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Mollehave, L.T.; Grand, M.K.; Kriegbaum, M.; Andersen, C.L.; Lind, B.S.; Vliet, N.A. van; ... ; Strandberg-Larsen, K.

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Clinical Research Article

Maternal Thyroid Function in Early Pregnancy and Offspring School Performance and Neurodevelopmental Disorders

Line Tang Møllehave,[1] Mia Klinten Grand,[2] Margit Kriegbaum,[2] Christen Lykkegaard Andersen,[2,3] Bent Struer Lind,[4] Nicolien Alien van Vliet,[5] Diana van Heemst,[5] and Katrine Strandberg-Larsen[6]

[1]Center for Clinical Research and Prevention, Bispebjerg and Frederiksberg Hospital, 2000 Frederiksberg, Denmark
[2]Department of Public Health, Research Unit for General Practice and Section of General Practice, University of Copenhagen, 1353 Copenhagen K, Denmark
[3]Department of Hematology, Rigshospitalet, 2100 Copenhagen Ø, Denmark
[4]Department of Clinical Biochemistry, Copenhagen University Hospital Hvidovre, 2650 Hvidovre, Denmark
[5]Department of Internal Medicine, Section of Gerontology and Geriatrics, Leiden University Medical Center, 2300 RC Leiden, the Netherlands
[6]Department of Public Health, Section of Epidemiology, University of Copenhagen, 1353 Copenhagen K, Denmark

Correspondence: Line Tang Møllehave, PhD, Center for Clinical Research and Prevention, Bispebjerg and Frederiksberg Hospital, Frederiksberg Hospital, Hovedvejen, Entrance 5, Nordre Fasanvej 57, DK-2000 Frederiksberg, Denmark. Email: line.tang.moellehave@regionh.dk.

Abstract

Context: Thyroid hormones are critical for neural development, and during the first trimester of pregnancy, the fetus relies fully on maternal thyroid hormone production.

Objective: To investigate the associations between maternal thyroid hormone levels in the first trimester with the child's school performance, risk of attention deficit hyperactivity disorder (ADHD), and autism spectrum disorder (ASD).

Methods: Information from the Copenhagen Primary Care Laboratory Pregnancy Database on first trimester TSH and free thyroxine measurements in mothers of children born in 2000 through 2014 were linked with information on the child's standardized test scores in school, ADHD (patient record diagnoses and medication), and ASD (patient record diagnoses) until the end of 2018. Associations of TSH and free thyroxine with the outcomes were individually assessed by linear mixed models and Cox regression models. The analyses were stratified by preexisting maternal thyroid disorders.

Results: TSH measurements were available for 17 909 mother-child dyads. Among those with children born in 2000 through 2009, 6126 had a standardized school test score and were analyzed for the association between maternal thyroid hormone levels and child's school performance, and no support for an association was found. The association between thyroid hormone levels and child's risk of ADHD and ASD were analyzed for the 17 909 dyads and with no support for an association between thyroid hormone levels and these neurodevelopmental disorders. Stratification by preexisting maternal thyroid disorders did not affect the results.

Conclusion: We found no evidence for associations between first trimester maternal thyroid hormone levels and child's school performance, or risk of ADHD or ASD.

Key Words: thyroid function, pregnancy, attention deficit hyperactivity disorder, autism spectrum disorder, neurodevelopment

Abbreviations: ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; CGPL, The Copenhagen General Practitioners' Laboratory; CopLab, Copenhagen Primary Care Laboratory; fT4, free thyroxine; GP, general practitioner; GW, gestational weeks; ICD, International Classification of Diseases; IQ, intelligence quotient; PY, person-years.

Thyroid hormones play a crucial role in metabolism, growth, and most developmental processes, especially in brain development (1). Fetal neural development begins at conception, but the fetus' own thyroid hormone production is not fully functional until 14 to 18 weeks of gestation (2). Therefore, the unborn child relies on the supply of maternal thyroid hormones during the first trimester of pregnancy. Previous studies have indicated associations between disturbances in maternal thyroid hormone homeostasis during early pregnancy and the child's cognitive performance and development of attention deficit hyperactivity disorder (ADHD) and autism spectrum disorder (ASD); however, the results have been conflicting.

The prevalence of thyroid dysfunction in pregnancy varies widely between populations because it is influenced by both environmental and individual factors, and methods of assessment varies. Clinically, thyroid dysfunction is classified as overt hypothyroidism (elevated TSH and decreased free thyroxine [fT4]) or hyperthyroidism (decreased TSH and elevated fT4), or the subclinical states (TSH outside the reference range and fT4 within the reference range). In a systematic review and meta-analyses published in 2018 by Thompson et al, findings from 37 observational studies were summarized, reporting maternal overt and subclinical hypothyroidism during pregnancy to be associated with an

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increased risk of indicators of intellectual disability, no effect on ADHD risk, and an unclear effect on autism risk (3). However, the included studies contained some heterogeneity (eg, the timing of thyroid hormone measurement and methods of outcome assessment) (3). Fewer studies have examined whether hyperthyroidism is associated with neural developmental outcomes. Two meta-analyses, in which individual participant data from 3 birth cohorts were included, demonstrated no independent association between low TSH and intelligence quotient (IQ) score. However, high levels of fT4 were associated with higher risk of autistic traits, but not with IQ or risk of ADHD (4, 5). Two randomized controlled trials have examined the effect of levothyroxine treatment in pregnant women with either high TSH or low fT4 or both on their offspring's cognitive function. None demonstrated a difference between treatment and control, which may be due to initiation of treatment in second trimester (6, 7), low statistical power (8), or no/weak association. The effect of thyroid hormones on proliferation, migration, and differentiation of neural cells (9, 10) underscores the biological plausibility of an effect of maternal thyroid dysfunction on cognitive performance and neurodevelopmental disorders. Further, findings from a Dutch birth cohort with neuroimaging data demonstrated an inverse U-shaped association between maternal TSH during pregnancy and total, as well as cortical gray matter volume in the child at 10 years of age. The association between TSH and brain morphology was most pronounced when TSH was measured in gestational week (GW) 8, whereas there was no association when TSH was measured after GW 14 (11).

Early measurement of thyroid function is paramount to examine the effect of maternal thyroid hormone on the child's neural development. However, in many previous studies, thyroid status was measured in the second trimester in large parts of the study populations. Further, cohort studies may be prone to differential self-selection into the studies and attrition before outcome assessment. Also, IQ testing and screening tools for neurodevelopmental disorders in young children may not be clinically relevant, whereas school performance and clinically assessed diagnoses of ADHD and ASD more closely reflect relevant consequences for the child.

In the present study, we attempt to overcome these limitations by investigating the association between first trimester (GW 1-12) thyroid dysfunction and the child's neural development by examining whether first trimester measures of TSH and fT4 are associated with standardized school test scores, or the neurodevelopmental disorders ADHD and ASD.

Material and Methods

Study Design and Population

The present study is a cohort study for which we retrieved data from the Copenhagen Primary Care Laboratory Pregnancy (CopPreg) Database, which has been described in detail previously (12). In short, the Copenhagen Primary Care Laboratory Pregnancy Database combines the Copenhagen Primary Care Laboratory (CopLab) Database with the Danish Medical Birth Register. The latter contains data on all births, live and stillbirths, by women with residence in Denmark and giving birth in Denmark (13). The CopLab database contains clinical test results from The Copenhagen General Practitioners' Laboratory (CGPL) from July 1, 2000, to December 31, 2015 (14). The laboratory was accredited and served all general practitioners (GPs) (n = 750) and other private specialists (n = 300) in the Copenhagen Municipality and the former Copenhagen County during this period. In Denmark, residents have direct access to primary care and hospital care at no cost, and approximately 98% of Danes are listed with a GP. We included 19 306 mother-child dyads with a first trimester (GW 1-12) TSH measurement and liveborn between 2000 and 2014 to ensure sufficient follow-up time. For analyses of neurodevelopmental disorders, children with nonsensical clinical information on TSH or missing information on covariates were excluded. For analyses of school test results, we further restricted to children born in 2000 through 2009 and having results from minimum 1 mandatory school test (**Fig. 1**). In Supplementary Fig. S1 (15), a Lexis diagram of the study population and ages for school tests and lower age limits for diagnosis of ASD and ADHD are presented.

According to Danish legislation, ethical approval is not required for studies based solely on registers. Data handling approval was obtained at the Faculty of Health Science, University of Copenhagen. All analyses were performed on servers at Statistics Denmark with pseudonymized personal identifiable data.

TSH Analyses

TSH was determined in serum by the commercially available ADVIA Centaur/CentaurXP TSH method (Bayer/Siemens, Tarrytown, NY) according to the instruction of the manufacturer. The results are traceable to the World Health Organization reference material IRP 80/558. The interserial (day to day) coefficient of variation was 6.3% (at level 0.43 mIU/L, n = 1480), 5.3% (at level 5.15 mIU/L, n = 2846), and 4.9% (at level 22.52 mIU/L, n = 1518). The TSH assay was subject to external quality control through participation in the Labquality external quality assessment service (Helsinki, Finland). The assessment schemes included 11 to 18 distributions annually. Each distribution comprised 1 to 4 samples. The results from Labquality confirmed the reliability of the assay, and the results from CGPL (n = 329, from 2002 to 2015) deviated less than 12% from the method mean (limits set by Labquality) in 95% of the results. The mean deviation from method mean was +4.5% (n = 303, from 2003 to 2015). The clinical decision limit for TSH reported back to the GPs from the CGPL was 0.2 mIU/L (low limit) and 5.0 mIU/L (high limit), independent of age and sex throughout the entire study period (16). Recent data have shown that the use of uniform limits for TSH normality for the entire first trimester are too simplistic because TSH is highly dynamic during first trimester, with higher levels earliest in pregnancy (GW 4-6) followed by a gradual decline reaching the lowest level in GW 10 to 12 (17, 18). For this reason, we categorized TSH into 6 categories using predefined cutoffs: < 0.05, 0.05-<0.1, 0.1-<2.5, 2.5-<5.0, 5.0-<10.0, and ≥10.0 mIU/L, according to Andersen et al (19). For the analyses of neurodevelopmental disorders, it was reduced to 3 categories by defining 0.1 to ≤ 2.5 mIU/L as normal, and values, respectively, below and above these thresholds as low and high levels. In case of several TSH measures in first trimester (=1091), these were averaged.

fT4 Analyses

fT4 was measured in individuals with TSH outside the range of 0.2 to 5.0 mIU/L or if fT4 was specifically requested by

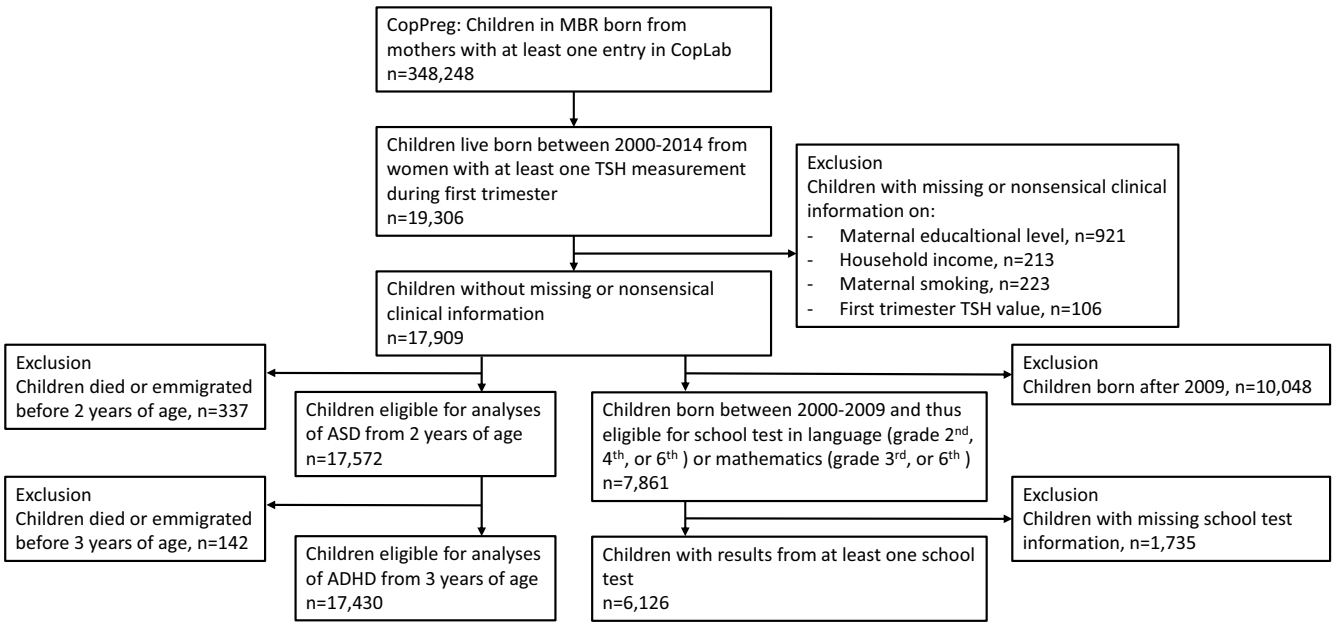

Figure 1. Flow chart of inclusion and exclusion in the study populations.

Abbreviations: ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; CopLab, Copenhagen Primary Care Laboratory Database; CopPreg, Copenhagen Primary Care Laboratory Pregnancy Database; MBR, Danish Medical Birth Register; TSH, thyroid stimulating hormone.

the GP. fT4 was determined in serum by the commercially available ADVIA Centaur/CentaurXP method (Bayer/Siemens, Tarrytown, NY) according to the instruction of the manufacturer. The interserial (day to day) coefficient of variation was 7.5% (at level 4.7 pmol/L) and 6.9% (at level 50.0 pmol/L). The free thyroxin assay was subject to external quality control through participation in the Labquality external quality assessment service (Helsinki, Finland). The assessment schemes included 12 to 20 distributions annually. Each distribution comprised 1 to 4 samples. The results from Labquality confirmed the reliability of the assay, and the results from CGPL (from 2002 to 2015) deviated <12% from the method mean (limits set by Labquality) in 94% of the results. The mean deviation from the method mean was 1.8% (n = 281, from 2004 to 2015). The normal range for fT4 test results reported back to the GPs was 10.0–22.0 pmol/L throughout the study period. As for TSH, we categorized fT4 into 6 predefined categories according to the cutoffs used by Andersen et al (19): < 10.0, 10-<11.0, 11.0-<12.0, 12.0-<19.0, 19.0-<20.0, and ≥20.0 pmol/L. In the analyses of neurodevelopmental disorders, fT4 was classified in 3 categories: low (<12.0 pmol/L), normal (12-18.9 pmol/L), and high (≥19 pmol/L).

School Performance

The Danish National Test program (20) was implemented in 2010, and all pupils attending any public school undergo mandatory tests for language in second (at approximately 8 years of age), fourth (10 years), and sixth grades (12 years), and for mathematics in third (9 years) and sixth grades (12 years) (Supplementary Fig. S1 (15)). We used data on tests from 2010 to December 31, 2018. In language, measured competences are on language comprehension, decoding, and reading comprehension. The mathematical competences include numbers and algebra, geometry, and applied mathematics. The tests are computer-based and adaptive, meaning that the test program draws new questions at difficulty levels based on the prior answers. The tests are initially computer-scored according to a Rasch model within each of the 3 profile areas of language and mathematics, respectively, and afterwards mapped by the Danish Ministry of Education into a single standardized test score between 0 and 100 (higher scores indicate better test results) for the student's performance in language and mathematics, respectively (21).

Neurodevelopmental Disorders: ADHD and ASD

Cases of ADHD were defined as a diagnosis of ADHD (International Classification of Diseases [ICD]-10: F90.x) and/or a redeemed prescription of ADHD medication (methylphenidate, modafinil, or atomoxetine [anatomical therapeutic chemical classification codes: N06BA04, N06BA07, N06BA09]) at age 3 years or older (22). Cases of ASD were defined as a diagnosis of ASD (ICD-10: F84.0, F84.1, F84.5, F84.8, or F84.9) recorded at 2 years of age or older (23) (Supplementary Fig. S1 (15)). Diagnoses were retrieved from the Danish National Patient Registry (24) and information on medication was retrieved from the Danish National Prescription Registry (25). The register-based psychiatric diagnoses have previously been validated and the registries are near complete (25-27).

History of Maternal Thyroid Dysfunction

Information on maternal history of thyroid disorders was retrieved by linkage to the Danish National Patient Registry (24) and the Danish National Prescription Registry (25). We identified thyroid disorders before pregnancy (ie, before the first day of the last menstrual period) as either a hospital diagnosis of hypo- and/or hyperthyroidism (up to 1995 coded with the eighth version of the ICD: 242.00-242.29, 243.99, and 244.00-244.09, and from 1995 and onwards coded with the 10th version, ICD-10: E00, E03.0-E03.9, E05.0-E05.9 and E89.0, with the exemptions of 244.02, E03.0A, E03.1B,

E03.4, E05.4, E05.8A, and E05.9A), or a redeemed prescription of either thyroid hormone or antithyroid medication (anatomical therapeutic chemical classification codes: H03A and H03B).

Covariates

From the Medical Birth Register, we retrieved information on maternal age at birth, preconception body mass index (available from 2003), smoking status in the first trimester, and parity, as well as information on the child's sex, year of birth, gestational age at delivery (classified as preterm [GW < 37] and term [GW ≥ 37]), birth weight, and Apgar score (13). Weight-for-gestational age was defined by sex-specific cutoffs (classified as large, normal, small, or unknown as defined by Marsal et al (28)).

By linkage to nationwide Danish administrative registers, we retrieved information on the following: equivalized disposable household income (classified as above or below the country mean for each calendar year). Maternal highest attained educational level (classified according to the International Standard Classification of Education codes as short [primary or upper secondary education], medium [vocational education or short-cycle higher education], and long [bachelor's or master's degree]). Both were defined for the year of birth. Maternal ethnicity (classified in accordance with Statistic Denmark's definition of country of origin as immigrant (Danish resident not born in Denmark, with neither parent born in Denmark), descendants (born in Denmark, but both parents being foreigners), or Danish origin.

Statistical Analyses

For the analyses of school performance according to maternal levels of TSH and fT4, respectively, we used linear mixed models with nested random effects to account for dependency between siblings and multiple school test results of the same child. The results are presented as the adjusted mean difference in standardized test scores and 95% CI between children of mothers with first trimester thyroid function in each of the 6 predefined categories (references: TSH 0.1-2.5 mUI/L or fT4 12-18.9 pmol/L). Tests for interactions between maternal thyroid function and school test subject (language vs mathematics), as well as between maternal thyroid function and grade were performed to decide whether to present subject-specific and grade-specific estimates.

For the analyses of ADHD and ASD, we used Cox proportional hazard models with a robust variance estimator to account for siblingship. Children's age was used as the underlying time scale, and children were followed from 3 and 2 years of age for ADHD and ASD, respectively, until date of diagnosis, death, emigration, or December 31, 2018, whichever occurred first. The results are presented as cause-specific hazard ratios with 95% CIs for the rates of ADHD or ASD, respectively, between children of mothers with low or high compared with normal first trimester thyroid function.

All analyses were performed unadjusted, as well as adjusted for the a priori selected confounders: maternal age, maternal educational level, household income, maternal ethnicity, maternal smoking, sex of the child, and year of birth. The analyses were carried out using the complete cases and missing observations in covariates were assumed to be missing at random. The adjusted models were validated against the observed data (29). For ADHD, ethnicity and sex, and for ASD, ethnicity and education were included as strata to ensure reasonable fulfilment of the proportional hazard assumptions. We stratified the analyses according to maternal history of thyroid disorder when data allowed such a division. Further, the analyses were stratified into early (GW 1-6) and late (GW 7-12) first trimester thyroid function measurements. All analyses were performed using R version 4.3.1.

Results

During 2000 through 2014, we identified 19 306 mother-child dyads with at least one TSH measurement during first trimester. For the analyses of TSH and school performance 6126 mother-child dyads were available after exclusions. For the analyses of TSH and neurodevelopmental disorders, 17 572 and 17 430 dyads were available for analyses of ASD and ADHD, respectively, after exclusions (**Fig. 1**). Excluded dyads were not different from the study populations apart from a higher proportion of immigrants (33% vs 21%) (data not shown). Characteristics of the study populations are presented in **Table 1**. The thyroid function measurements were performed throughout the first trimester with the most measurements per week in GW 6-10 (Supplementary Figure S2a and S2b (15)).

School Performance

There was no association between level of maternal TSH measured during first trimester and the children's mean standardized test scores (**Fig. 2**). The results were not materially different among children of mothers with and without a preexisting thyroid disorder (Supplementary Fig. S3 (15)) or for TSH measurements performed in early (GW 1-6) or late (GW 7-12) first trimester (Supplementary Fig. S4 (15)).

Data on maternal fT4 and school performance was available for 1206 dyads. There was no statistically significant association between fT4 measured during first trimester and mean standardized test scores (**Fig. 3**). The results were not materially different among children of mothers with and without a preexisting thyroid disorder (Supplementary Fig. S5 (15)) or for fT4 measurements performed in early or late first trimester (Supplementary Fig. S6 (15)).

Neurodevelopmental Disorders

ADHD was diagnosed at median 8 years (interquartile range, 6-10) and ASD was diagnosed at median 6 years (interquartile range, 4-9) of age. The overall rate of ADHD was 33/10 000 person-years (PY) and for ASD 29/10 000 PY. Our data were compatible with no association between low or high maternal TSH levels and the rate of ADHD. Likewise, no association appeared between low or high maternal TSH level and the rate of ASD, despite point estimates below and above 1 for the groups with low and high maternal TSH, respectively (**Table 2**).

Data on maternal fT4 and neurodevelopmental disorders were available for 3388 dyads. Among these the overall rate of ADHD was identical, but slightly higher for ASD 33/10 000 PY. Again, data were compatible with no association between maternal fT4 level and the risk of ADHD as well as ASD (**Table 2**).

Table 1. Characteristics of the study populations

Characteristic		ASD		School test	
		N	%	N	%
Total		17,572	100	6126	100
Maternal age (y)	14-24	1383	8	565	9
	25-29	4843	28	1808	30
	30-34	6789	39	2366	39
	35-56	4557	26	1387	23
Gestational age	Term	16,452	93	5721	93
	Preterm	1120	7	405	7
Educational level	Long	4979	28	1303	21
	Medium	7453	42	2876	47
	Short	5140	29	1947	32
Household income	Above average	8454	48	2874	47
	Below average	9118	52	3252	53
Maternal ethnicity	Danish origin	13163	75	4642	76
	Immigrant	3634	21	1263	21
	Descendant	775	4	221	4
Parity	Multi	8373	48	2986	49
	Nulli	8971	51	3011	49
	Unknown	228	1	129	2
Maternal smoking	No	16,304	93	5459	89
	Yes	1268	7	667	11
Maternal BMI (kg/m^2)	10–18.4	796	5	251	4
	18.5–25	10,215	58	2821	46
	25–61	4007	23	1119	18
	Unknown	2554	15	1935	32
Child sex	Female	8536	49	2982	49
	Male	9036	51	3144	51
Apgar score	0-5 points	93	1	30	0
	6-8 points	340	2	129	2
	9-10 points	17,065	97	5939	97
	Unknown	74	0	28	0
Birthweight for gestational age	Large	1485	8	561	9
	Normal	13785	78	4808	78
	Small	2204	13	739	12
	Unknown	98	1	18	0

Abbreviations: ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; BMI, body mass index.

Discussion

In this prospective cohort study, with clinical measurements of maternal thyroid function in first trimester of pregnancy we demonstrate no notable signals of associations between TSH or fT4 with the child's standardized school test scores, or risk of ADHD and ASD.

School Performance

Few previous studies have examined the association between maternal thyroid function and school performance. Although school performance and IQ are closely correlated, they are measures of different factors. School performance can be considered a more relevant or "real-life" outcome than IQ and is highly predictive of the final exit examination score (21). However, school performance outcomes are influenced by factors such as homework discipline, major life events, and resources of the school system that can affect learning possibilities or performance. This may have affected the results of the current study toward the null and obscured small differences because we deem these factors to be most likely nondifferential and independent on the maternal thyroid functioning.

Noten et al examined the association of thyroid function measured at median GW 12.9 with national mathematics and language test scores at 5 years in n = 1196 mother-child dyads from a birth cohort in Amsterdam, Netherlands. They, as us, demonstrated no association with TSH. In subanalyses, they found a higher risk of suboptimal performance in mathematics tests in children of mothers with hypothyroxinemia; however, this association attenuated after adjustment, and was no longer statistically significant after inverse-probability weighing (30). In the Northern Finland Birth Cohort, Päkkilä et al evaluated the association between thyroid function measured at mean GW 10.7 and school performance evaluated by the teacher at 8 years of age (n = 4357) and self-evaluated at 16 years of age (n = 5078). No association between maternal thyroid function and school performance at 8 years was found. Some indication of an association of subclinical hypothyroidism and hyperthyroidism with lower self-rated mathematics performance at 16 years was observed (31). In line with our study, Nelson et al did not find an association between maternal first trimester thyroid function (median GW 10) and the children's school performance evaluated at 5 national tests from 4 to 16 years of age in n = 4615 children from the ALSPAC cohort in the United Kingdom (32).

Taken together, our study in combination with the previously mentioned studies provide little support for an association of maternal thyroid function with school performance. It is notable that the study of Noten et al finds an association before, but not after, adjustment for attrition by inverse-probability weighing. In most previous studies of maternal thyroid function and child neural development, the participants are self-selected into the cohorts and with no outcome assessment in those lost to follow-up. Both mechanisms are likely sources of bias, which are not present in the current study. However, in this study, measurement of thyroid function depended on the GP's clinical judgment because this was not—and still is not—part of the mandatory clinical testing regime for pregnant women. Thus, indication bias (ie, bias originating from the rationale for ordering the clinical tests) is a serious limitation, and therefore we believe it to be crucial to distinguish women with and without known thyroid disorder as done in the present study. According to clinical guidelines, a risk-based screening strategy for thyroid dysfunction in pregnant women is recommended; however, a recent study has found that clinical practice does not comply with these guidelines (33).

Several studies have examined the association between maternal thyroid function and IQ. An association between low fT4 and decreased IQ seems to be more consistent than other measures of thyroid function (4, 19), and fT4 may be a better indicator of maternal thyroid function than TSH during the first trimester of pregnancy (2). Our data demonstrated a very weak signal of an inverse U-shaped association between fT4 and school performance but was not close to statistically significant. However, analyses of fT4 in the present study are hampered by lack of power, as indicated by the wide CIs. Furthermore, fT4

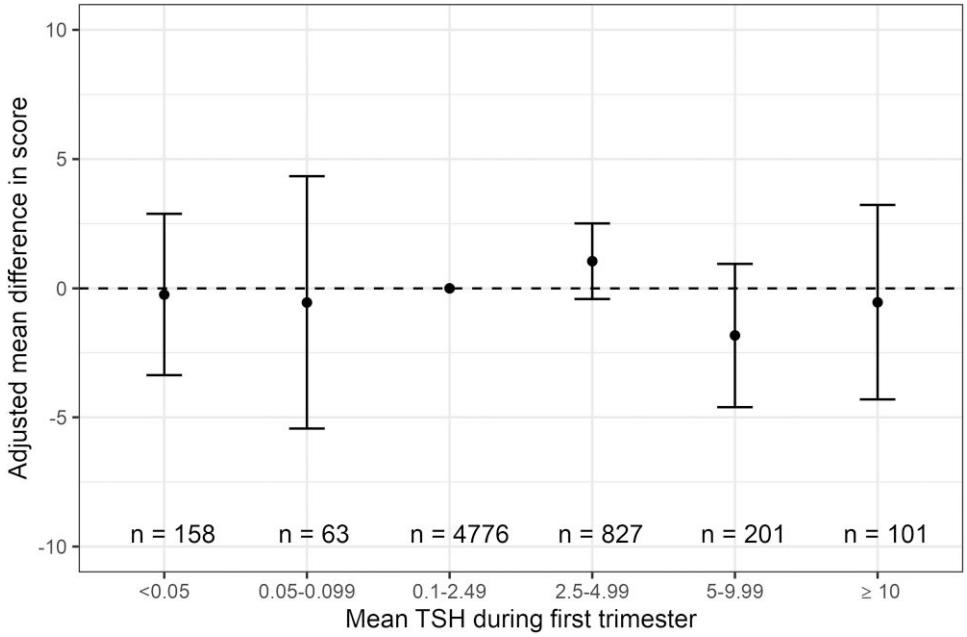

Figure 2. Differences in standardized test scores at the school tests according to mean maternal level of TSH during the first trimester of pregnancy. Data are presented as adjusted means with standard errors. Adjusted for: maternal age, maternal highest attained educational level, household income, maternal ethnicity, smoking, sex of child, and year of birth. Results were not materially different among children of mothers with and without a preexisting thyroid disorder (Supplementary Fig. S3 (15)) or for TSH measurements performed in early (GW 1-6) or late (GW 7-12) first trimester (Supplementary Fig. S4 (15)).

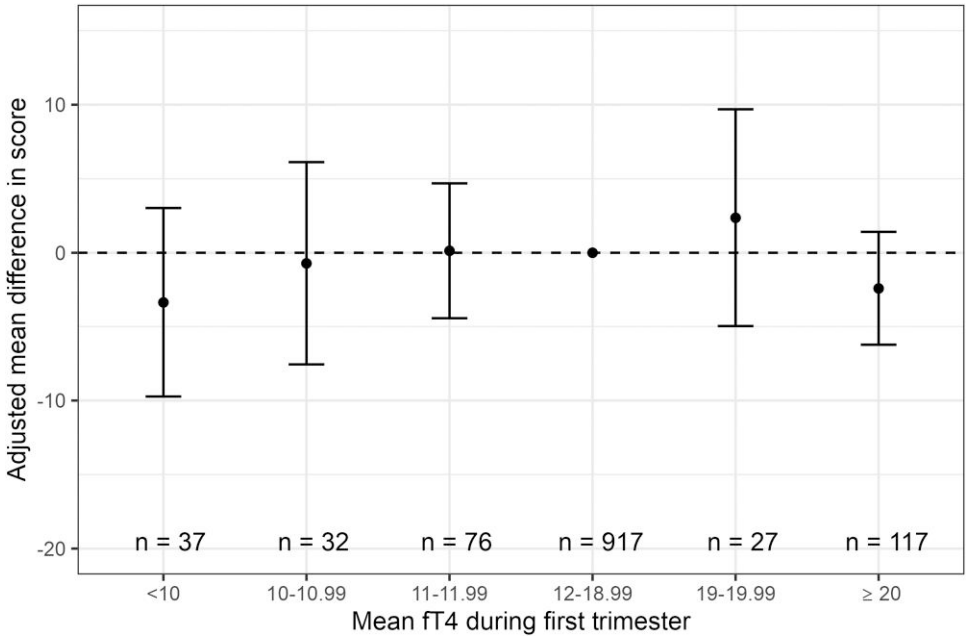

Figure 3. Differences in standardized test scores at the school tests according to the mean maternal level of fT4 during the first trimester of pregnancy. Data are presented as adjusted means with standard errors. Of the total 1206 in this analysis, 558 of the mothers had a history of thyroid disorder. Results were not materially different among children of mothers with and without a preexisting thyroid disorder (Supplementary Fig. S5 (15)) or for TSH measurements performed in early (GW 1-6) or late (GW 7-12) first trimester (Supplementary Fig. S6 (15)).

was only measured on indication and therefore the population with fT4 measurements is highly selected with preexisting thyroid disease in approximately half of the women and TSH outside the reference limits in most women. Therefore, the population is not representative for the general population; however, the measures of association may still be applicable.

Neurodevelopmental Disorders

Findings on an association between maternal thyroid function and neurodevelopmental disorders have been somewhat inconsistent in previous studies. Despite indications of an association between maternal thyroid function and ADHD in individual studies, the review and meta-analyses of Thompson et al found

Table 2. Hazard ratios for ASD or ADHD according to the mean level of maternal thyroid hormones during the first trimester of pregnancy

TSH level	Low	Normal	High
ADHD			
Hazard ratio (95% CI)	0.84 (0.40-1.74)	1 (ref)	1.19 (0.90-1.57)
Cases	9	262	66
Person-years	3.352	80.808	17.756
ASD			
Hazard ratio (95% CI)	0.84 (0.43-1.62)	1 (ref)	1.16 (0.88-1.51)
Cases	9	267	69
Person-years	3.860	94.683	20.873
fT4 Level	**Low**	**Normal**	**High**
ADHD			
Hazard ratio (95% CI)	0.90 (0.41-1.99)	1 (ref)	1.18 (0.53-2.66)
Cases	7	48	9
Person-years	2.137	15.165	2.329
ASD			
Hazard ratio (95% CI)	1.18 (0.57-2.44)	1 (ref)	1.12 (0.55-2.29)
Cases	9	57	10
Person-years	2.424	17.737	2.757

Adjusted for: maternal age, maternal highest attained educational level, household income, maternal ethnicity, smoking, sex of child, and year of birth. Analyses of the association with TSH were performed in n = 17430 dyads for ADHD and in n = 17572 dyads for ASD and analyses of the association with fT4 were performed in n = 3292 dyads for ADHD and in n = 3320 dyads for ASD.
Abbreviations: ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder.

no effect of hypothyroidism or low fT4 (3). In a recent study in almost 400 000 Israeli children, Rotem et al found an indication of an increased risk of ADHD in children of mothers with a diagnosis of hypothyroidism. However, because this association was not affected by levothyroxine treatment and was still found in mothers with a hypothyroid diagnosis and normal thyroid hormone levels, the authors suggest that the association between hypothyroidism and ADHD may not be fully mediated through thyroid hormone levels (34). In the Norwegian Mother, Father and Child Cohort Study, in a case-cohort study including 298 cases and 554 controls, Engel et al demonstrated an increased risk of ADHD diagnoses in children of mothers with fT4 in the lowest and highest quintiles assessed at median GW 17. Insufficient iodine intake seemed to amplify the risk, whereas there was no association with TSH (35). Thus, there is some indication that fT4 may directly or indirectly be more relevant than TSH in the association with ADHD; however, our study had limited power for these analyses.

Rotem et al found maternal diagnosis of hyper- and hypothyroidism to be associated with ASD but, as with ADHD, did not find this to be mediated through maternal thyroid hormone levels (36). In a study of n = 397 201 dyads from the United States, Getahun et al found an increased risk of ASD in children of women with a diagnosis of hypothyroidism before or during pregnancy. Thyroid hormone levels were only available for women with a diagnosis of hypothyroidism, and in women diagnosed during pregnancy, the risk of ASD was only elevated in those with high TSH and/or low fT4 but not in those with normal thyroid hormone levels (37). Also in the United States, Lylall et al examined a range of biomarkers and genetic factors suspected to be associated with ASD (38). In 78 cases with a diagnosis of ASD and 149 controls, higher maternal TSH (GW 15-19) was associated with lower risk of ASD (39).

Andersen et al conducted a case-cohort study of 2276 cases and 7624 controls in a similar Danish population but, in contrast to our data, demonstrated an increased risk of ASD diagnosis in hypo- and hyperthyroid mothers and an indication of ADHD in low fT4 (40). In their study, a large proportion of the thyroid function measurements were taken in second trimester, and the children were followed from 1 month after birth and until 7 to 13 years. Thus, some of the difference may be explained by the differences in follow-up because it may be speculated that children of mothers with a clinically diagnosed thyroid disease are more likely to be diagnosed early, and especially girls experience diagnostic delay (41).

Strengths and Limitations

The main strengths of our study are, first, the relatively large sample size, although the study still had limited power for fT4 and subgroup analyses. Second, the measurements of maternal thyroid function are all taken in first trimester of pregnancy (ie, before the fetus develops its own thyroid function and the thyroid hormone levels thus represents the true exposure of the fetus). Third, there was no self-selection into the study or dropout before outcome assessment. The population of women with thyroid function measurements during first trimester of pregnancy can be considered a high-risk group because the GP may already suspect thyroid dysfunction, also reflected by the relatively large proportion with preexisting thyroid disease. Thus, despite indication bias the population might be more relevant to study for these outcomes. Fourth, the biochemical analyses were all performed in one highly standardized laboratory with good quality control and uniform procedures during the study period. Fifth, the school tests have good reliability (eg, test-retest reliability and Person Separation Index score for reliability >0.8 in all profile areas of language and mathematics), standardization of the test scores from 1 to 100 limits teacher bias (42), and follow-up was available for all children attending public schools. Sixth, the diagnoses of neurodevelopmental disorders in the Danish registers have high validity (26, 27) and follow-up was available for all children because of register linkage using the unique personal identifier, assigned to all Danish citizens. Seventh, it is a strength that the measurement of thyroid function and the school testing, or diagnoses of neurodevelopmental disorders were assessed by independent observers. Information was available for most relevant confounders and obtained from national registers with little risk of bias, but we acknowledge that there may still be residual and unmeasured confounding. For example, no information was available on maternal iodine status or use of dietary supplements containing iodine, which may affect neurodevelopmental outcomes (43, 44). Last, the potential capacity of the Danish school and welfare system to attenuate effects of thyroid dysfunction on neurodevelopmental outcomes and other potential confounding factors may differ between countries and thus limit the generalizability.

Conclusions

Based on the current and previous studies, we cautiously suggest that, although studies have demonstrated that maternal

thyroid dysfunction during early pregnancy is associated with impairments of offspring IQ, this does not seem to be similar for school performance at ages 8 to 12 years. Further, an association between maternal thyroid dysfunction and clinically diagnosed ADHD or ASD was not confirmed in our study.

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Author Contributions

K.S.L., M.K.G., N.A.V., and D.H. conceived and designed the study. M.K.G. performed the data management and analyses, and L.T.M. and M.K. verified the findings. K.S.L., M.K.G., and L.T.M. interpreted the results. B.S.L. contributed with information on CopLab methods and material. L.T.M. wrote the draft manuscript. All authors reviewed the results and approved the final version of the manuscript.

Disclosures

The authors have nothing to disclose.

Data Availability

Restrictions apply to the availability of some or all data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

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