

# Free Thyroxine Levels After Very Preterm Birth and Neurodevelopmental Outcomes at Age 7 Years

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## KEY WORDS

preterm infants, thyroxine, hormones, cognitive outcome, brain volumes

## ABBREVIATIONS

AUC—area under the curve  
 CI—confidence interval  
 CNS—central nervous system  
 FSIQ—Full Scale IQ  
 fT<sub>4</sub>—free thyroxine  
 GA—gestational age  
 IPPV—intermittent positive pressure ventilation  
 T<sub>4</sub>—thyroxine  
 THOP—transient hypothyroxinemia of prematurity  
 THs—thyroid hormones  
 TSH—thyroid stimulating hormone  
 VPT—very preterm

Dr Scratch conceptualized and designed the study, analyzed the data, and drafted the initial manuscript; Dr Hunt collected the blood specimens for hormone analysis, was involved in initial cerebral MRI interpretation, assisted with the conceptualization and design of the study, supervised data collection at all time points, and reviewed and revised the manuscript; Dr Thompson supervised the collection and analysis of the MRI scans and reviewed and revised the manuscript; Ms Ahmadzai performed the post-acquisition analysis of the MRI scans and reviewed and revised the manuscript; Dr Doyle assisted with the conceptualization and design of the study, supervised data collection at all time points, and reviewed and revised the manuscript; Dr Anderson assisted with the conceptualization and design of the study, supervised data collection at all time points, and reviewed and revised the manuscript; and all authors approved the final manuscript as submitted.

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**WHAT'S KNOWN ON THIS SUBJECT:** Preterm infants have transiently lowered thyroid hormone levels during the early postnatal period. Past research suggests that low thyroid hormone levels are related to cognitive and developmental deficits in children born preterm.



**WHAT THIS STUDY ADDS:** Contrary to expectations, in this study of children born <30 weeks' gestation, higher concentrations of free thyroxine over the first 6 weeks of life were associated with poorer cognitive function at 7 years of age.

## abstract



**BACKGROUND AND OBJECTIVES:** Preterm infants commonly have transient hypothyroxinemia of prematurity after birth, which has been associated with deficits in general intellectual functioning, memory, attention, and academic achievement. However, research has predominantly focused on thyroxine levels in the first 2 weeks of life and outcomes are limited to the preschool period. Our objective was to evaluate the relationships between free thyroxine (fT<sub>4</sub>) levels over the first 6 weeks after very preterm (VPT) birth with cognitive functioning and brain development at age 7 years.

**METHODS:** A total of 83 infants born VPT (<30 weeks' gestation) had fT<sub>4</sub> concentrations measured postnatally and 2- and 6-week area under the curve (AUC) summary measures were calculated. Follow-up at age 7 years included a neuropsychological assessment and brain MRI. Univariable and multivariable regression modeling was used where AUC for fT<sub>4</sub> was the main predictor of neurodevelopmental outcome at age 7 years.

**RESULTS:** Multivariable modeling revealed that higher, not lower, postnatal fT<sub>4</sub> levels (2-week AUC) were associated with poorer cognitive performances at age 7 years on tasks of verbal learning ( $P = .02$ ), verbal memory ( $P = .03$ ), and simple reaction time ( $P < .001$ ). A similar pattern of results was found when the 6-week AUC was examined. No significant associations between postnatal fT<sub>4</sub> levels and brain volumes at age 7 years were identified.

**CONCLUSIONS:** Results are contradictory to previous observations and suggest that after adjustment for confounders, higher postnatal fT<sub>4</sub> levels in VPT infants, rather than lower levels, may be a marker of adverse neuropsychological development in childhood. *Pediatrics* 2014;133:e955–e963

Very preterm (VPT; <32 weeks' gestation) children are at increased risk for a range of neuropsychological deficits, including impairments in general intellect,<sup>1</sup> language,<sup>2</sup> visuoperceptual reasoning,<sup>3</sup> learning and memory,<sup>4,5</sup> attention,<sup>6</sup> and executive functioning.<sup>7</sup> Furthermore, neuroimaging studies of preterm infants and children have reported volumetric reductions in gray and white matter tissue,<sup>8</sup> as well as specific brain regions (cerebellum, basal ganglia, amygdala, hippocampi, and corpus callosum).<sup>9</sup> Identifying mechanisms underpinning these negative outcomes is critical to improve the management and outcomes of these infants.

Transient hypothyroxinemia of prematurity (THOP) is a condition that primarily affects preterm infants born <30 weeks' gestation and is characterized by low levels of circulating thyroid hormones (THs), despite normal levels of the pituitary hormone, thyroid stimulating hormone (TSH). During the first week of life, TH levels, for which thyroxine (T<sub>4</sub>) is the body's main circulating TH, drop to a nadir below cord levels. The depth of the nadir and length of time before THOP resolves is related to gestational age (GA). This condition usually resolves within 2 to 3 weeks with progressive maturation of the hypothalamic-pituitary-thyroid axis.<sup>10,11</sup> As no consensus exists for THOP reference ranges, prevalence rates vary by study definition, with 35%<sup>12</sup> to 85%<sup>13</sup> of VPT infants reported to be affected.

THs are critical for normal central nervous system (CNS) development,<sup>14</sup> including cerebral neurogenesis (during early prenatal life)<sup>15</sup>; neural migration and differentiation, axonal and dendritic growth, and synaptogenesis<sup>16,17</sup>; gliogenesis (late fetal life until 6 months postnatally); dendritic arborization<sup>18</sup>; and myelogenesis (second trimester to 2 years postnatally).<sup>19</sup>

Consistent with this literature, the low levels of T<sub>4</sub> associated with THOP have been found to be related to neurodevelopmental deficits in children born preterm, such as delayed development of motor, cognitive, language, and educational skills.<sup>20–24</sup>

Research to date has predominantly focused on TH levels in the first 2 weeks of life, and in most studies outcome assessments have been limited to infancy and the preschool period. The current study aimed to examine the relationship between fT<sub>4</sub> over the first 6 weeks after VPT birth and cognitive functioning and brain development at age 7 years. Based on previous research it was hypothesized that low fT<sub>4</sub> levels would be negatively associated with cognitive performance and brain volumes at age 7 years in a group of children born VPT.

## METHOD

### Study Design

Participants (*n* = 99) were born <30 weeks' GA between June 2002 and December 2003 at the Royal Women's Hospital (Melbourne, Victoria, Australia). Infants who had significant genetic or congenital abnormalities likely to affect brain growth or development were excluded. At age 7 years, 83 were assessed (the "assessed group"). Approval for the study was obtained from the Human Research Ethics Committees of the Royal Women's Hospital and the Royal Children's Hospital. Written consent was obtained from parents.

### Procedure and Measures

Whole blood (0.5 mL) was collected from the cord at delivery, then on days 1, 4, 7, 14, 21, 28, and 42. Specimen collection was timed with routine morning blood sampling (7 to 9 AM). Whole blood was centrifuged at 3000 rpm for 5 minutes. Plasma was collected and stored at -70°C; hormone assays were

performed in batches. Free T<sub>4</sub> was measured directly by competitive analog immunoassay using a chemiluminescent substrate on the Immulite 2000 (Siemens AG, Healthcare Sector, Erlangen, Federal Republic of Germany). The level of fT<sub>4</sub> was interpolated from a stored standard curve calibrated for fT<sub>4</sub> concentrations.<sup>25</sup> The intra- and inter-assay coefficients of variation were acceptable (intra-assay = 5.1%; inter-assay range = 7.5% to 5.7%). Neonatal fT<sub>4</sub> data were expressed as area under the curve (AUC) values as a summary measure of hormone exposure over 2 designated time periods: (1) birth to day 14, and (2) birth to day 42.

Additional perinatal variables of interest included GA (weeks), sex, and duration of intermittent positive pressure ventilation (IPPV), which was used as a general marker of illness. At term equivalent age, T<sub>1</sub> and T<sub>2</sub> images were acquired with a 1.5 Tesla MRI scanner at the Royal Children's Hospital (Signa LX Echospeed System; General Electric, Milwaukee, WI). A validated global cerebral abnormality scoring system was rated by a neonatal neurologist who was blinded to TH status, with higher values reflecting more severe pathology.<sup>26</sup>

The 7-year follow-up included a detailed neuropsychological assessment and neuroimaging. Neuropsychological measures for the current study were selected to represent a broad range of cognitive domains (Table 1). Children who attempted a task but were too impaired to perform or had difficulty comprehending task instructions were assigned the lowest possible raw score for a subtest, or in the case of reaction time measures, the highest score recorded to signify a longer reaction time. The Social Risk Index parent questionnaire was also administered with higher scores representing greater social disadvantage.<sup>27</sup>

**TABLE 1** Neuropsychological Measures

Test Battery	Description
WASI	The 4-subtest version of the WASI was administered to estimate general intellectual functioning (FSIQ). Normative mean = 100, SD = 15.
CELF-IV	The Core Language Index from the CELF-IV was administered and used as a measure of general language ability. Normative mean = 100, SD = 15.
WMTBC	Three subtests from the WMTBC were administered to assess elements of immediate and working memory: (1) Digit Recall involves the child repeating a sequence of numbers, assessing immediate verbal memory; (2) Backward Digit Recall involves the child repeating a presented sequence of numbers in reverse order, assessing verbal working memory; and (3) Block Recall requires the child to recall a sequence of blocks that the examiner taps on a standardized board, assessing spatial immediate memory. Raw scores for each task were used in the analyses.
CVLT-C	The CVLT-C is a verbal learning and memory task in which the child is required to recall a list of 15 words, which is presented 5 times. Raw total learning score (total words recalled from trial 1 to 5) and long delay recall score were used as measures of verbal learning and verbal memory, respectively.
CMS	Dot Locations subtest from the CMS was administered to assess spatial learning and memory. The child is required to learn and remember the spatial location of an array of dots, which is presented 3 times. Raw total learning score (total score from trials 1 to 3) and long delay recall score were used as measures of spatial learning and spatial memory, respectively.
TEA-Ch	Raw scores for 4 selected subtests were used to assess different attention domains: (1) Sky Search (number of correct targets) assesses selective attention; (2) Score! (number of correct trials) assesses sustained attention; (3) Creature Counting (number of correct trials) assesses shifting attention; and (4) Sky Search Dual Task (proportion of correct counting games and identified targets) assesses divided attention.
TOL	The TOL is a measure of planning, organization, and problem solving. The total raw score was used, which is a summary score for the 12 items where higher points are awarded for quicker completion times and points subtracted for planning errors.
<i>CogState Research</i> <sup>58</sup>	Two subtests from the CogState Research battery were selected to assess processing speed: (1) Detection assesses simple reaction time; and (2) Identification assesses choice reaction time. The variable of interest for these 2 tasks was the mean of the log10 transformed reaction times for correct responses where lower scores indicated a better performance.

CELF-IV, Clinical Evaluation of Language Fundamentals, 4th ed, Australian Standardized Edition<sup>51</sup>; CMS, Children's Memory Scale<sup>54</sup>; CVLT-C, California Verbal Learning Test – Children's Version<sup>53</sup>; TEA-Ch, Test of Everyday Attention for Children<sup>55</sup>; TOL, Tower of London<sup>56,57</sup>; WASI, Wechsler Abbreviated Scale of Intelligence<sup>50</sup>; WMTBC, Working Memory Test Battery for Children<sup>52</sup>

Whole brain images were acquired at age 7 years on a 3 Tesla Siemens Magnetom Trio (Tim system) scanner, at the Royal Children's Hospital, Melbourne, Australia ( $n = 65$ ). Reasons for drop-out included: failing the mock scan ( $n = 7$ ), being too impaired to participate ( $n = 4$ ), parents not consenting to MRI ( $n = 3$ ), failing to attend ( $n = 1$ ), refusal by the child ( $n = 1$ ), cochlear implant ( $n = 1$ ), and extreme movement artifact ( $n = 1$ ). Structural T<sub>1</sub>-weighted images were used for the purpose of the current study (flip angle = 9°, repetition time = 2.27 ms, echo time = 2.27 ms, field of view = 210 × 210 mm, matrix = 256 × 256, 0.8 mm<sup>3</sup>

isotropic voxels). Images were visually inspected and those that were considered of unacceptably poor quality due to artifact were excluded ( $n = 13$ ). Data were processed by a single operator on Linux workstations using the automated FreeSurfer imaging processing suite (stable release version 4.4.0, <http://surfer.nmr.mgh.harvard.edu>). FreeSurfer output was inspected and manually edited as required. Cortical and cerebellar gray and white matter, thalamus, caudate, putamen, pallidum, hippocampus, and amygdala volumes were estimated for each hemisphere, and volumes from both hemispheres combined. The total volumes for the

caudate, putamen, and pallidum were combined to represent basal ganglia volume. Cerebellar volume was the sum of cerebellar white and gray matter. Total brain tissue was the combined volumes of all brain structures, excluding cerebrospinal fluid.

## Data Analysis

Data were analyzed by using Stata 12 (StataCorp, College Station, TX). Lost to follow-up analyses used *t* tests or Mann-Whitney U (continuous data) and  $\chi^2$  (categorical data). Regression models were employed by using AUC for  $\text{fT}_4$  as the main predictor of outcome with models fitted in 2 ways: (1) cord to day 14 levels (14-day AUC), and (2) cord to day 42 (42-day AUC). Cognitive models were fitted controlling for GA, sex, social risk, duration of IPPV, and global neonatal brain abnormality score. Imaging model covariates included GA, sex, duration of IPPV, and global neonatal brain abnormality.

To account for the non-independent effect of twins/triplets, robust standard errors using the Huber/White/sandwich method were used for all analyses. Standardized  $\beta$  coefficients ( $\beta$ ) (cut-points: 0.10 = weak, 0.30 = moderate, 0.50 = strong) were used as measures of the magnitude of effect. Also, the proportion of variance explained by an independent predictor ( $R^2$  for unadjusted analyses;  $sr^2$  for adjusted analyses) was investigated and expressed as a percentage, where larger values were more desirable.

## RESULTS

### Sample and Hormone Characteristics

Table 2 outlines the sample characteristics of the assessed and imaged groups. The mean age of the assessed group was 7.5 years (range, 6.8–8.3 years). There were no characteristic

differences between those who did and did not attend (data not shown). Sample characteristics for those children who had a useable MRI scan at age 7 years are also presented in Table 2; those in the imaged group had higher Full Scale IQ (FSIQ) scores than the non-imaged group (mean difference = 10.8; 95% confidence interval (CI) = 3.0 to 18.6;  $P = .007$ ).

Importantly, there were no differences in  $fT_4$  levels between the assessed group and those lost to follow-up. The  $fT_4$  profile of the assessed cohort included a slight elevation in mean concentration over the first 24 hours of life, followed by a decrease to a nadir at day 4 to 7. Recovery from this transient state was noted over the next 2 to 3 weeks with concentrations rising slightly above cord concentrations by day 42 (see Fig 1). Those born less mature and sicker in infancy (with longer durations of assisted ventilation) were exposed to lower levels of  $fT_4$  over both the first 2 (GA:  $b$ -coefficient [95% CI] = 21.9 [17.9 to 25.4],  $P < .001$ ; duration of IPPV:  $b$ -coefficient [95% CI] =  $-0.05$  [ $-0.07$  to  $-0.04$ ],  $P < .001$ ) and 6 (GA:  $b$ -coefficient [95% CI] = 47.0 [36.7 to 57.4],  $P < .001$ ; duration of IPPV:  $b$ -coefficient [95% CI] =  $-0.11$  [ $-0.15$  to  $-0.07$ ],  $P < .001$ ) weeks of life.

### Cognitive Results

No relationships between  $fT_4$  and cognitive performance were observed at a univariable level. On multivariable analysis, higher levels of  $fT_4$  were associated with poorer performances on verbal learning, verbal memory, and simple reaction time tasks (Table 3). The 14-day AUC independently accounted for between 6.5% and 13% of the variance for these outcomes, with moderate to strong effect sizes observed.

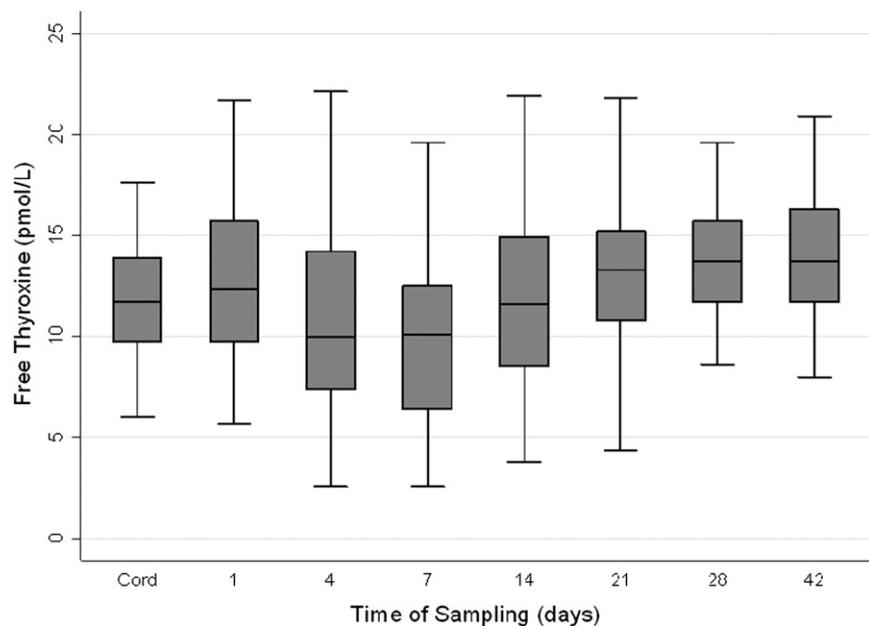
As expected, the pattern of results obtained when the 42-day AUC was

**TABLE 2** Perinatal, Demographic, and Hormone Characteristics

	7-Year Cognitive Group ( $n = 83$ ) <sup>a</sup>	7-Year Imaging Group ( $n = 52$ ) <sup>a</sup>
Child characteristics		
GA (wk), M (SD)	27.3 (1.7)	27.4 (1.6)
Birth weight (g), M (SD)	975 (235)	992 (216)
Males, $n$ (%)	35 (42.2)	19 (36.5)
Singleton, $n$ (%)	49 (59.0)	27 (51.9)
Perinatal medical factors		
Small for GA, $n$ (%)	2 (2.4)	2 (3.8)
Sepsis, $n$ (%)	34 (41.0)	21 (40.4)
Patent ductus arteriosus, $n$ (%)	40 (48.2)	23 (44.2)
Necrotizing enterocolitis, $n$ (%)	9 (10.8)	4 (7.8)
Bronchopulmonary dysplasia, $n$ (%)	26 (31.7)	15 (28.8)
Antenatal corticosteroids, $n$ (%)	76 (91.6)	50 (96.2)
Postnatal corticosteroids, $n$ (%)	10 (12.0)	4 (7.7)
IPPV (hours), median (25th and 75th percentile)	98 (7, 431)	88 (9, 376.5)
Cystic periventricular leukomalacia, $n$ (%)	1 (1.2)	1 (1.9)
Grade III/IV intracranial hemorrhage, $n$ (%)	3 (3.6)	1 (1.9)
MRI brain injury, median (25th and 75th percentile)	5 (3, 8)	5 (3, 8)
Neonatal free thyroxine characteristics		
14-day AUC (pmol)	154.1 (47.7)	159.9 (46.8)
42-day AUC (pmol)	525.4 (114.4)	535.0 (117.0)
7-year measures		
Social risk score at age 7 years, median (25th and 75th percentile)	2 (1, 3)	2 (0, 3)
FSIQ, M (SD)	95.5 (17.8)	99.5 (12.7) <sup>b</sup>

<sup>a</sup> Some sample sizes are less than the total sample owing to missing data.

<sup>b</sup> Those in the image analysis group had higher FSIQs at age 7 years than those without imaging data ( $P = .007$ ).



**FIGURE 1**

Boxplot of the  $fT_4$  profile from cord to day 42 for the assessed group. Values depicted as the median (solid line within box), 25th to 75th percentiles (margins of the box), and range of the data. Outliers were removed.

examined was less profound than the 14-day AUC analyses. However, a similar profile was observed with higher levels

of  $fT_4$  over the first 6 weeks of life related to poorer cognitive performance (Table 3).

**TABLE 3** Relationship Between Free Thyroxine 14-Day AUC/42-Day AUC and 7-Year Cognitive Outcomes

Cognitive outcomes	14-Day AUC (unadjusted; n = 83 <sup>a</sup> )			14-day AUC (adjusted; n = 79 <sup>a</sup> )			42-day AUC (unadjusted; n = 83 <sup>a</sup> )			42-day AUC (adjusted; n = 79 <sup>a</sup> )		
	$\beta$	R <sup>2</sup> (%)	p	$\beta$	sr <sup>2</sup> (%)	p	$\beta$	R <sup>2</sup> (%)	p	$\beta$	sr <sup>2</sup> (%)	p
General intellectual ability												
FSIQ	0.17	2.9	0.19	-0.08	0.3	0.69	0.09	0.8	0.45	-0.09	0.4	0.61
Language												
General language ability	0.02	0.03	0.90	-0.25	2.7	0.17	-0.03	0.09	0.81	-0.20	2.0	0.25
Verbal working memory, learning, and memory												
Immediate verbal memory	-0.03	0.09	0.83	-0.40	6.6	0.06	-0.05	0.3	0.57	-0.23	2.6	0.13
Working memory	-0.003	<0.001	0.98	-0.15	0.9	0.48	-0.01	0.01	0.93	-0.04	0.1	0.81
Verbal learning	-0.04	0.2	0.72	-0.42	7.5	0.02	-0.08	0.7	0.42	-0.30	4.5	0.04
Verbal memory	-0.06	0.4	0.61	-0.40	6.5	0.03	-0.13	1.6	0.28	-0.35	6.2	0.06
Spatial working memory, learning, and memory												
Immediate spatial memory	-0.07	0.5	0.61	-0.31	4.1	0.11	-0.08	0.7	0.44	-0.12	0.7	0.43
Spatial learning	-0.09	0.9	0.51	-0.26	2.8	0.17	-0.05	0.2	0.60	0.03	0.04	0.85
Spatial memory	-0.11	1.3	0.44	-0.21	1.9	0.21	-0.07	0.5	0.49	0.06	0.2	0.62
Attention and executive functioning												
Selective attention	-0.03	0.1	0.80	-0.33	4.4	0.09	-0.11	1.1	0.35	-0.28	4.0	0.06
Sustained attention	0.005	<0.001	0.97	-0.15	0.9	0.35	0.08	0.7	0.46	0.10	0.4	0.56
Shifting attention	0.19	3.7	0.08	0.11	0.5	0.50	0.17	2.9	0.13	0.12	0.8	0.46
Divided attention	0.06	0.4	0.58	-0.07	0.2	0.68	0.04	0.2	0.71	-0.02	0.01	0.90
Planning	0.008	0.01	0.95	-0.19	1.6	0.29	-0.02	0.06	0.82	-0.13	0.8	0.43
Speed of information processing and reaction time												
Simple reaction time <sup>b</sup>	0.19	3.66	0.13	0.56	13.0	<0.001	0.22	4.7	0.05	0.40	7.7	0.002
Complex reaction time <sup>b</sup>	-0.0003	<0.001	0.99	0.24	2.4	0.10	0.05	0.2	0.67	0.21	2.1	0.16

Covariates for adjusted analyses included GA, gender, social risk, duration of IPPV, and neonatal brain injury.

<sup>a</sup> Some sample sizes are less than the total sample owing to missing data.

<sup>b</sup> Task is reverse-scored, where higher scores signify a slower reaction time.

### Brain Volume Results

Despite a consistent pattern of results from the cognitive models, no unadjusted or adjusted associations were found between early postnatal  $fT_4$  levels and measures of 7-year brain volume (Table 4). Consequently,  $fT_4$  levels only explained a limited percentage of variance within the models (14-day AUC, 0.01%–1.7%; 42-day AUC, 0.1%–4.0%).

## DISCUSSION

In the current study the concentrations of  $fT_4$  over the first 6 weeks of life in infants <30 weeks' GA were similar to previous reports, but the surprising finding was that higher concentrations rather than lower concentrations of  $fT_4$  were associated with poorer cognitive function at age 7 years. The association between  $fT_4$  and cognitive outcome was not explained by effects on brain volumes at age 7 years.

### Free Thyroxine Concentrations Over the First 6 Weeks of Life

The daily mean (SD) of  $fT_4$  ranged from 9.9 (4.1) to 14.0 (3.0) pmol/L over the 8 sampling days, with the lowest levels observed on day 7, consistent with other reports and with the phenomenon of THOP.<sup>28,29</sup> For example, 1 study investigated a group of 128 infants born 23 to 29 weeks' gestation whose  $fT_4$  profile included a gradual decrease from 15 pmol/L at <5 hours of age, reached a nadir of 9.7 pmol/L on day 7, and then increased to 11.0 pmol/L on day 14.<sup>29</sup> Both GA and increased duration of IPPV (marker of illness) affected  $fT_4$  levels in the current study, consistent with past reports.<sup>11,29</sup> Of note, 2 more recent studies observed this GA/illness trend only when total  $T_4$ , not  $fT_4$  levels, were considered.<sup>28,30</sup>

### Free Thyroxine and Cognition

There was little evidence that low postnatal  $fT_4$  levels over different

periods of exposure were associated with cognitive deficits at age 7 years across a broad range of neuropsychological domains in children born VPT. Contrary to expectations, higher  $fT_4$  levels were related to adverse cognitive performances on tasks of verbal learning, verbal memory, and simple reaction time, after adjustment for confounding variables. Given the sparse nature of the literature, the best support for the aforementioned findings rests in studies of neonatal thyrotoxicosis and results from TH supplementation trials. Neonatal thyrotoxicosis is rare, usually arising in mothers who have Graves' disease in which the exposure to elevated levels of THs appears to be detrimental.<sup>31</sup> Although long-term outcome studies following this condition are limited and mixed, elevated rates of intellectual and psychological impairment<sup>32,33</sup> have been reported. Furthermore, in a model using rats made hyperthyroid during the first 24 days of life, the

**TABLE 4** Relationship Between Free Thyroxine 14-Day AUC/42-Day AUC and 7-Year Imaging Outcomes

Imaging outcomes	14-Day AUC (unadjusted; n = 52 <sup>a</sup> )			14-Day AUC (adjusted; n = 52 <sup>a</sup> )			42-Day AUC (unadjusted; n = 52 <sup>a</sup> )			42-Day AUC (adjusted; n = 52 <sup>a</sup> )		
	$\beta$	R <sup>2</sup> (%)	p	$\beta$	sr <sup>2</sup> (%)	p	$\beta$	R <sup>2</sup> (%)	p	$\beta$	sr <sup>2</sup> (%)	p
Total brain tissue	0.06	0.4	0.73	-0.01	<0.001	0.96	-0.06	0.4	0.68	-0.13	0.9	0.42
Cortical gray matter	0.04	0.2	0.82	-0.07	0.2	0.69	-0.10	1.1	0.54	-0.22	2.6	0.22
Cortical white matter	0.01	0.01	0.95	-0.08	0.3	0.64	-0.10	1.0	0.47	-0.17	1.6	0.24
Cerebellum	0.03	0.1	0.83	-0.18	1.2	0.26	-0.04	0.2	0.76	-0.16	1.5	0.29
Thalamus	-0.08	0.7	0.51	-0.11	0.5	0.41	-0.14	2.0	0.34	-0.09	0.4	0.60
Basal ganglia	-0.04	0.2	0.77	-0.18	1.3	0.22	-0.15	2.2	0.23	-0.25	3.4	0.12
Hippocampus	-0.008	0.01	0.96	-0.01	<0.001	0.96	-0.09	0.8	0.54	-0.10	0.6	0.55
Amygdala	0.04	0.2	0.77	-0.13	0.6	0.55	-0.03	0.1	0.84	-0.14	1.1	0.40

Covariates for adjusted analyses included: GA, gender, duration of IPPV, and neonatal brain injury.

<sup>a</sup> Some sample sizes are less than the total sample owing to missing data.

animals performed less well on a learning task as adults compared with controls.<sup>34</sup> It is important to note that the levels of fT<sub>4</sub> observed in our study did not approach levels consistent with those seen in children who have neonatal thyrotoxicosis. In the same way as causation for deficit following THOP has been extrapolated from understanding mechanisms in congenital hypothyroidism, relative elevation of fT<sub>4</sub> may be on the spectrum of maldevelopment linked to neonatal thyrotoxicosis.

With regard to TH supplementation trials, 1 study included a randomized, placebo-controlled, double-blind trial of T<sub>4</sub> supplementation that involved 200 infants born <30 weeks' gestation and 3 follow-up investigations.<sup>35</sup> At age 2 years, T<sub>4</sub>-treated infants born <27 weeks' gestation scored 18 points higher on a general development examination than those in the placebo group. However, for those in the T<sub>4</sub>-treated group born at 27 weeks' gestation or later, a detrimental effect of the supplementation was found, with the T<sub>4</sub>-treated group scoring 10 points lower than their placebo counterparts.<sup>35</sup> At the 5.7- and 10.5-year follow-ups a similar gestational difference was noted. In another study T<sub>4</sub> supplementation was positively associated with outcome in children born <29 weeks' gestation (especially in the 25/26-week GA group), but more

developmental problems were observed in those T<sub>4</sub>-treated children born >29 weeks' gestation.<sup>36</sup> Although these findings support the notion that elevated T<sub>4</sub> levels may be problematic for later outcome in VPT subgroups, it is important to note that conclusions were based largely on a set of posteriori analyses, which have innate limitations.

Further support for the finding that elevated fT<sub>4</sub> levels may be of concern rests in an endocrine model of critical illness.<sup>37–39</sup> This model outlines the hypothetical response of the endocrine system to both acute and chronic illness phases. In summary, the acute phase is considered transient, adaptive, and beneficial, and hormone variations during this time are thought to promote the redirection of energy to vital organs such as the brain. The chronic phase is a harmful, maladaptive response of the body's natural defense mechanisms. Thyroid hormones have been monitored over these phases and have been noted to drop significantly in the first hours after illness onset with the magnitude of the drop reflecting the severity of illness. Moreover, TSH concentrations remain unchanged, suggesting that the feedback mechanisms are altered at the hypothalamic-pituitary level. If illness remains prolonged, THs continue to be reduced, but are then accompanied by diminished TSH secretion.

The application of this model to the preterm population is interesting. It suggests that the low levels of THs, without a reduction in TSH, may be a protective reaction to illness, with the body potentially treating preterm birth itself as an acute illness state. Findings from the current study further support this premise in that low fT<sub>4</sub> levels in our group of VPT infants were not associated with adverse later childhood outcomes. In contrast, higher fT<sub>4</sub> levels fall outside the expected endocrine response to acute illness.

It is possible that our cohort of VPT infants may have been healthier than groups examined in past studies. Many of the published studies began before the late 1990s when advances in perinatal, neonatal, and obstetric care, such as increased use of antenatal corticosteroids and surfactant therapy, were not regularly used.<sup>40,41</sup> It is therefore possible that experiencing chronic illness endocrine patterns during early postnatal life may affect later outcome.

### Free Thyroxine and Brain Volumes

In the current study, there was little evidence of an association between fT<sub>4</sub> exposure over the first weeks of life and brain volumes at age 7 years in children born VPT. This was unexpected, especially for white matter volume. THs are important for (1) the maturation of oligodendrocytes<sup>42</sup>; (2) the accumulation of oligodendrocytes

within white matter tracts<sup>43</sup>; (3) the regulation and production of components of myelin such as basic myelin protein<sup>43</sup>; and (4) the compaction of myelin within the axonal sheath.<sup>44</sup> The role of THs in myelination also appears to be time-dependent, with different brain regions affected at different developmental stages.<sup>45</sup>

There are limited MRI studies investigating the impact of TH status on brain morphology. In one study, MRI and a subjective rating system were used to investigate myelination and CNS morphology in infants who had congenital hypothyroidism before TH replacement therapy.<sup>46</sup> No differences in myelination patterns were found between those who had congenital hypothyroidism and control subjects; both groups also had normal MRI examinations. In another study of very low birth weight infants, cerebral white matter damage diagnosed by cranial ultrasound was twice as common in infants exposed to low total T<sub>4</sub> levels compared with peers exposed to higher total T<sub>4</sub> levels.<sup>47</sup>

A limitation of our study was use of a direct immunoassay to measure fT<sub>4</sub>

levels; immunoassays can produce variable results, with a tendency to overestimate fT<sub>4</sub> at high protein concentrations and underestimate fT<sub>4</sub> at low protein concentrations, which tend to be found in sick infants.<sup>48</sup> Therefore, direct dialysis methods are now considered to be the “gold standard” for measuring TH levels.<sup>49</sup>

The current study is the first of its kind to comprehensively examine the association of postnatal fT<sub>4</sub> and brain volumetrics in childhood. Myelination in the human brain is largely complete by 2 years of postnatal age. It is possible that an early delay in myelination did occur as a result of disturbed thyroid levels, but that imaging at 7 years of age missed the window in which to detect this delay. If there was any scope for catch-up in myelination, then this may well have occurred by 7 years of age, explaining the absence of association between fT<sub>4</sub> levels and global white matter volumes. Therefore, more research is needed to truly understand this relationship, especially given the findings from the

cognitive models detailed above. Finally it must be noted that our imaged group had higher FSIQ scores than those who attended 7-year follow-up and did not have a usable MRI scan. This bias may have skewed our imaging data by not allowing examination of those children who had more severe cognitive difficulties who were unable to participate in scanning procedures.

## CONCLUSIONS

The fT<sub>4</sub> profile observed in our group of VPT infants was consistent with the transiently altered levels commonly reported within the literature. However, novel elements of our study included the use of brain volumetrics and the unexpected relationship between higher postnatal fT<sub>4</sub> levels and reduced cognitive performances at age 7 years. Conclusions supported an endocrine model of critical illness and suggest that future investigations should potentially be directed toward those infants exhibiting unexpected endocrine patterns after VPT birth.

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[www.pediatrics.org/cgi/doi/10.1542/peds.2013-2425](http://www.pediatrics.org/cgi/doi/10.1542/peds.2013-2425)

doi:10.1542/peds.2013-2425

Accepted for publication Jan 8, 2014

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PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

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**FINANCIAL DISCLOSURE:** The authors have indicated they have no financial relationships relevant to this article to disclose.

**FUNDING:** Supported by Australia's National Health and Medical Research Council (project grants 237117, 491209, and Senior Research Fellowship to Dr Anderson, 628371), National Institutes of Health (R01, HD058056), and the Victorian Government's Operational Infrastructure Support Program. Funded by the National Institutes of Health (NIH).

**POTENTIAL CONFLICT OF INTEREST:** The authors have indicated they have no potential conflicts of interest to disclose.