Thyroid status and its association with cognitive functioning in healthy boys at 10 years of age

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Abstract

Objective: Thyroid hormones (THs) are crucial for the correct maturation of the CNS and the neurodevelopment of the child. We aimed to investigate the association of TSH and free thyroxine (FT₄) levels with cognitive functioning in children from the INMA-Granada cohort studied during their follow-up at the age of 9–11 years.

Design: We evaluated 300 children from the original cohort, which comprised 668 eligible mother–son pairs recruited at birth from 2000 to 2002 in Granada (Spain).

Methods: FT₄ and TSH concentrations were measured, and cognitive development was assessed using neuropsychological tests ($n=187$). Children with chronic disease related to thyroid function and/or cognitive development were excluded.

Results: Median TSH and FT₄ levels were 3.1 μ IU/ml and 1.2 ng/dl respectively. In multivariable regression analyses adjusted for maternal and child characteristics, children with TSH levels in the top tertile had worse verbal comprehension and immediate and long-term recall. Children with FT₄ levels in the top tertile had better attention and lower impulsivity and were at a lower risk of scoring below the 20th percentile in intelligence quotient (OR=0.24; 95% CI=0.08–0.74; $P=0.013$) and in abstract reasoning ability (OR=0.28; 95% CI=0.09–0.88; $P=0.029$).

Conclusion: Our findings indicate that circulating THs and TSH may in the top tertile have an impact on cognitive functions; thus, higher TSH slightly but significantly increased the risk of a lower score in certain neuropsychological tests.

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Introduction

Thyroid hormones (THs) mediate the normal development of the brain, regulating neuronal cytoarchitecture and growth (1), myelinogenesis (2), synapse formation, and the production of neurotransmitter systems. These processes are highly sensitive to TH deficiency, and the damage caused by TH deprivation can be irreversible (2). The extent of damage depends on the magnitude and duration of the deficiency and the development period in which it occurs (3). The consequences of severe

hormone deficiency include a lower intelligence quotient (IQ) and impaired neuromotor, visuospatial, memory, verbal, and attention skills (4, 5, 6). A lesser degree of the same cognitive deficits have also been observed in cases of mild iodine deficiency (7, 8, 9, 10). Although the mechanisms by which THs affect neurological structures are not yet well known, some authors have proposed that they act predominantly on the hippocampus, prefrontal cortex, caudate nucleus, and auditory

pathways, affecting memory, attention, and language functions (4, 11).

Few studies have addressed the association between mild-to-moderate alterations of thyroid function in children and their subsequent neuropsychological functioning (6, 10), and there are almost no studies investigating the neurodevelopment of healthy children with TH or thyrotropin (TSH) levels that only slightly differ from the 'normal' range, except for two studies from the Spanish Environment and Childhood 'Infancia y Medio Ambiente (Childhood and Environment) (INMA)' mother-child cohort study (4, 8).

Our working hypothesis was that neurodevelopment is an ongoing process of acquiring skills and capabilities that are not consolidated until the age of 18 years (12), and that each period during childhood is associated with the development of specific abilities. The aim of this study was to investigate the association of TSH and free thyroxine (FT₄) levels with cognitive function in children from the INMA-Granada cohort at their follow-up at 9–11 years of age.

Materials and methods

Study population

The INMA Project is a multicentre Spanish population-based study of a mother-child cohort designed to assess the effect of prenatal environmental exposure and diet on growth, development, and health from early fetal life until adolescence (13). From 2000 to 2002, 668 eligible mother-son pairs registered at the San Cecilio University Hospital were enrolled after delivery, establishing the INMA-Granada cohort, which also served to assess the prevalence of urogenital malformations (cryptorchidism and hypospadias). The inclusion and exclusion criteria were published previously (14). The follow-up of the children at the age of 9–11 years (during the period of 2010–2012) included interviews with the parents, assessment of the growth and neuropsychological status of the children, and biological sample collections (blood and urine). All families in the INMA-Granada cohort were contacted and invited to participate. This study was approved by the Ethics Committee of San Cecilio University Hospital and signed informed consent was obtained from the participants' families.

Neuropsychological measures

Evaluation of neurodevelopment was assessed using a comprehensive neuropsychological battery of tests at the

Monitoring and Early Stimulation Unit of the hospital by a neuropsychologist trained to administer the tests and interpret scores for specific neuropsychological domains.

- i) General cognitive intelligence: assessed with the Kaufman Brief Intelligence Test (K-BIT) (15), in which the composite IQ is based on verbal and non-verbal scale scores.
- ii) Language: evaluated with the verbal scale of the K-BIT (15), which includes two subtests: i) verbal knowledge, which measures receptive vocabulary with a task in which the child names graphically displayed objects and ii) general knowledge, and riddles, which measures expressive reasoning with a task in which children must deduce words based on a definition of the word and some of the letters it contains.
- iii) Attention: assessed with the continuous performance test (CPT) (16), which measures sustained and selective attention and impulsivity with a task in which the child responds by pressing any key on the keyboard, as quickly as possible, each time the letter 'A' appears in yellow ('go' condition). The main dependent variables are: hits (press any key in 'go' condition), commission errors (press any key in 'no go' condition), omission errors (no key pressed in 'go' condition), and attention index.
- iv) Verbal memory: evaluated with the Complutense-Spain Madrid verbal learning test (TAVECI) (17), which assesses different memory and learning processes, including immediate recall, short- and long-term recall, and recognition. The test involves the examiner reading a list of 15 words five times, with the child stating the words recalled after each time and then after intervals of 10 and 20 min.
- v) Visual-motor coordination: assessed using part A of the trail making test (TMT) (18). The task involves connecting consecutive numbers in an alternating sequence as quickly as possible. The main dependent variable is time (s).
- vi) Processing speed: measured by the sum of the results of two subtests (symbol search and coding) from the Wechsler Intelligence Scales for Children-Quarter Edition (WISC-IV) (19). The first task is to identify, as quickly as possible, whether or not figures are included in a series of figures. In the second task, the child fills in spaces under numbers with corresponding symbols following an established model. Both tasks must be completed within a maximum of 2 min.
- vii) Executive function: divided into four components: updating, inhibition, shifting, and abstract reasoning (20).

- i) Updating measurements: with two components:
 - a) Working memory: assessed using the letter-number sequencing subtest from the WISC-IV (Spanish version), in which the child listens to and then repeats a mixed set of numbers and letters, first saying the numbers in order from the lowest to the highest and then the letters in alphabetic order (19) and b) verbal fluency: assessed using the categorical verbal fluency test (FAS), in which children are instructed to say as many names of animals as they know during 1 min. No grammatical variations or repetitions are counted. The number of animals correctly named is the dependent variable (21).
- ii) Inhibition: with two components: a) the Spanish children's version of the Stroop Color and Word Test (STROOP) (22), which measures cognition inhibition with a procedure in which the child is asked to name colored words (condition 1), read color-words printed in black ink (condition 2), and name the color of the words, which are printed in colors that conflict with their meaning, e.g. the word 'blue' appears in red ink (condition 3, inhibition). The dependent variable was the interference score, calculated from the results for the three conditions using a specific formula and b) the go/no-go task (23), which measures motor inhibition with a task in which the child responds to certain stimuli presented on the computer screen while inhibiting the response to distracter stimuli, with hit and false-alarm rates being the main dependent variables.
- iii) Shifting: measured by part B of the TMT (18). The task involves connecting consecutive numbers and letters in an alternating sequence as quickly as possible, with time (s) being the main dependent variable.
- iv) Abstract reasoning (matrix analogies test): measured with the non-verbal scale of the K-BIT (15), a multiple-choice test that presents stimuli in a matrix format. The child selects a picture or abstract design that best completes a visual pattern following a visual analogy. The dependent variable is the number of correct responses.

Thyroid function

Thyroid function was assessed by measuring the concentration of TSH and FT₄ in serum samples by

chemiluminescence assay (Eclcsys System, Roche Diagnostics). The limit of detection (LD) was 0.005 μ IU/ml for TSH and 0.023 ng/dl for FT₄. The reference range criteria proposed for children (6–11 years) by the Manual of Reference Intervals for children and Adults – <http://www.katrangilab.org/UploadFolder/Files/Thyroid%20Reference%20data%20Roche.pdf> – are 0.60–4.84 μ IU/ml for TSH and 0.97–1.67 ng/dl for FT₄. The pediatrician examined the thyroid of each child, finding no palpable gland and confirming the absence of a thyroid disorder in all cases.

Covariates

Information on parental socio-demographic characteristics, smoking during pregnancy, breastfeeding, and children's age, weight, and height at 9–11 years was gathered at the follow-up visit. The children's place of residence was classified as urban (city of Granada, 236 000 inhabitants), sub-urban (towns of > 20 000 inhabitants in city residential belt), or rural (<20 000 inhabitants). We estimated the general cognitive ability of parents by assessing their verbal reasoning ability using the Similarities subtest of the Weschler Adult Intelligence-Third Edition (WAIS-III) (24).

Three hundred families (44.9% participation rate) gave their written informed consent for this follow-up. Out of these 300 children, 18 were excluded because of the presence of chronic disease related to thyroid function and/or cognitive development, including diabetes ($n=1$), hyperthyroidism ($n=1$), attention deficit hyperactivity disorder (ADHD) ($n=7$), language disorder ($n=1$), Asperger syndrome ($n=2$), Noonan syndrome ($n=1$), Tourette syndrome with ADHD ($n=1$), Charcot-Marie-Tooth syndrome with ADHD ($n=1$), cerebral palsy ($n=1$), Spina Bifida ($n=1$), and brain tumor surgery ($n=1$). Only 187 of the 300 families (62.3%) agreed to the drawing of a blood sample from their child. Finally, data on thyroid hormone concentrations, neuropsychological tests, and covariates were available for 176 out of 300 (58.7%) subjects.

Statistical analysis

We performed a descriptive analysis of the characteristics of children and their parents. The relationship between quantitative variables was studied using the Spearman's correlation test, and the association between quantitative and categorical variables using the Mann-Whitney U test. The Kruskal-Wallis test, followed by Dunn's *post hoc* test, was also performed. The relationship between groups of categorical variables was explored using the χ^2 test.

Table 1 Characteristics of the study population (I).

	<i>n</i>	Mean	s.d.	Median	Min	Max
Child variables						
Age (years)	176	9.8	0.3	9.7	9.0	11.2
BMI (kg/m ²)	176	18.7	3.5	18.0	13	29
TSH (μIU/ml)	176	3.3	1.5	3.1	0.0	8.6
FT ₄ (ng/dl)	155	1.2	0.1	1.2	1.0	2.8
Maternal variables						
Age (years)	176	39.9	4.8	40.0	27	54
Verbal reasoning ^a	153	14.8	4.9	14.0	3	29
Paternal variables						
Age (years)	175	42.2	5.1	42.0	28	61
Verbal reasoning ^a	82	16.3	4.9	17.0	2	32

Min, minimum; Max, maximum. Parent's age at follow-up.

^aVerbal reasoning, measured by similarities subtest of WAIS-III.

The association of FT₄ and TSH hormone levels with neuropsychological test scores was studied using linear and logistic regression models. TSH and FT₄ levels were categorized in tertiles.

Neuropsychological test results were analyzed as continuous variables based on the raw scores, because standardized scores for the Spanish child population were not available for all tests. In addition, logistic regression models were constructed to estimate the risk of obtaining scores above the 80th percentile (test: TMTA and TMTB) or below the 20th percentile (other tests) as a function of hormone levels. These percentiles were selected to enhance the detection of low or subclinical performance, as proposed by Jacobson & Jacobson (25).

In the linear and logistic regression models, we calculated unadjusted and adjusted coefficients. Linear regression models included child's age (years), BMI (kg/m²), the mother's education (higher education/secondary/up to primary), and smoking during pregnancy. The logistic regression models included child's age and smoking during pregnancy.

The potential influence of boys with TSH and FT₄ levels outside the reference ranges (*n*=23, 13%) was assessed by performing a sensitivity analysis in which these children were excluded from the multivariable models.

The significance level was set at $P \leq 0.05$, following the recommendations of Rothman (26) for the evaluation of exposure–outcome relationships in the public health setting. They argue that application of the Bonferroni's correction in multiple comparisons increases the risk of missing new relationships of potential interest whose importance can be tested more reliably by replication. Data analyses were performed using SPSS v20.0 (IBM) and R statistical computing environment v3.0.0 (<http://www.r-project.org/>).

Results

Tables 1 and 2 show the characteristics of the study population. At the follow-up, the median age of children was 9.7 years and their median BMI was 18.0 kg/m², the median age was 40 years for mothers and 42 years for fathers, 22% of mothers and 19% of fathers had higher education, 18% of participating families lived in urban areas, 21% of mothers reported smoking during pregnancy, and 83% had breastfed the children. Median serum TSH and FT₄ levels in the children were 3.1 μIU/ml and 1.2 ng/dl respectively. A negative correlation was observed between TSH and FT₄ levels, although statistical significance was not reached ($r_s = -0.113$, $P = 0.163$). The FT₄

Table 2 Characteristics of the study population (II).

	<i>n</i>	%
Area of residence		
Urban	31	17.6
Semi-urban	114	64.8
Rural	31	17.6
Breastfeeding		
Yes	146	83.0
No	30	17.0
Smoking during pregnancy ^a		
Yes	36	20.5
No	140	79.5
Maternal education		
University	38	21.6
Secondary school	58	33.0
Up to primary	80	45.5
Paternal education		
University	33	18.8
Secondary school	58	33.0
Up to primary	85	48.3

^aBy mothers.

value was above the upper limit of the reference range (1.67 ng/dl) in one child. The TSH value was above the upper reference range limit (4.84 μ IU/ml) in 20 children (20/176, 11%), and the highest value was 8.60 μ IU/ml in one child and the TSH value was below the lower limit of the reference range (0.60 μ IU/ml) in two children (2/176, 1.1%). None of these children had a diagnosis of thyroid disorder, despite having thyroid hormone levels outside the reference range.

No differences were found in the majority of cognitive functions and paternal characteristics between the children with and without hormone measurements (176 vs 124 subjects). However, differences were found in: short-term recall, measured with the TAVECI test (median (P25, P75) of 11.0 (10.0, 13.0) vs 12.0 (10.0, 13.0), $P=0.044$ respectively); father's verbal reasoning (16.5 (13.0, 19.0) vs 14.0 (10.0, 18.0), $P=0.03$ respectively); and area of residence (urban (17.6% vs 35.2% respectively, $P=0.001$)

and semi-urban (64.8% vs 45.1% respectively, $P=0.001$)). As reported above, TSH or FT₄ levels were outside reference range limits in 23 children (13%).

Bivariate analyses showed that TSH concentrations were 0.78 μ IU/ml higher in children whose mothers smoked during pregnancy than in children of those who did not (95% CI=0.22, 1.33; $P=0.006$) and that FT₄ concentrations were higher in children of mothers with higher cognitive ability, with an increase of 0.007 ng/dl for each additional point on the mother's intelligence test score (95% CI=0.001, 0.014; $P=0.026$). Remaining parent and child variables were not significantly associated with TSH or FT₄ parameters.

Table 3 exhibits the raw cognitive function scores, indicating the main parameters in each test. The mean (s.d., range) raw IQ score of the children was 219.2 (22, 121–276) points and their standardized IQ was 109.0 (12.6, 52–141) points.

Table 3 Children's scores in neuropsychological tests.

Cognitive function ^a	Test	n	Mean	Median	s.d.	Range
General cognitive	K-BIT (IQ) ^b	176	219.2	220.0	22.0	121 to 276
Verbal expression and comprehension	K-BIT					
	Full verbal ^b	176	51.5	52.0	5.0	36 to 64
Sustained attention	CPT					
	Correct detection ^b	171	63.4	64.0	5.4	43 to 70
	Commission errors ^c	171	9.7	7.0	11.9	0 to 110
	Omissions ^c	171	6.5	6.0	5.3	0 to 27
	Attention Index ^b	171	0.6	0.6	0.2	–0.4 to 0.9
Verbal memory	TAVEC					
	Immediate Recall Trial 1 ^b	176	7.0	7.0	1.7	4 to 11
	Immediate Recall Trial 5 ^b	176	12.0	12.0	1.9	7 to 15
	Learning curve ^b	176	1.2	1.3	0.5	–1 to 3.4
	Short-term recall ^b	176	11.2	11.0	2.3	4 to 15
	Long-term recall ^b	176	11.6	12.0	2.4	4 to 15
	Hits in recognition ^b	176	14.5	15.0	0.9	9 to 15
Visual-motor coordination	TMT-A time (s) ^c	175	28.7	26.0	10.9	9 to 86
Processing speed	WISC-IV					
	VP ^b	175	97.6	99.0	11.1	64 to 119
Executive functions						
Working memory	WISC-IV					
	Letter-number sequencing ^b	176	17.4	17.0	2.8	10 to 25
Verbal fluency	FAS ^b	176	17.1	17.0	4.2	7 to 27
Impulsivity/inhibition	STROOP					
	Words ^b	174	113.6	114.0	10.3	86 to 141
	Colors ^b	174	79.0	79.0	8.4	55 to 98
	Words and Colors ^b	174	48.6	48.5	7.0	30 to 70
	Interference ^b	174	2.4	2.2	5.2	–12.1 to 15.5
	GO NO GO					
	Hit rate ^b	165	0.9	1.0	0.0	0.5 to 1.0
	False-alarm rate ^c	166	0.0	0.0	0.0	0.0 to 0.2
Shifting	TMT-B time (s) ^c	175	61.0	52.0	35.2	23 to 360
Abstract reasoning	Matrix analogies ^b	176	31.1	31.0	4.7	18 to 44

^aFor all tests, direct scores were used in the analysis.

^bHigher score indicates better cognitive function.

^cHigher score indicates worse cognitive function.

Table 4 Association between TSH levels ($\mu\text{IU/ml}$) and cognitive development in children from INMA-Granada cohort ($n=176$). β , linear regression coefficient; S.E.M. (reference group: 2nd TSH tertile (2.54–3.77 $\mu\text{IU/ml}$). Significant values ($P<0.05$) are in bold.

Cognitive functions ^a	Crude model						Adjusted model					
	TSH tertiles ($\mu\text{IU/ml}$)						TSH tertiles ($\mu\text{IU/ml}$)					
	1st (0.05–2.53)			3rd (3.78–8.60)			1st (0.05–2.53)			3rd (3.78–8.60)		
	β	S.E.M.	P	β	S.E.M.	P	β	S.E.M.	P	β	S.E.M.	P
Intelligence quotient ^b	–3.85	4.10	0.349	–3.81	4.04	0.348	–4.63	3.79	0.224	–4.82	3.69	0.193
Verbal comprehension ^b	–0.52	0.64	0.412	–1.11	0.63	0.080	–0.51	0.62	0.408	–1.34	0.60	0.027
Attention												
Impulsivity ^c	2.03	2.25	0.369	0.76	2.23	0.734	1.65	2.32	0.479	0.77	2.27	0.734
Attention index ^b	–0.01	0.04	0.776	0.00	0.04	0.919	–0.01	0.04	0.789	0.00	0.04	0.993
Verbal memory ^b												
Immediate recall ^d	–0.64	0.36	0.078	–0.74	0.36	0.042	–0.62	0.36	0.090	–0.80	0.35	0.026
Long-term recall	–0.93	0.44	0.037	–0.82	0.44	0.065	–1.03	0.45	0.023	–0.96	0.43	0.030
Visual-motor coordination ^c	–3.45	2.04	0.093	–0.55	2.00	0.782	–2.83	2.06	0.171	–0.11	1.99	0.956
Processing speed ^b	0.47	2.08	0.821	–1.09	2.05	0.593	–0.11	2.12	0.957	–1.33	2.06	0.518
Executive functions												
Working memory ^b	0.08	0.53	0.867	–0.40	0.52	0.436	–0.13	0.52	0.805	–0.54	0.51	0.286
Verbal fluency ^b	0.43	0.79	0.586	–1.22	0.78	0.119	0.45	0.80	0.566	–1.30	0.77	0.097
Impulsivity/inhibition												
Interference ^b	–0.34	0.99	0.726	–0.14	0.97	0.886	–0.20	1.02	0.839	–0.17	0.99	0.863
Hit rate ^b	0.01	0.00	0.158	0.01	0.00	0.229	0.01	0.00	0.163	0.01	0.00	0.252
False-alarm rate ^c	–0.00	0.01	0.800	–0.01	0.01	0.316	–0.00	0.01	0.842	–0.01	0.01	0.278
Shifting ^c	–2.73	6.61	0.680	1.42	6.50	0.827	–2.70	6.75	0.689	2.06	6.55	0.753
Abstract reasoning ^b	–0.33	0.88	0.707	–0.62	0.87	0.476	–0.28	0.82	0.733	–0.75	0.80	0.352

^aWe show the most relevant abilities measured. For all tests, direct scores were used in the analysis.

^bA higher score indicates better cognitive function.

^cA higher score indicates worse cognitive function.

^dImmediate Recall Trial 5 (TAVECI). Linear regression model adjusted for age of children, BMI (kg/m^2), smoking during pregnancy, and maternal schooling.

In the model that included all of the boys, the population was categorized in tertiles of TSH levels (1st (0.05–2.53 $\mu\text{IU/ml}$); 2nd (2.54–3.77 $\mu\text{IU/ml}$); and 3rd (3.78–8.60 $\mu\text{IU/ml}$)) and FT_4 levels (1st (1.00–1.21 ng/dl); 2nd (1.22–1.32 ng/dl); and 3rd (1.33–2.84 ng/dl)), taking the 2nd tertile as a reference in all cases, and the association between TSH levels and cognitive development was investigated (Supplementary Table 3, see section on supplementary data given at the end of this article). This relationship was examined by constructing multi-variable linear regression models (Table 4). Unadjusted analysis showed negative associations between 3rd tertile of TSH levels and neuropsychological test scores in comparison with reference values (2nd tertile), which were statistically significant for immediate verbal recall ($P=0.04$). Negative associations were also found between 1st tertile of TSH levels and neuropsychological test scores in comparison with reference values (2nd tertile), and the association was statistically significant for long-term verbal recall ($P=0.04$) (Fig. 1). In the adjusted multi-variable model, these associations remained significant and, additionally, the 3rd tertile of TSH levels was found to be significantly associated with long-term verbal recall

Figure 1

Association of TSH hormone levels with cognitive function using the long-term recall (raw score), verbal comprehension (raw score), and IQ (typical score).

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and with verbal comprehension (Table 4). No relevant changes in the β coefficients were found when the analyses were performed only in the group with hormone values within reference ranges ($n=153$, 87%) in comparison with the model with the whole study population ($n=176$) (Supplementary Tables 1 and 2, see section on supplementary data given at the end of this article). The long-term verbal recall ability was not significant for the third tertile in the smaller sample ($n=153$) for the adjusted model (Supplementary Table 2).

We also explored the association between TSH levels and cognitive development by multivariable logistic regression analysis, but obtained no significant results (data not shown).

The relationship between FT₄ levels and cognitive development was investigated (Supplementary Table 4, see section on supplementary data given at the end of this article). This association was examined using multivariable linear regression models (Table 5). Unadjusted analysis indicated that the IQ score, verbal expression/comprehension, attention index, and verbal fluency abilities were positively and significantly associated with

the 3rd tertile of FT₄ in comparison with the reference 2nd tertile, being a negative association for impulsivity ability (Fig. 2). When the model was adjusted for child age and BMI, maternal educational level, and smoking during pregnancy, the associations with impulsivity and attention index remained statistically significant (Table 5). No relevant changes in the regression coefficients were found when the multivariable analyses were repeated excluding the boys with FT₄ values outside the reference range (data not shown).

Multivariable logistic regression models for FT₄ levels and cognitive development revealed that children with FT₄ levels in the 3rd tertile had a lower risk of a score <P20 in the verbal expression and comprehension test (OR=0.36; 95% CI=0.14, 0.94; $P=0.038$), and children with FT₄ levels in the 3rd tertile had a lower risk of a score >P80 in the motor inhibition test (OR=0.32; 95% CI=0.11, 0.92; $P=0.036$) vs those with levels in the 2nd tertile. After adjusting for child age and smoking during pregnancy, children with FT₄ levels in the 3rd tertile had a lower risk of a score <P20 in IQ (OR=0.24; 95% CI=0.08, 0.74; $P=0.013$) and in the abstract reasoning ability (OR=0.28;

Table 5 Association between FT₄ levels (ng/dl) and cognitive development in children from INMA-Granada cohort ($n=176$). β , linear regression coefficient; s.e.m. (reference group: 2nd FT₄ tertile (1.22–1.32 ng/dl). Linear regression model adjusted for age of children, BMI (kg/m²), maternal schooling, and smoking during pregnancy. Significant values ($P<0.05$) are in bold.

	Crude model						Adjusted model					
	FT ₄ tertiles (ng/dl)						FT ₄ tertiles (ng/dl)					
	1st (1.00–1.21)			3rd (1.33–2.84)			1st (1.00–1.21)			3rd (1.33–2.84)		
	β	s.e.m.	P	β	s.e.m.	P	β	s.e.m.	P	β	s.e.m.	P
Cognitive functions^a												
Intelligence quotient (IQ) ^b	7.76	4.44	0.083	9.12	4.37	0.039	5.15	4.07	0.208	6.06	4.09	0.141
Verbal expression and comprehension ^b	1.52	1.01	0.136	2.26	1.00	0.026	0.87	0.97	0.374	1.46	0.98	0.140
Attention												
Impulsivity ^c	–4.16	2.47	0.094	–5.78	2.46	0.020	–3.75	2.54	0.142	–5.38	2.58	0.039
Attention Index ^b	0.07	0.04	0.100	0.121	0.04	0.012	0.07	0.04	0.135	0.10	0.05	0.037
Verbal memory ^b												
Short Term Recall	–0.21	0.47	0.657	0.26	0.46	0.579	–0.31	0.47	0.512	0.34	0.47	0.474
Long Term Recall	–0.35	0.49	0.481	–0.07	0.48	0.886	–0.45	0.49	0.366	–0.10	0.50	0.835
Visual-motor coordination ^c	1.76	2.22	0.429	0.15	2.19	0.946	2.30	2.20	0.298	1.00	2.23	0.653
Processing speed ^b	–0.90	2.26	0.691	–1.15	2.24	0.608	–1.25	2.30	0.587	–2.03	2.32	0.382
Executive functions												
Working memory ^b	0.41	0.58	0.482	–0.09	0.57	0.863	0.34	0.57	0.550	–0.27	0.57	0.639
Verbal fluency ^b	0.58	0.83	0.481	1.88	0.82	0.023	0.44	0.85	0.606	1.56	0.85	0.069
Impulsivity/inhibition												
Interference ^b	1.28	1.05	0.227	0.89	1.05	0.396	1.10	1.08	0.309	0.73	1.10	0.505
Hit rate ^b	0.00	0.01	0.550	0.00	0.01	0.795	0.00	0.01	0.547	–0.00	0.01	0.770
False-alarm rate ^c	–0.00	0.01	0.629	–0.01	0.01	0.350	–0.00	0.01	0.541	–0.01	0.01	0.318
Shifting ^c	–5.95	7.30	0.416	–3.03	7.23	0.675	–3.78	7.41	0.610	1.12	7.50	0.881
Abstract reasoning ^b	1.45	0.93	0.124	1.69	0.92	0.069	0.80	0.86	0.354	0.88	0.87	0.311

^aWe show the most relevant abilities measured. For all tests, direct scores were used in the analysis.

^bA higher score indicates better cognitive function.

^cA higher score indicates worse cognitive function.

Figure 2

Association of FT₄ hormone levels (in tertiles) with cognitive function using the impulsivity (raw score), attention index (raw score), verbal expression and comprehension (raw score), and IQ (typical score). A higher score indicates better cognitive function, except for impulsivity variable, in which higher score indicates worse cognitive function.

95% CI=0.09, 0.88; $P=0.029$) vs those with levels in the 2nd tertile (data not shown).

Discussion

The present results in a group of healthy 10-year-old boys indicate that small deviations of TSH and FT₄ levels from the accepted normal reference ranges may play a role in the modulation of cognitive functions. Thus, TSH levels around the upper reference range limit were negatively associated with verbal comprehension and immediate and long-term recall, while FT₄ levels around the upper limit were positively associated with attention and negatively associated with impulsivity. In addition, boys with FT₄ levels in the upper tertile had a lower risk of poor IQ scores and poor abstract reasoning ability in comparison with children with FT₄ levels in the 2nd tertile.

The differences in cognitive abilities between TSH and FT₄ tertiles, although statistically significant, were small, which was expected given the non-pathological status of our study population in relation to both thyroid and cognitive functions. However, our aim was to establish the ranges of hormone (TSH and FT₄) values associated with an increase in the risk of having fewer scores in

the neuropsychological test and to identify the specific capacities affected for a closer follow-up of these boys. Moreover, we were able to identify children who tended to show lower scores in cognitive abilities and distinguish between 'normal' and 'low or subclinical' responses (25).

TH is known to be important in neurodevelopment, especially during periods in which the CNS is developing and maturing (2, 27, 28). Our findings indicate that there may be a relatively narrow interval of optimal TH concentrations that should be identified in different populations in order to develop strategies for preventing associated adverse health effects, especially those related to neurofunctioning. It appears that the 'normal' reference ranges for TH levels may not be adequate to detect children at risk of low cognitive development.

Many authors have claimed that TH reference ranges are not the right tools to establish euthyroidism or dysthyroidism in individuals, indicating that each individual may have a unique set point for hypothalamic–pituitary–thyroid axis function (29). Furthermore, the traditional methodology for determining TSH reference limits does not reflect possible differences in TSH distribution among groups according to environmental influences or their ethnic/genetic composition (30). In fact, the reference range of TSH concentrations is controversial, especially in relation to therapeutic decision making (31), with some authors recommending a reduction in the upper limit to 2.5 μ IU/ml (32, 33, 34) and others associating serum levels of 5–10 μ IU/ml with an increased risk of progression to overt hypothyroidism (35).

Our observations are consistent with previous results in the same mother–child cohort (INMA-Granada). For instance, a cross-sectional study nested in the same cohort showed lower general cognitive and quantitative capacities and executive function in 4-year-old boys with higher neonatal TSH levels (4.19–17.0 μ IU/ml) (8). In addition, 4-year-old boys and girls in the INMA cohort with neonatal TSH concentrations in the range of 2.43–5.01 μ IU/ml evidenced impaired memory and verbal skills and higher attention deficit and hyperactivity/impulsivity symptoms in comparison with those with lower TSH concentrations (4). In the same study, children with higher FT₄ levels had a decreased risk of poor attention scores, in agreement with the present observations (4).

Very few studies have investigated the association between THs and neuropsychological functioning in healthy pre-pubertal children from the general population, and most of them have focused on the consequences of maternal thyroid dysfunction (1, 2, 7, 27, 28). Other authors have studied specific subpopulations

with overt thyroid diseases or in high-risk environments, including children with congenital hypothyroidism (6), neurological disorders, or thyroid hormone resistance syndrome or those living in iodine-deficient areas (2, 4, 8).

Numerous environmental agents can produce marginal reductions of circulating THs. Although it is well documented that severe developmental hypothyroidism is profoundly detrimental to neurodevelopment, less information is available on the effects of modest TH deficits or on the impact of low-level TH disruptions induced by environmental contaminants, including endocrine-disrupting chemicals (EDCs) (36). There is increasing epidemiological evidence on the influence of environmental exposure to low doses of EDCs in some human diseases and disabilities (36, 37).

Our research group has previously reported that the functioning of TSH and FT₄ may be affected by factors such as exposure to EDCs (38), and our results may indicate a non-monotonic response between these hormones and cognitive functions. Thus, poor child cognitive function was significantly associated with higher TSH levels but not with intermediate levels, following an inverse U-shaped curve. The relationship between higher FT₄ levels and better neuropsychological scores showed a similar but inverted pattern. This U-shaped dose–response curve has also been observed for the relationship between iodine intake and thyroid disorders (39), finding an increased risk of thyroid disease in individuals with iodine intake below or above the recommended range. Non-monotonic responses and low-dose effects are remarkably common in studies of natural hormones and some EDCs (37).

The neurodevelopment of children has been associated with maternal higher education (40), the duration of breastfeeding, and various maternal lifestyle factors during pregnancy, including their diet (especially fish intake) and tobacco habit (41, 42). Thus, smoking during pregnancy has been related to changes in maternal and fetal thyroid function (43) and to reduced iodine concentrations in breast milk (44). In this study, TSH concentrations were higher in the offspring of mothers who smoked during pregnancy than in the children of non-smoking mothers ($P=0.006$).

Obesity is another important factor associated with the neurodevelopment of children (45), and TSH levels are consistently found to be higher in obese children than normal-weight children (46). However, no significant relationship was found between TSH levels and BMI in this study ($r_s=0.123$, $P=0.105$). One recent review has reported that 10–23% of obese children have moderately elevated TSH levels (range of 4–10 $\mu\text{IU/ml}$) and normal or

slightly elevated FT₄ levels. The fact that weight loss is associated with the normalization of TSH may suggest that hyperthyrotropinemia is a consequence rather than a cause of obesity (46).

Our study has several limitations. The subtle effects on cognitive abilities observed may have been mediated by other socio-cultural, economic, or genetic variables that were not controlled in this study, e.g. birth weight, dietary intake of iodine, or exposure to environmental contaminants. In addition, no data were available on other hormone biomarkers such as tri-iodothyronine (T₃), anti-thyroid peroxidase antibodies, and/or thyroglobulin antibody, and our population only included boys. Finally, the cross-sectional design of the study prevented evaluation of the variability of hormonal values over time, which is an important issue (32).

The strengths of our study include its novelty, given the absence of published research on these relationships in healthy children. Moreover, the sample size was relatively large, the children were followed over a 10-year interval, and data were available for multiple covariates since their birth. Finally, it contributes the results of a comprehensive neuropsychological test battery at the age of 9–11 years, a time window that allows a wide range of cognitive functions to be examined with sensitive and specific tests.

In summary, this study revealed that scores for some cognitive functions in healthy 10-year-old boys were reduced in those with higher TSH levels, and scores for some other cognitive functions were increased in those with higher FT₄ levels. These findings suggest that circulating TH values at the limit of reference ranges may have an impact on cognitive functions, slightly but significantly increasing the risk of a lower score in certain neuropsychological tests. It is also important to determine whether these effects on cognitive functions persist over the longer term and have an adverse impact on educational performance.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EJE-14-0093>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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