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Supplementary appendix

Methods

In a subset of 2397 children cord blood TSH and FT4 were available, and in 2460 children TSH and FT4 levels were available at time of IQ testing. A comparison between the children who had cord blood, or late thyroid function available and those who had missing data showed no differences between maternal TSH, FT4 or TPOAb positivity. However, there was a difference in child IQ between the groups (mean increase of 1.3 and 1.0 points for cord blood and late thyroid function, respectively). Children with an IQ ≤ 50 were excluded because the IQ test outcome does not go below 50 and these children scored poorly on motivation, concentration, collaboration and/or understanding of instructions.

Serum measurements

Between April 2002 and January 2006, serum samples were obtained from 6398 women during early pregnancy. Due to measurement error (laboratory) , TSH, FT4 or TPOAb were obtained in 6065 mothers. The intra- and interassay coefficients of variation were <4.1% for TSH at a range of 3.97-22.7 mU/L and <5.4% for FT4 at a range of 14.3-25.0 pmol/L (for Vitros ECI; Ortho Clinical Diagnostics, Rochester, NY). Maternal TPOAbs were measured using the Phadia 250 immunoassay (Phadia AB, Uppsala, Sweden) and considered positive when >60 IU/ml. Maternal total human chorionic gonadotropin (hCG) levels (same sample as thyroid function) were analyzed in serum using an Immulite XPi system (Siemens Healthcare Diagnostics, Deerfield, IL, USA), details of which have been described previously.[1] Child TSH and FT4 levels at time of IQ measurement were determined using an electrochemiluminescence immunoassay on the Cobas e601immunoanayzer (Roche Diagnostics, Germany). The intra- and interassay coefficients of variation were 1.1 – 3.0 % for TSH at a range of 0.4 – 0.04 mU/L and 1.6 – 5.0 % for FT4 at a range of 1.6 -24.1 pmol/L. Corresponding maternal FT4 MoM values [2] for the 1^{st} – 10^{th} percentile were 0.64, 0.67, 0.70, 0.73, 0.74, 0.75, 0.76, 0.77, 0.79 and 0.80 respectively. For the 90^{th} – 99^{th} percentile MoM values were 1,28, 1.31, 1.33, 1.35, 1.38, 1.40, 1.45, 1.49, 1.56 and 1.70, respectively.

Determinants and covariates

Gestational age at blood sampling was defined using fetal ultrasound data on crown-rump length or biparietal diameter for pregnancy dating.[3] Information on maternal age, smoking status, education level and ethnicity was obtained by questionnaires during pregnancy. Ethnicity was determined by country of origin and was defined according to the classification of Statistics Netherlands.[4] Maternal ethnicity was categorized according to the major ethnic groups in Rotterdam, the Netherlands (Dutch, Moroccan, Turkish, Surinamese, Indonesian, Cape Verdian or Antillean) and the remaining women were grouped into Asians, other Western or other non-Western. Maternal smoking status was classified as no smoking, smoking until known pregnancy, and continued smoking during pregnancy. Maternal education level consisted of five categories: no education finished/primary school, secondary phase one, secondary phase two, higher education phase one, higher education phase two. Weight and length were measured at intake and were used to calculate body mass index (BMI). Information on fertility treatment, delivery, pregnancy outcome, date of birth, birth anthropometrics, and the gender of the child were obtained from community midwives, obstetricians, and hospital registries. Medical and obstetrical history were assessed by questionnaires and answers were crosschecked by certified medical doctors. Information about initiation of breastfeeding was collected from delivery reports; data on continuation of breastfeeding was obtained from postal maternal self-report questionnaires at 2, 6 and 12 months post partum, details have been described elsewhere.[5] Child behavior checklist/1½–5 (CBCL/1½–5) was used to obtain a standardized parental rating of child's emotional and behavioral problems. The CBCL/1½–5 contains 99 problem items, scored on seven syndromes that were derived by factor analyses: Emotionally Reactive, Anxious/Depressed, Somatic Complaints, Withdrawn, Sleep Problems, Attention Problems, and Aggressive Behavior.[6]

MRI data

Exclusion of women with fertility treatment (N=4) did not change the results for total gray matter volume or cortex volume. An overview of the neuroimaging component, including participant selection, has been described elsewhere.[7] An inversion recovery fast spoiled gradient recalled sequence was performed 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, matrix 256 × 256, ASSET factor of 2, and an isotropic resolution of 0.9 × 0.9 × 0.9 mm^3. Before scanning took place, children were familiarized with the scanning environment during a mock scanning session. Volume outcomes are given in cm^3.

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). Research staff that performed MRI ratings are unaware of any test outcomes, including maternal thyroid function during pregnancy. The next step of quality assurance took place after the images were processed through the FreeSurfer pipeline[8] (http://surfer.nmr.mgh.harvard.edu/) and consisted of a visual inspection of the segmentation quality and surface reconstruction of the data. All images were rated on a six-point scale (from poor 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. All outcomes were measured using the FreeSurfer software. The corpus callosum was also automatically segmented and quantified into a volume by summing its voxels in the 5 midline slices, additional quality checks were made by two independent staff members to be certain of data validity for the corpus callosum measurements but an additional stringent quality assessment did not change the results (data not shown).

Image processing

Cortical reconstruction and volumetric segmentation were performed with the FreeSurfer image analysis suite, which has been described in detail elsewhere.[8] Cortical gray matter volume is extracted from the surface reconstructions of both the left and right hemispheres. Total gray matter volume consists of the surface-based measures of cortical gray matter volume described above, plus the volume-based measures of all subcortical structures and cerebellar gray matter. FreeSurfer morphometric procedures have been demonstrated to show good test-retest reliability across scanner manufacturers and across field strengths.[9]

Novel potential confounders

All analyses were additionally adjusted for hCG, TPOAbs and child thyroid function based on the physiologic background. hCG plays an important role in fetal and placental development and may thus affect brain development, while it is also a well-known stimulator of maternal thyroid function during pregnancy. A few small studies reported an association between maternal thyroperoxidase antibody (TPOAb) status and child neurocognitive development but it is unknown if these effects are replicable and whether this is due to an effect on thyroid function or due to the autoimmunity itself.[10-12] Third, maternal hypothyroxinemia may be associated with lower TH levels during childhood and the chronic exposure to subnormal TH levels may influence postnatal brain development and subsequently child IQ levels.[13]

Possible confounding effects of hCG, TPOAbs or child thyroid function were analyzed by additionally adjusting for hCG levels, TPOAbs (continuous or positivity) or child TSH or FT4 levels (at birth and at time of IQ measurement). Additionally, we investigated possible mediation or confounding by child head size (at birth and at IQ measurement), placental factors (including placental blood flow and placental angiogenic factors) and blood flow in the fetal brain (at approximately 25 weeks) for all significant associations but this did not change the results.

Statistical analyses

Figures show back-transformed and/or adjusted axis values (covariates set to mean levels or most appearing category). To investigate if the trend between maternal TSH and mean IQ levels is an independent effect, maternal TSH and FT4 levels were added to the model. We investigated possible differences in gestational time period by adding a product interaction term for TSH or FT4 and gestational age at blood sampling to the model. Cut-off levels for maternal FT4 were investigated using multivariate ANOVA, with $<90^{th}$ percentile or $>10^{th}$ percentile as reference group and cut-off values increasing or decreasing per percentile. For all analyses, model fit and assumptions were assessed by plotting model residuals, evaluating (adjusted) R-squared and/or the le Cessie - van Houwelingen - Copas - Hosmer unweighted sum of squares test. *P*-values shown in figures 2, 3, supplement 1, 3 and 4 test the *null*

hypothesis that the mean population IQ is similar for every level of TSH/FT4. For variables with missing data, multiple imputation according to the Markov Chain Monte Carlo method was used (all but TPOAb levels (missing N=243), due to large differences per imputation), five databases were pooled for analyses.

1. Korevaar TI, Steegers EA, de Rijke YB, Schalekamp-Timmermans S, Visser WE, Hofman A, Jaddoe VW, Tiemeier H, Visser TJ, Medici M, Peeters RP. Reference ranges and determinants of total hCG levels during pregnancy: the Generation R Study. *European journal of epidemiology* 2015.
2. Bestwick JP, John R, Maina A, Guaraldo V, Joomun M, Wald NJ, Lazarus JH. Thyroid stimulating hormone and free thyroxine in pregnancy: expressing concentrations as multiples of the median (MoMs). *Clin Chim Acta* 2014;**430:**33-7.
3. Verburg BO, Steegers EA, De Ridder M, Snijders RJ, Smith E, Hofman A, Moll HA, Jaddoe VW, Witteman JC. New charts for ultrasound dating of pregnancy and assessment of fetal growth: longitudinal data from a population-based cohort study. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology* 2008;**31:**388-96.
4. Jaddoe VW, van Duijn CM, Franco OH, van der Heijden AJ, van Iizendoorn MH, de Jongste JC, van der Lugt A, Mackenbach JP, Moll HA, Raat H, Rivadeneira F, Steegers EA, Tiemeier H, Uitterlinden AG, Verhulst FC, Hofman A. The Generation R Study: design and cohort update 2012. *European journal of epidemiology* 2012;**27:**739-56.
5. van Rossem L, Oenema A, Steegers EA, Moll HA, Jaddoe VW, Hofman A, Mackenbach JP, Raat H. Are starting and continuing breastfeeding related to educational background? The generation R study. *Pediatrics* 2009;**123:**e1017-27.
6. Achenbach TM, Rescorla L. *ASEBA school-age forms & profiles*: Aseba Burlington; 2001.
7. White T, El Marroun H, Nijs I, Schmidt M, van der Lugt A, Wielopolki PA, Jaddoe VW, Hofman A, Krestin GP, Tiemeier H, Verhulst FC. Pediatric population-based neuroimaging and the Generation R Study: the intersection of developmental neuroscience and epidemiology. *European journal of epidemiology* 2013;**28:**99-111.
8. Fischl B. FreeSurfer. *Neuroimage* 2012;**62:**774-81.
9. Han X, Jovicich J, Salat D, van der Kouwe A, Quinn B, Czanner S, Busa E, Pacheco J, Albert M, Killiany R, Maguire P, Rosas D, Makris N, Dale A, Dickerson B, Fischl B. Reliability of MRI-derived measurements of human cerebral cortical thickness: the effects of field strength, scanner upgrade and manufacturer. *Neuroimage* 2006;**32:**180-94.
10. Li Y, Shan Z, Teng W, Yu X, Li Y, Fan C, Teng X, Guo R, Wang H, Li J, Chen Y, Wang W, Chawinga M, Zhang L, Yang L, Zhao Y, Hua T. Abnormalities of maternal thyroid function during pregnancy affect neuropsychological development of their children at 25-30 months. *Clin Endocrinol* 2010;**72:**825-9.
11. Wasserman EE, Pillion JP, Duggan A, Nelson K, Rohde C, Seaberg EC, Talor MV, Yolken RH, Rose NR. Childhood IQ, hearing loss, and maternal thyroid autoimmunity in the Baltimore Collaborative Perinatal Project. *Pediatr Res* 2012;**72:**525-30.
12. Pop VJ, de Vries E, van Baar AL, Waelkens JJ, de Rooy HA, Horsten M, Donkers MM, Komproe IH, van Son MM, Vader HL. Maternal thyroid peroxidase antibodies during pregnancy: a marker of impaired child development? *J Clin Endocrinol Metab* 1995;**80:**3561-6.
13. Pakkila F, Mannisto T, Surcel HM, Ruokonen A, Bloigu A, Pouta A, Hartikainen AL, Vaarasmaki M, Jarvelin MR, Suvanto E. Maternal thyroid dysfunction during pregnancy and thyroid function of her child in adolescence. *J Clin Endocrinol Metab* 2013;**98:**965-72.

Supplemental Table 1 Descriptive statistics of women with gestational FT4 levels >95th percentile.

		>95th percentile		>5th and <95th percentile	
		Median	**(95% range)**	**Median**	**(95% range)**
TSH	(mU/L)	0.60	(0.00 – 2.81)	1.39	(0.16-4.46)
FT4	(pmol/L)	22.5	(20.8 – 41.8)	14.9	(11.3-19.8)
hCG	(IU/L)	62,052	(16,902 – 142,862)	44,247	(12,384-103,252)
Gestational age[a]		12.5	(9.1 – 16.8)	13.2	(9.8-17.4)
Maternal age[d]		30.4	(19.8 - 39.5)	30.8	(20.1-39.1)
BMI		23.5	(17.5 - 33.2)	23.5	(18.8-35.5)
Parity[c]					
0		118	(59.3)	2064	(59.7)
1		64	(32.2)	1012	(29.3)
2		14	(7.0)	283	(8.2)
>2		3	(1.5)	98	(2.8)
Smoking[c,e]					
Non-smokers		151	(75.9)	2593	(75.0)
Stopped smokers		22	(11.1)	346	(10.0)
Smokers		26	(13.1)	518	(15.0)
Education level[c,e]					
None/Primary		292	(7.6)	261	(7.5)
Secondary phase 1		487	(12.7)	443	(12.8)
Secondary phase 2		1203	(31.3)	1072	(31.0)
Higher phase 2		884	(23.0)	798	(23.1)
Higher phase 1		974	(25.3)	883	(25.5)
Ethnicity[c,e]					
Dutch		98	(49.2)	1988	(57.5)
Moroccan		11	(5.5)	178	(5.1)
Turkish		9	(4.5)	242	(7.0)
Surinamese		26	(13.1)	253	(7.3)
Cape Verdian		7	(3.5)	136	(3.9)
Dutch Antilles		4	(2.0)	64	(1.9)
Indonesian		4	(2.0)	107	(3.1)
Asian		5	(2.5)	76	(2.2)
Other western		24	(12.1)	290	(8.4)
Other non-western		11	(5.5)	123	(3.6)
Birth weight (g)		3340	(2331 – 4489)	3455	(2240-4450)
Child gender[c] (boys %)		79	(40.1)	1709	(49.4)

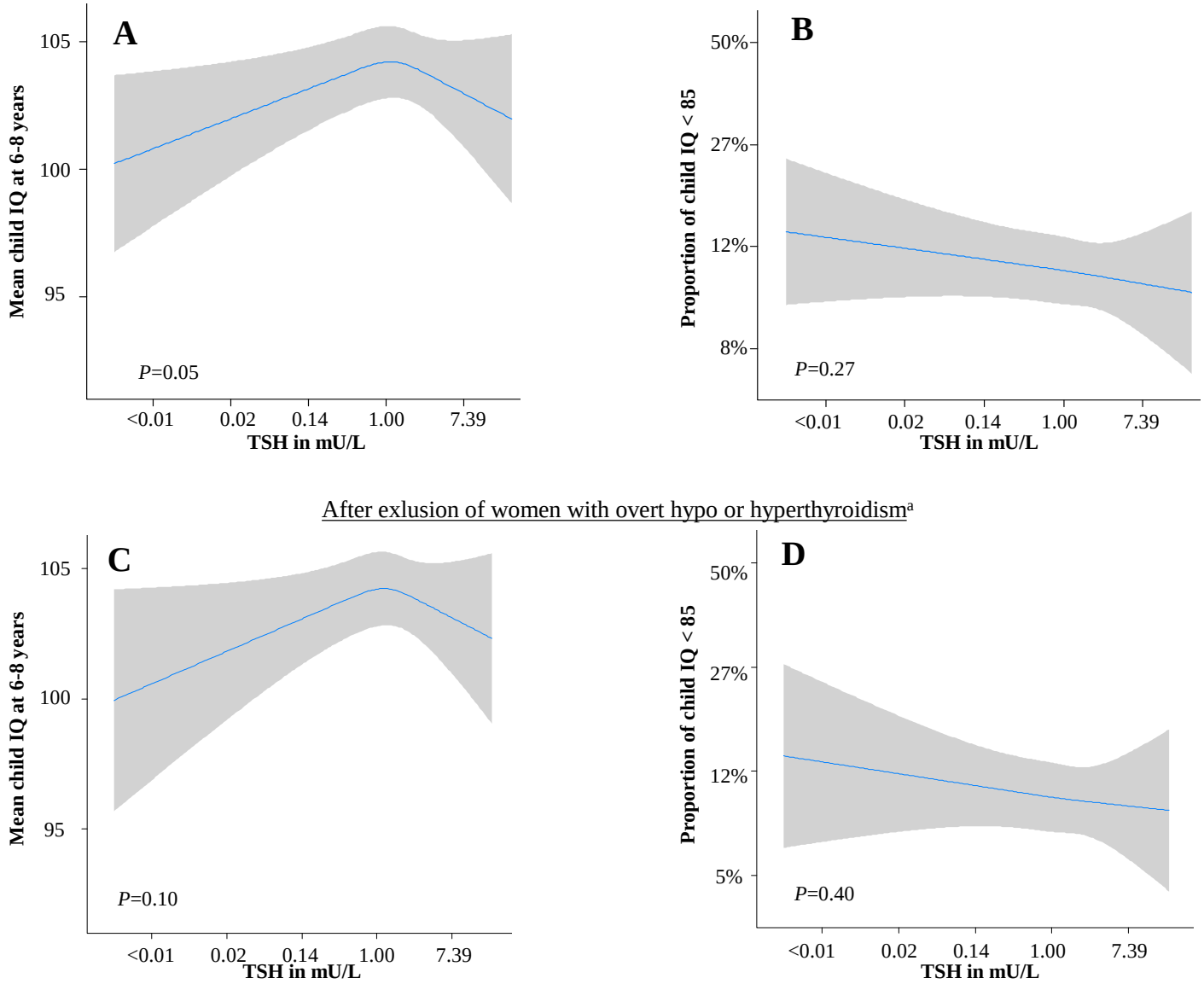
[a] At time of blood sampling; data shown as median in weeks
[b] Data shown as mean in (SD)
[c] Data shown as n (%)
[d] Data shown as median in years
[e] Data shown after imputation of missing data (see methods).

Supplemental Table 2. Associations of maternal TSH, FT4 with mean child IQ.

	IQ	
Variables in model	Beta ± SE[a]	*P*
TSH	-0.194 ± 0.280	0.50
TSH^2	-0.144 ± 0.059	0.02
FT4	33.81 ± 12.25	0.009
FT4^2	-6.235 ± 2.210	0.007

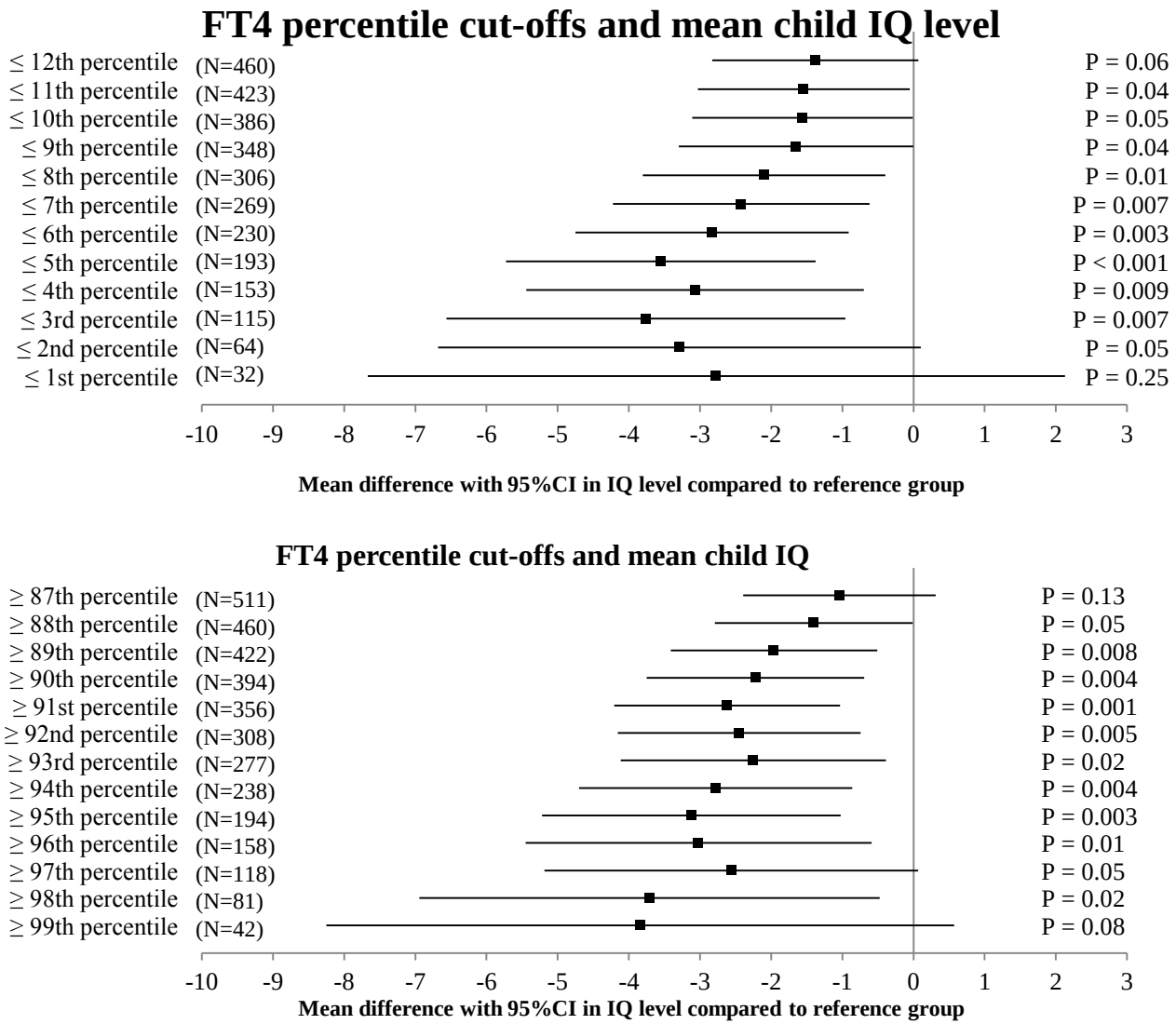
[a] Reported beta and standard error are increase in cm^3 per log increase in TSH or FT4. TSH^2 and FT4^2 refer to addition of a squared TSH/FT4 variable in the model. Analyses were performed using linear regression models in N=3839 mother-child pairs. TSH and FT4 values were transformed by the natural logarithm. Analyses were adjusted for gestational age at blood sampling, maternal age, smoking, BMI, parity, education level, ethnicity, fetal sex and birth weight.

Supplemental Figure 1. The association between maternal TSH and offspring IQ.



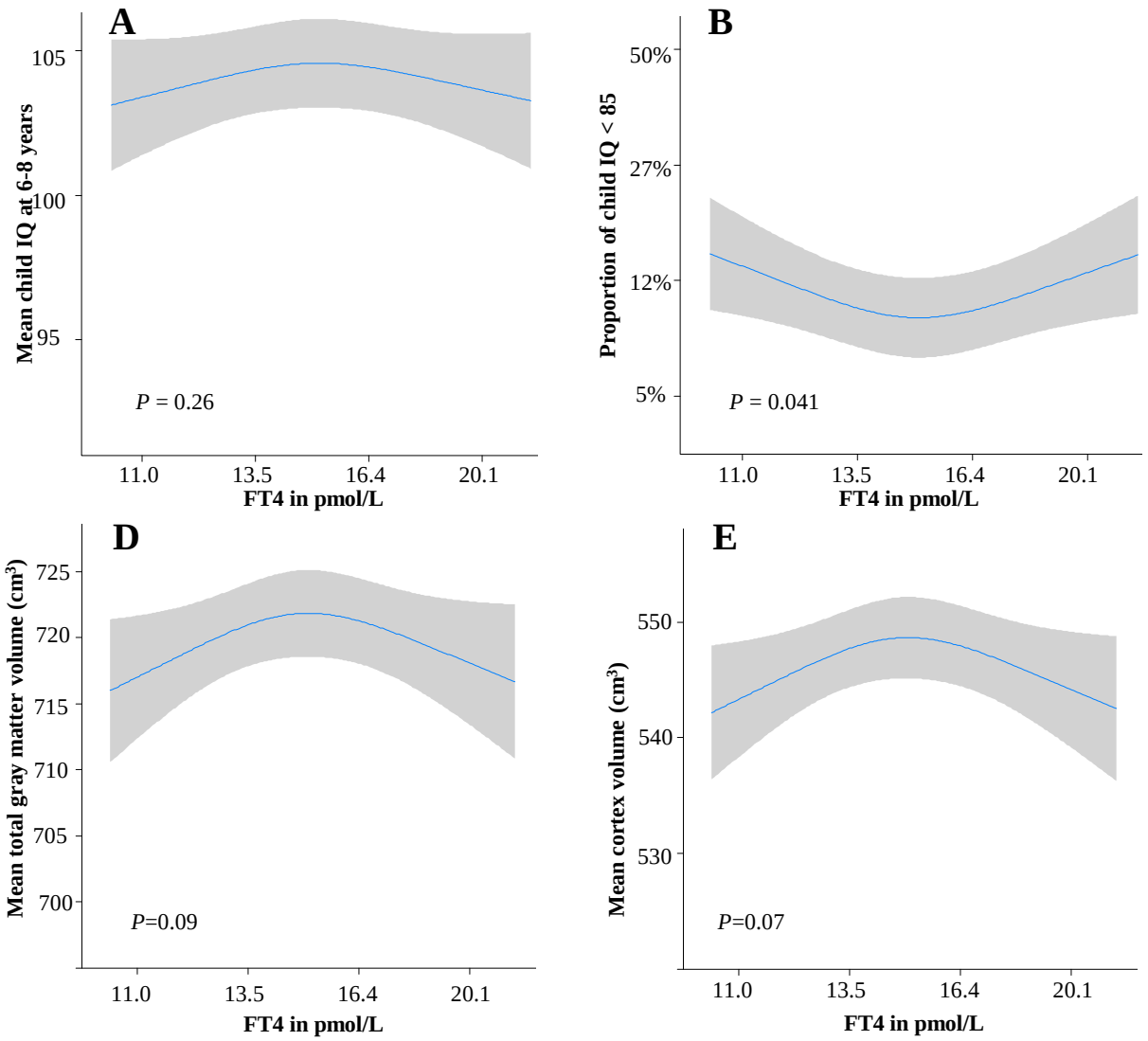
[a] Overt hypo and hyperthyroidism is defined as the biochemical diagnosis made during pregnancy based on the central 95% reference range as advocated by international guidelines.
Plots show the association between maternal TSH levels during pregnancy and child IQ in the whole population (A,B) and after exclusion of women with overt thyroid disease (C,D) as predicted mean with 95 percent confidence interval. Analyses were performed with R statistical package using the RMS package amongst singleton pregnancies after exclusion of women with IVF treatment (N=76) or women with known thyroid disorders or thyroid interfering medication usage (N=89) and were adjusted for gestational age at blood sampling, hCG, maternal age, smoking, BMI, parity, education level, ethnicity, fetal gender and birth weight.
See Table S2 for effect estimates of curve linear regression models.

Supplemental Figure 2. Sensitivity analyses on cut-off values for low or high FT4 and decreased offspring IQ.



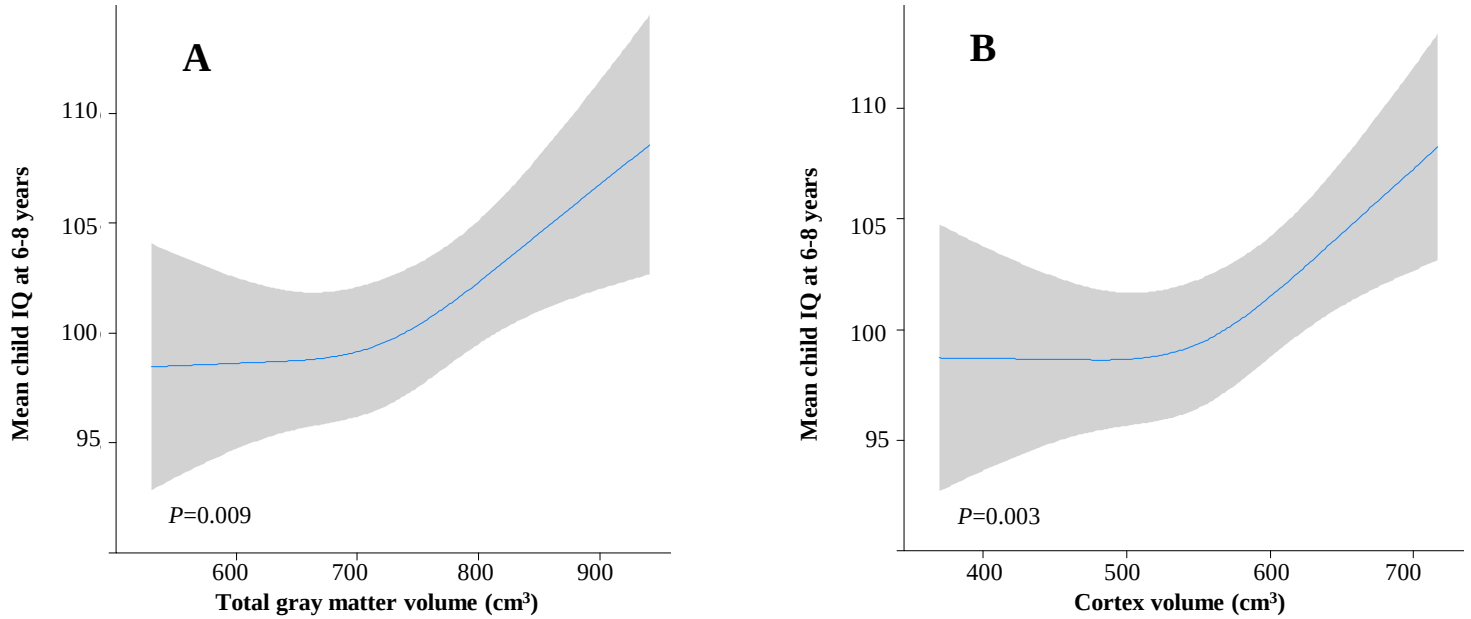
Plots show the mean difference between different percentile-cut-off points for maternal FT4 levels during pregnancy and the reference category of 10th - 90th percentile with 95 percent confidence interval. Analyses were performed amongst singleton pregnancies after exclusion of women with IVF treatment (N=76) or women with known thyroid disorders or thyroid interfering medication usage (N=89) and were adjusted for gestational age at blood sampling, maternal age, smoking, BMI, parity, education level, ethnicity, fetal gender and birth weight.

Supplemental Figure 3. The association of maternal FT4 within the normal range with offspring IQ and gray matter.



Plots A and B show the association between maternal FT4 levels during pregnancy within the normal range and child IQ as predicted mean with 95 percent confidence interval (N=3602). Plots C and D show the association between maternal FT4 levels during early pregnancy within the normal range and child MRI brain morphology outcomes as predicted mean with 95 percent confidence interval (N=613).

Supplemental Figure 4. The association between child total gray volume or cortex volume and IQ.



Plots show the association between child total gray volume (A) or cortex volume (B) and IQ as predicted mean with 95 percent confidence interval. Analyses were performed with R statistical package using the RMS package amongst singleton pregnancies after exclusion of women with IVF treatment (N=76) or women with known thyroid disorders or thyroid interfering medication usage (N=89) and were adjusted maternal age, BMI, parity, education level, child age, gender and birth weight.

Supplemental Table 3. Associations between maternal TSH, FT4 and child brain morphology assessed by MRI scanning.

	Total brain volume		**Gray matter volume**		**Cortex volume**		**White matter volume**		**Hippocampal volume**	
Variables in model	Beta ± SE[a]	*P*	Beta ± SE[a]	*P*	Beta ± SE[a]	*P*	Beta ± SE[a]	*P*	Beta ± SE	*P*
TSH	84.3 ± 27.2	0.002	59.5 ± 17.4	0.0007	54.3 ± 15.3	0.0004	23.8 ± 10.5	0.02	193 ± 213	0.37
TSH^2	-44.7 ± 13.0	0.0006	-31.5 ± 8.32	0.0002	-29.4 ± 7.32	0.00007	-12.7 ± 5.02	0.01	-85.8 ± 102	0.40
FT4	22.6 ± 15.2	0.14	220 ± 97.2	0.02	208 ± 85.6	0.02	12.0 ± 58.6	0.84	236 ± 1185	0.84
FT4^2	-40.2 ± 26.0	0.12	-38.4 ± 16.6	0.02	-36.3 ± 14.6	0.01	-2.78 ± 10.0	0.78	-43.1 ± 202	0.83
	Analyses adjusted for total brain volume									
Variables in model	Beta ± SE	*P*	Beta ± SE[a]	*P*	Beta ± SE[a]	*P*	Beta ± SE[a]	*P*	Beta ± SE	*P*
TSH	N/A		4.09 ± 4.57	0.37	6.52 ± 4.88	0.18	N/A[b]		-160 ± 187	0.39
TSH^2	N/A		1.78 ± 2.21	0.42	-3.83 ± 2.36	0.11	N/A[b]		104 ± 90.4	0.25
FT4	N/A		81.8 ± 24.7	0.0001	87.6 ± 26.4	0.001	N/A[b]		-688 ± 1019	0.50
FT4^2	N/A		-13.8 ± 4.21	0.001	-14.9 ± 4.51	0.001	N/A[b]		120 ± 174	0.49

[a] Reported beta and standard error are increase in cm^3 per log increase in TSH or FT4.
[b] Not applicable due to high collinearity between total brain volume and white matter volume.
TSH^2 and FT4^2 refer to addition of a squared TSH/FT4 variable in the model.
Analyses were performed using linear regression models in N=646 mother-child pairs. TSH and FT4 values were transformed by the natural logarithm. Analyses were adjusted for gestational age at blood sampling, maternal age, BMI, child sex, birth weight, gestational age at birth and age at time of MRI.