation for TSH were 2.5%–4.1% and 1.0%–1.2%. We defined hypothyroxinemia as fT_4 below the fifth percentile of the sample and TSH levels within the reference range for pregnancy (n = 129, 7.3% of the sample) (4). The fifth percentile in our sample corresponded to fT_4 = 10.99 pmol/L. We defined maternal subclinical hypothyroidism as fT_4 within the normal range and high TSH (n = 522 with TSH cutoff of 2.5 mIU/L and n = 255 with TSH cutoff of 3.0 mIU/L). We measured maternal TPO-Abs (Phadia 250 immunoassay; Phadia) and defined positive TPO-Abs by plasma concentrations of 100 IU/mL or greater.

Thyroid parameters were measured after delivery, and parents were not informed about the results of the tests, except one clinical case that was excluded from this study.

Children's nonverbal IQ

At the age of 6 years, the children were invited to visit the Generation R research center. During this visit, children's nonverbal IQ was assessed using two subtests of a well-validated Dutch nonverbal intelligence test: Snijders-Oomen Niet-verbale intelligentie test, revisie (23). The two subsets were mosaics, which assesses spatial visualization abilities, and categories, which assesses abstract reasoning abilities. In a different sample of 626 children aged 4½-7½ years, the correlation between total scores derived from the mosaics and categories subsets and IQ scores derived from the complete test was $r = 0.86$ (P. J. Tellegen, personal communication). Raw test scores were converted into nonverbal IQ scores using normal values tailored to exact age.

Imaging

MRIs were acquired using a GE Discovery MR750 3.0 Tesla scanner (General Electronics Healthcare) with an eight-channel head coil. The high-resolution, T1-weighted image was collected using an inversion recovery fast spoiled gradient recalled sequence with the following parameters: repetition time = 10.3 msec, echo time = 4.2 msec, inversion time = 350 msec, number of excitations = 1, flip angle = 16° repetition time and echo time are correct. Instead of TI please write 'inversion time' and instead of NEX please write 'number of excitations', readout bandwidth = 20.8 kHz, matrix 256 × 256, imaging acceleration factor of 2, and an isotropic resolution of $0.9 \times 0.9 \times 0.9$ mm^3. Before scanning took place, children were familiarized with the scanning environment during a mock scanning session (21).

Image quality assurance was performed in two steps. The first step was a visual inspection of the image quality of the T1 sequence prior to preprocessing the data. All images were rated on a six-point scale (unusable to excellent). The next step of quality assurance took place after the images were processed through the FreeSurfer pipeline (http://surfer.nmr.mgh.harvard.edu/) and consisted of a visual inspection of the segmentation quality of the data. All images were rated on a six-point scale (not constructed to excellent). The T1 data that were rated as unusable or poor were not used nor were the data from the children whose FreeSurfer output was not constructed or were rated as poor for both hemispheres.

Image processing

Cortical reconstruction and volumetric segmentation were performed with the FreeSurfer image analysis suite, which has been described in detail elsewhere (24). Cortical thickness was calculated as the closest distance from the gray/white boundary to the gray/cerebrospinal fluid boundary at each vertex on the tessellated surface (25). The surface-based map was smoothed using a 10-mm, full-width half-maximum Gaussian kernel prior to the surface-based analyses. FreeSurfer morphometric procedures have been demonstrated to show good test-retest reliability across scanner manufacturers and across field strengths (26).

Covariates

Potential confounders were selected a priori and on the basis of background knowledge about the causal structure of the study question (7, 8, 27). Information on birth date, sex, and birth weight was obtained from registries. Gestational age at birth was established using an ultrasound examination during pregnancy. Birth order, maternal age, education, marital status, ethnicity, household income, and history of smoking were assessed by questionnaires. The child's ethnic background was defined based on the country of birth of both parents. Maternal education was defined by the highest completed education. Maternal smoking was assessed at enrollment and in mid- and late pregnancy. Maternal weight and length were measured at enrollment and were used to calculate body mass index. Maternal C-reactive protein (CRP) concentration was analyzed in plasma samples in early pregnancy by using an immunoelectrochemiluminescence assay on the Architect System (Abbott Diagnostics) (28). We used the Brief Symptom Inventory, a validated self-report questionnaire, to measure maternal psychopathology during pregnancy (29). Head circumference was measured during the visit at the research center at the age of 6 years.

When the children were at 2.5 years of age, their mothers reported on their expressive language using the Language Development Survey (30). The Child Behavior Checklist, preschool version (CBCL1½-5) was collected when the children were 6 years of age to obtain standardized parental ratings of the children's behavior and emotion (30). The CBCL1½-5 is a validated instrument to measure early behavioral and emotional problems in children. At the same age, we also collected an 18-item short form of the Social Responsiveness Scale (SRS) to assess autistic behaviors. This questionnaire obtained parent observations of their child's social behavior in a naturalistic setting (31).

Statistical analyses

The children with data on maternal fT_4 levels in pregnancy and nonverbal IQ were included in the main analyses (n = 3727). For the subsample with imaging data, we included 652 children with maternal fT_4 data and good-quality MRI. The percentage of missing data for covariates were below 10% except for maternal psychopathology in pregnancy (15.3%) and household income (17.7%). The percentage of missing values for maternal TSH was 1.4%. We used an independent-sample *t* test and χ^2 statistics to explore whether the nonresponse was selective. Missing values were imputed using multiple imputations. Thirty copies of the original data set were generated, with missing values replaced by values randomly generated from the predictive distribution on the basis of the correlation between the variable with missing values and other variables.

Maternal thyroid function during early pregnancy was the determinant in all analyses. Maternal TSH and fT_4 levels were divided by their SD so that their associations with nonverbal IQ could be interpreted as SD increments or decrements of the predictor. We used linear regression models to explore the relation between maternal thyroid function and a child's nonverbal IQ and brain volumetric measures. Models were adjusted for child's sex and age (models for brain measures only), child's ethnicity and birth order, maternal age, body mass index, marital status, maternal history of smoking and education, maternal psychopathology in pregnancy, household income, time of blood sampling in pregnancy, and total brain volume (models for brain measures only). Models were additionally adjusted for maternal CRP levels in pregnancy, a general marker of inflammation, to account for an existing nonthyroid disease in the mother. Previous findings of the same cohort showed relations of maternal hypothyroxinemia with children's expressive language delay and autistic symptoms (4, 7). Furthermore, we showed that early age language development was a weak predictor of nonverbal IQ at age 6 years (32). In this study, we additionally adjusted for these factors to explore the effect of maternal hypothyroxinemia on children's nonverbal IQ above and beyond early age language development or autistic symptoms.

Šidák correction was applied to correct for multiple comparisons of the association between hypothyroxinemia and six volumetric brain measures. Due to high correlations between volumetric brain measures (r = 0.31–0.99), we performed an adjustment to the Šidák test, by adjusting for the number of effective independent tests performed instead of total number of tests performed (5.2 vs 7) (significance level = 0.01) (33).

Animal studies suggest sex-specific effects of induced hypothyroxinemia on the cerebellum in the offspring (13). Therefore, we explored the statistical interaction between sex and maternal thyroid function in relation to nonverbal IQ and brain measures. We also reran the analyses in a sample excluding the children with SRS scores in the top fifth percentile to explore the effect of hypothyroxinemia on nonverbal IQ independent of autistic symptoms (7). The mean nonverbal IQ score of children with high SRS was significantly lower than the children with low SRS (92.9 vs 103.7, $P <$ .001). Also, we performed analyses in subsamples excluding the children's of mother who were TPO-Ab positive in pregnancy (n = 176) or those who received medication for a thyroid-related condition (n = 30). Because the imaging subsample was overrepresented by children with internalizing and externalizing problems (21), we performed a sensitivity analyses by rerunning all analyses in a sample excluding children with scores in the clinical range for all CBCL1½-5 scales (n = 492).

We performed surface-based general linear model vertex-wise cortical analyses using the FreeSurfer in-built module QDEC to investigate the relationship between maternal hypothyroxinemia and cortical thickness and surface area (www.surfer.nmr.mgh.harvard.edu). QDEC allows users to perform inter-subject/group averaging and inference on the morphometry data produced by the FreeSurfer processing stream. Age during scanning and sex were included as covariates in the analyses. To correct for multiple testing (for all brain vertices), a Monte Carlo null-Z simulation was performed using a threshold of 1.3 ($P < .05$).

Results

Table 1 shows the characteristics of the participants. Among the children exposed prenatally to hypothyroxinemia, 40% (52 of 129) had nonverbal IQ in the lowest quartile of the sample (nonverbal IQ < 92). This percentage was 26% (930 of 3598) in nonexposed children. We did not find a relation between maternal fT_4, TSH, and TPO-Ab positivity in early pregnancy and children's nonverbal IQ at the age of 6 years (Table 2). However, the children of mothers with hypothyroxinemia in early pregnancy had an IQ 4.3 points lower than the children of mothers with normal thyroid status [95% confidence interval (CI) −6.68, −1.81; P = .001]. When we adjusted the analyses for maternal CRP, we found similar effect size (B = −4.32, 95% CI −6.83, −1.82). Furthermore, additional adjustment for expressive language at 2.5 years or autistic symptoms did not significantly alter the effect size (B = −4.72, 95% CI −7.97, −1.47; and B = −4.33, 95% CI −6.83, −1.83). We reran the analysis in a subsample of children with imaging data and also in samples of children excluding those with high autistic symptoms or samples excluding the children of mothers with TPO-Ab positivity or a history of thyroid-related medication, the observed association remained essentially unchanged (Supplemental Table 1). Analyses with different cutoff values for TSH had similar results. There was no interaction between sex and hypothyroxinemia in the prediction of children's nonverbal intelligence. We did not find a relationship between maternal subclinical hypothyroidism and the children's nonverbal IQ at the age of 6 years (data not shown).

Maternal hypothyroxinemia in early pregnancy was not associated with total brain volume in the children (B adjusted for covariates = 14850, 95% CI −27578, 57278, P = .49). Table 3 summarizes the results on the association between maternal hypothyroxinemia and children's MRI brain volumetric measures. We did not find a relationship between maternal hypothyroxinemia in early pregnancy and global brain measures except that the chil-

Table 1. Participant Characteristics

	Sample With Data on Maternal Thyroid Function and IQ (n = 3727) Maternal Hypothyroxinemia		Sample With Data on Maternal Thyroid Function and Magnetic Resonance Brain Imaging (n = 652) Maternal Hypothyroxinemia	
	No (n = 3598)	Yes (n = 129)	No (n = 625)	Yes (n = 27)
Maternal characteristics				
Age at enrollment, y	30.4 (3.5)	31.2 (4.8)	30.6 (4.9)	31.1 (3.8)
Body mass index at enrollment, kg/m^2	24.4 (4.3)	27.0 (5.1)	24.4 (4.3)	26.6 (4.0)
Education, %				
Primary school	20.0	34.2	19.7	28.0
Secondary school	53.5	52.1	51.9	60.0
High school	26.5	13.7	28.4	12.0
Psychopathology score in pregnancy	0.15 (0.06, 0.33)	0.19 (0.08, 0.37)	0.17 (0.06, 0.35)	0.19 (0.08, 0.51)
Smoking, %				
Never	76.4	69.9	75.5	70.4
Stopped with pregnancy	8.5	9.8	7.2	7.4
Continued in pregnancy	15.1	20.3	17.3	22.2
Household income, %				
<€1200	6.1	14.4	5.7	8.7
>€1200 and <€2000	15.5	24.7	13.6	34.8
>€2000	78.5	60.8	80.7	56.5
Marital status, married/with partner, %	89.2	79.1	86.3	76.0
Thyroid function in pregnancy				
Time of sampling, wk	13.4 (1.9)	14.4 (2.0)	13.4 (2.0)	14.0 (2.0)
TSH, mU/L	1.37 (0.86, 2.08)	1.26 (0.84, 1.76)	1.35 (0.84, 1.99)	1.31 (1.06, 1.79)
fT_4, pmol/L	14.88 (13.28, 16.85)	10.32 (9.86, 10.74)	14.88 (13.28, 16.97)	10.44 (10.05, 10.90)
TPO-Ab, yes	4.8	2.3	5.6	3.7
Child characteristics				
Age at visit, y	6.2 (0.5)	6.0 (0.4)	7.9 (1.0)	7.8 (0.9)
Sex, boy, %	49.5	50.0	50.7	55.6
First born, %	59.7	48.4	62.6	44.4
Ethnic background, %				
Dutch	60.5	42.9	72.8	55.6
Other Western	9.3	8.7	5.8	7.4
Non-Western	30.3	48.4	21.4	37.0
Birth weight, g	3428 (547)	3480 (648)	3450 (544)	3523 (704)
Gestational age at birth, wk	39.3 (40.1, 41.0)	40.1 (39.0, 41.0)	40.3 (39.3, 41.1)	40.4 (39.3, 41.3)
Head circumference at age 6 y, cm	51.4 (1.6)	51.3 (1.5)	51.3 (1.6)	51.3 (1.1)
Behavioral problem score at 6 y	6.00 (2.00, 11.00)	7.00 (3.00, 13.00)	8.00 (3.00, 14.00)	9.00 (4.25, 17.94)
Emotional problem score at 6 y	4.00 (2.00, 8.00)	5.14 (2.53, 10.50)	5.00 (2.00, 11.50)	8.00 (4.25, 11.50)
IQ score at 6 y	101.7 (15.1)	94.6 (13.8)	102.7 (14.6)	95.7 (15.9)

Numbers are mean (SD) for variables with normal distribution, median (quartile range) for not normally distributed variables, and percentages for categorical variables.

dren exposed prenatally to hypothyroxinemia had larger cerebellar white matter volumes compared with the healthy controls [29 517 (630) mm^3 vs 28 182 (129) mm^3, $P = .04$]. However, the association was not significant after adjustment for multiple testing (significance level 0.01). Maternal hypothyroxinemia in early pregnancy did not predict children's cortical thickness and surface area at age 8 years. Excluding the children with clinical scores of the CBCL1½-5 scales did not influence the results (data not shown).

Table 2. Maternal Thyroid Function in Early Pregnancy and Child's IQ at 6 Years

	Child's IQ at 6 y (n = 3727)		
	B	**95% CI**	***P* Value**
TSH, per SD	0.04	−0.17, 0.26	.70
fT_4, per SD	−0.24	−0.61, 0.14	.21
Severe hypothyroxinemia	−4.32	−6.68, −1.81	.001
TPO-Abs, positive	−0.24	−2.37, 1.90	.88

Models were adjusted for child's ethnic background and birth order and maternal age, body mass index, marital status, maternal history of smoking, educational levels, maternal psychopathology in pregnancy, household income, and time of blood sampling in pregnancy. Severe hypothyroxinemia (n = 129): 0.03 less than TSH less than 2.5 mIU/L and fT_4 less than 10.99 pmol/L (less than the fifth percentile).

Discussion

In this large-scale prospective population-based study, we showed that the children born to hypothyroxinemic mothers had significantly lower nonverbal IQ scores if compared with nonexposed children. This magnitude of effect is considered clinically significant (one third of SD decrease in nonverbal IQ at the population level) and very similar to an effect size associated with breast-feeding, which led to a change in policy and recommendations for pregnant women (34). Our finding supports the previous reports, which showed adverse cognitive outcomes for maternal hypothyroxinemia in smaller samples (35) or in studies with a shorter follow-up period (2, 4). Observational studies, which reported no association between maternal hypothyroxinemia and children's cognition, either assessed maternal thyroid function in the second half of pregnancy or did not have a long-term follow-up of children (36, 37). The results of the only published randomized clinical trial have shown no effect of levothyroxine treatment on cognitive functioning in children of hypothyroxinemic mothers in comparison with the control group (8). In this trial, treatment in pregnant women was initiated relatively late, and the outcome of the trial was the IQ at 3 years of age.

To observe any effect of treatment on the cognitive function of the children born to hypothyroxinemic mothers, randomized trials should target the very early stages of pregnancy or even preconceptual period. Furthermore, IQ assessment in children, as young as 3 years, may not be a sensitive measure to detect adverse consequences of maternal mild thyroid dysfunction. Yet we cannot entirely rule out that the suboptimal thyroid function (with normal TSH) and cognitive deficits in the child might both be the result of an underlying supervening illness in the mother. Future studies are needed to establish the causality of the observed association between hypothyroxinemia and children's cognitive impairments. Moreover, although speculative, a common underlying condition in the fetus may lead to low thyroid function in the mother as well as causing cognitive impairments in the child.

The observation that maternal hypothyroxinemia and children's IQ but not maternal subclinical hypothyroidism and child's IQ are related implies that fT_4 measurement in pregnant women may be of great importance. However, current guidelines recommend using TSH for assessment of thyroid function in high-risk pregnant women (22). Further investigations are needed to examine whether high-risk pregnant women and their children would benefit from a measurement of fT_4 and subsequent intervention in pregnancy.

Our knowledge about the underlying neurobiology of the link between low maternal thyroid hormone and children's neurodevelopment comes mainly animal studies (9, 10, 38). In humans, the extensive work of Rovet and her colleagues (16, 18, 19) in clinical samples has revealed

Table 3. Maternal Severe Hypothyroxinemia in Early Pregnancy and Magnetic Resonance Brain Imaging in Children at age 8 yr. The Generation R Study (*n* = 652)

Outcomes: Brain Morphology Predictor: Maternal Severe Hypothyroxinemia	**Estimated Mean**				
	Exposed Prenatally	**Healthy Controls**	**B**	**95% CI**	***P* Value[a]**
Total gray volume, mm^3	719 492 (3392)	722 045 (694)	−2554	−9349, 4241	.46
Cortical gray volume, mm^3	547 540 (3628)	551 525 (742)	−3985	−11253, 3284	.28
Cerebral white matter volume, mm^3	380 227 (3191)	380 362 (653)	−135	−6527, 6257	.97
Cerebellar gray matter volume, mm^3	110 707 (1609)	109 316 (329)	1391	−1831, 4613	.40
Cerebellar white matter volume, mm^3	29 517 (630)	28 182 (129)	1335	73, 2598	.04
Corpus callosum volume, mm^3	2722 (68)	2722 (14)	1	−135, 137	.99
Hippocampal volume, mm^3	7926 (137)	8082 (28)	−157	−432, 118	.26

Models were adjusted for child's sex and age at the time of imaging, ethnic background and birth order, maternal age, body mass index, marital status, maternal history of smoking, educational levels, maternal psychopathology in pregnancy, household income, time of blood sampling in pregnancy, and total brain volume. Severe hypothyroxinemia (n = 27): 0.03 < TSH < 2.5 mIU/L and fT_4 < 10.99 pmol/L (less than the fifth percentile).

[a] Significant level for *P* value was .01, adjusted for the number of effective independent tests performed.

brain structural and functional abnormalities in relation to clinical low thyroid function. Nevertheless, brain morphological changes in response to milder forms of low maternal thyroid function have never been studied in humans. This study is the first attempt to explore the relation between maternal hypothyroxinemia in early pregnancy and brain morphological differences in school-age children. In this relatively large sample of 8-year-old children, we found no evidence for brain morphological differences between children exposed to hypothyroxinemia in early pregnancy and healthy controls.

There are several explanations for this finding. First, despite our prior hypothesis, structural alternations resulting from hypothyroxinemia may not be global but rather involves subregions of the brain. For example, Samadi et al (16) showed that adolescents whose mothers had insufficiently treated hypothyroidism in pregnancy obtained a smaller genu and combined anterior section and a larger splenium and combined posterior section in corpus callosum compared with controls. Second, previous evidence on the morphological brain abnormalities in children exposed to hypothyroidism comes from clinical samples with more severe abnormalities in thyroid hormone levels (14). Thus, the differences are likely more subtle and obscured by the individual variations in the growth and development of the brain. Third, our observation of children's IQ at 6 years of age confirms the adverse effect of maternal hypothyroxinemia. This important phenotypic change could be the consequence of impaired connectivity between different brain regions and thus resides at a scale that is beyond the resolution of structural MRI. Further investigation is required to explore structural abnormalities in brain subregions and to investigate impairments in functional connectivity in children exposed to maternal hypothyroxinemia in utero.

In this study, we primarily observed a larger cerebellar white matter volume in the children of hypothyroxinemic mothers compared with unexposed children. This association was no longer significant after adjustment for the effective number of tests and thus should be interpreted with caution. This observation is in contrasts with the notion of bigger is better in the neuroscience of human intelligence (39). However, in the same cohort, we previously showed that maternal hypothyroxinemia in early pregnancy predicted larger fetal and infant head size (40). Future studies are needed to replicate our cerebellar white matter finding and examine possible mechanisms.

The present study had several strengths, including a large sample size, assessment of maternal thyroid function in early pregnancy, obtaining an objective measure of children's nonverbal IQ, considering various maternal lifestyle and health-related confounding factors, and a relatively long-term follow-up period for the children. Moreover, we performed brain MRI in a large subsample of the study. However, there are also several limitations to the study. First, we did not have any measure of neonatal or child thyroid function. Also, maternal thyroid parameters were assessed only once during pregnancy. Second, the number of children exposed to maternal hypothyroxinemia was relatively small in the imaging substudy, and it is likely that we were underpowered to detect subtle brain structural abnormalities in exposed children. Third, thyroid-related medication could potentially influence the observed relation between maternal hypothyroxinemia and children's nonverbal intelligence. However, our sensitivity analyses in the pregnant women with no history of thyroid medication revealed no effect of medication on the observed association. Furthermore, due to the observational design of the study, we cannot entirely rule out the possibility of residual confounding. Randomized clinical trials are needed to establish the causality of the association between maternal thyroid dysfunction and children's neurodevelopmental delay.

Acknowledgments

The general design of Generation R Study is made possible by financial support from the Erasmus Medical Center, Rotterdam; the Erasmus University Rotterdam; The Netherlands Organization for Health Research and Development (ZonMw); The Netherlands Organization for Scientific Research (NWO); the Ministry of Health, Welfare, and Sport; and the Ministry of Youth and Families. The authors gratefully acknowledge the contribution of general practitioners, hospitals, midwives, and pharmacies in Rotterdam.

The measurements of thyroid parameters have been made possible by a generous financial gift of Ortho Clinical Diagnostics, Rochester, New York; the Jan Dekker/Ludgardine Bouwman Foundation; Fa. Merck; and Foundation Zeist.

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This work was supported by NWO ZonMw TOP Project Number 91211021 and through the NWO Brain, Cognition (Hersenenen Cognitie) Project Number 433-09-228. The work of H.T. and A.G. was supported by Research Grant FP7/2008-2013 from the European Community's 7th Framework Programme under Grant Agreement 212652 (NUTRIMENTHE project, "The Effect of Diet on the Mental Performance of Children").

Disclosure Summary: The authors have nothing to disclose.

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