Maternal iodine status, thyroid function during pregnancy, and child neurodevelopment

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Jodium status van de moeder, schildklierfunctie tijdens de zwangerschap en hersenontwikkeling van het kind

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Contents

Chapter 1

General introduction

General introduction

The concept of the Developmental Origins of Health and Disease postulates that early developmental events shape our future health and well-being because our organs and its functions undergo programming during embryonic and fetal life [1]. The brain is one of the organs that starts developing soon after conception. Brain development is complexly regulated by neurotransmitters and hormones, such as thyroid hormone, which is produced by the thyroid gland positioned in the front of the neck [2]. The fetus is highly dependent on the placental transfer of thyroid hormone from the mother before mid-gestation, because the fetus is not able to produce sufficient amounts of thyroid hormone itself yet [3]. Before the fetal thyroid gland is fully functional, the brain is already expressing nuclear thyroid hormone receptors, which suggests a prominent role of thyroid hormone for brain development [2]. Exposure of the fetus to insufficient concentrations of thyroid hormone may disturb brain developmental processes such as the migration, the proliferation, and the differentiation of neuronal cells [4,5]. An impressive example of the importance of thyroid hormone for fetal brain development is cretinism. Individuals with this severe condition suffered from intellectual disability, hearing and speech problems, disorders of stance and gait, hypothyroidism, and/or stunted growth. A high rate of mothers that gave birth to cretins also had abnormal thyroid enlargement, so called goiter, which made it likely that these women had a malfunctioning thyroid gland [6].

> *"Goiter is a disease which, when once acquired and not cured, can be transmitted even to the third and fourth generation of posterity, therefore people with this disease should not be permitted to indulge in parenthood"* – Quote from the Boston Medical Surgical Journal from 1918 [7].

Diffuse goiter can develop when the thyroid hormone concentration in the circulation is too low. This low concentration is "sensed" by the hypothalamus, which subsequently increases the thyroid-releasing hormone (TRH) production. TRH acts on the thyrotrophic cells of the anterior lobe of the pituitary gland and stimulates the secretion of thyroid stimulating hormone (TSH). High stimulation of the thyroid by TSH can cause the thyroid to grow with the goal to restore normal concentrations of thyroid hormone in the circulation. When TSH binds to the TSH receptor on thyroid follicular cells, the thyroid gland will synthesize and secrete two types of thyroid hormones: thyroxine (T4) and the biologically active triiodothyronine (T3). T4 and T3 circulate around in serum either bound to thyroid transport proteins or in free form (FT4 and FT3) and T3 exerts thyroid hormone action by further binding to nuclear receptors in target organs. Once the thyroid hormone concentration in the circulation reaches normal values, the release of both TRH and TSH will be inhibited. This way, a homeostatic balance of thyroid hormone concentrations in serum is reached in healthy persons.

Research from the 19th and 20th century revealed that iodine is an important trace element required for the production of thyroid hormones [8]. Historically, goiter was more prevalent in areas where drinking water had a low concentration of iodine. Hence, low iodine intake was identified as the cause of goiter. Because of this relationship, the role of iodine deficiency in the etiology of endemic cretinism was investigated. For example, a double blinded controlled trial, in which families were randomized either to a placebo or iodine treatment, found that the prevalence of goiter and cretinism was lower in those families that received an injection of iodized oil [9]. However, timing of treatment mattered. Some women that were treated in the third trimester gave birth to a cretin, while no cretins were born to mothers treated before or in early pregnancy. Thyroid dysfunction in children with cretinism could also be reversed by iodine supplementation [10]. The hypothesis therefore is that endemic cretinism is caused by an inadequate thyroid hormone transfer from mother to fetus during pregnancy, that iodine deficiency is an important risk factor, and that timely iodine intervention can prevent severe iodine deficiency disorders in the child [11]. For this reason, thyroid function tests are performed in early pregnancy and recommendations were made for higher iodine intake in pregnancy, and for routinely checking the thyroid axis in all newborns.

> *"On a worldwide basis, iodine deficiency is the single most important preventable cause of brain damage"* – ICCIDD/UNICEF/WHO [12]

Thyroid dysfunction in pregnancy is relatively common due to a change in thyroid physiology, especially in case of iodine deficiency [13]. The current guidelines recommend treating pregnant women with overt hypothyroidism, which is characterized by a high TSH and a low FT4 concentration, with levothyroxine [14]. This recommendation is based on evidence from observational studies that revealed associations of overt hypothyroidism with severe pregnancy complications [15,16], including fetal death. Untreated overt hypothyroidism has also been associated with a 7-point lower IQ score in the offspring [17]. Universal screening and treatment of women with milder forms of thyroid dysfunction is, however, not routinely performed. Subclinical hypothyroidism, characterized by high TSH and a normal FT4 concentration, has been associated with adverse pregnancy outcomes in thyroid peroxidase positive women; see an overview of studies elsewhere [14]. Depending on the TSH concentration and/or the thyroid peroxidase antibody (TPOAb) status, treatment is considered [14,18]. The evidence for an association of subclinical hypothyroidism with child neurodevelopmental outcomes is inconsistent. By contrast, isolated hypothyroxinemia, characterized by a low FT4 and a normal TSH concentration, has been associated with a variety of adverse neurodevelopmental outcomes, such as lower IQ or a delay in cognitive functioning [19–23], worse psychomotor development [21,24], schizophrenia [25], autism spectrum disorder or autistic traits [26–28], and attention-deficit hyperactivity disorder (ADHD) or related symptoms [17,28–30]. Despite the evidence from observational studies, universal screening to detect low FT4 concentrations in pregnant women

and treatment is not recommended [14], because the existing randomized controlled trials failed to show beneficial effects of levothyroxine treatment of women with hypothyroxinemia on cognitive development of the offspring [31,32]. An observational study embedded in the Generation R cohort, which involves an iodine-sufficient population, showed in addition to a low maternal FT4 concentration, that also a high FT4 concentration during pregnancy was associated with a lower child IQ score and lower gray matter volume and cortex volume in the offspring [19]. Though this is in line with findings from animal studies [33–38], it has not yet been replicated in other cohort studies and it is not known whether associations differ in countries with a different iodine status.

Reference ranges of thyroid function tests are established to identify women with abnormal thyroid function. The international thyroid guidelines advise that reference ranges should be based on the 2.5th and 97.5th percentile of the population with an optimal iodine intake [14]. However, there is insufficient evidence to determine whether these reference ranges are affected by iodine status. Iodine nutrition in populations is most frequently assessed by a measurement of the urinary iodine concentration (UIC) in a single spot urine sample. According to the classification of the World Health Organization, a population with a median UIC below 150 µg/L is considered iodine deficient. Mild-to-moderate iodine deficiency during pregnancy is still a common problem [39,40] and has been associated with lower scores of verbal IQ, reading accuracy, and reading comprehension [41], poorer spelling [42,43], reduced receptive and expressive language skills [44], worse executive function [45], internalizing and externalizing problems [46], poorer fine mother skills [46], and higher ADHD symptom scores [47]. However, not all prospective birth cohort studies show an association between UIC and child neurodevelopmental outcomes [48–50]. Differences in results between studies might be related to methodological differences (e.g., selected reference group and available data on confounders), the age of assessment of the neurodevelopmental outcome of interest, the timing of the iodine measurements, and the severity of iodine deficiency in the population. What remains unknown is whether the association of maternal iodine status with child neurodevelopmental outcomes varies during different periods of gestation and what the consequences of iodine excess are for child neurodevelopment.

Objectives

The aims of this thesis are:

1. To explore the determinants of iodine status during early pregnancy in populations of differing iodine status.
2. To assess the association between maternal iodine status and maternal thyroid function during pregnancy in a mild-to-moderate iodine deficient population and to determine variation in thyroid function reference ranges according to iodine status.

3. To assess the association maternal iodine status with childhood IQ, autistic traits, and ADHD symptoms in populations of differing iodine status.
4. To assess the association of maternal thyroid function with childhood IQ, autistic traits, and ADHD symptoms in populations of differing iodine status.

Setting

Most studies presented in this thesis are embedded within the EUthyroid project entitled "Towards the elimination of iodine deficiency and preventable thyroid-related diseases in Europe". For this three-year Horizon 2020 project, which started in June 2015, data were harmonized and combined from three major European prospective birth cohort studies: INfancia y Medio Ambiente (INMA, Spain), Generation R (the Netherlands), and Avon Longitudinal Study of Parents and Children (ALSPAC, United Kingdom). These cohort studies had detailed information on maternal iodine status, thyroid function during pregnancy, and child neurodevelopmental outcomes and were selected because of the differing iodine status in these populations, ranging from iodine sufficiency to mild-to-moderate iodine deficiency. Not part of the EUthyroid project was the Swedish Environmental Longitudinal, Mother and child, Asthma and allergy (SELMA) cohort. This cohort study had information on maternal iodine status and more extensive data on thyroid function tests during pregnancy (e.g., TSH, (F)T4, (F)T3, and markers of thyroid autoimmunity) than INMA, Generation R, or ALSPAC. Data on child neurodevelopmental outcomes in SELMA could however not be used. These four cohort studies were designed to study the role of early environmental or genetic causes of normal and abnormal development from fetal life onwards. Combining individual-participant data of multiple cohorts permits hypothesis testing on a larger scale, may overcome difficulties associated with individual studies (e.g., low statistical power, range restriction), and may increase our confidence in the generalization of the results when it replicates the findings of individual studies. Hence, this thesis may provide an opportunity to generate a trustworthy foundation for conclusions and scientific progress.

INMA

INMA is a network of birth cohorts in Spain of which three birth cohorts were included in this thesis: Valencia, Sabadell, and Gipuzkoa [51]. Pregnant women were recruited in Valencia between November 2003 to June 2005, in Sabadell from July 2004 to July 2006, and in Gipuzkoa from April 2006 to January 2008 during the first pre-natal visit. INMA was used for the analysis of the first, third, and fourth aim of this thesis.

Generation R

The Generation R study is a population-based birth cohort study in Rotterdam, the Netherlands [52]. Pregnant women with a delivery date from April 2002 until January 2006 were eligible for participation. Enrollment was possible throughout gestation. Generation R was used for the analysis of the first, third, and fourth aim of this thesis.

ALSPAC

ALSPAC is a population-based birth cohort in Avon, United Kingdom [53,54]. Pregnant women with an expected date of delivery between April 1991 and December 1992 were eligible for inclusion (phase I). After pregnancy, additional recruitment phases (II and III) took place to enroll those who would have fitted the original eligibility criteria. All mother-child pairs that were recruited during pregnancy were used for the analysis of the first, third, and fourth aim of this thesis.

SELMA

The SELMA study is a population-based longitudinal prospective cohort study in the county of Värmland, Sweden [55]. Pregnant women were recruited from September 2007 to March 2010 during the first prenatal visit at an antenatal care center around week 10 of pregnancy. Women beyond week 22 in their pregnancy were excluded from the SELMA study. This study was only used for the second aim of this thesis.

Outline

Chapter 2 explores the determinants of iodine status in populations of differing iodine status. Chapter 3 focuses on the association between maternal iodine status and maternal thyroid hormone concentrations in a mild-to-moderate iodine deficient pregnant population from the SELMA study and variation in reference ranges by iodine status is investigated. In chapter 4, we study the associations of maternal iodine status during pregnancy with child neurodevelopmental outcomes. Chapter 4.1 describes the association of maternal iodine status with child IQ, while chapter 4.2 evaluates the association of maternal iodine status with child ADHD and autistic traits. Chapter 5 evaluates the association of maternal thyroid function with child neurodevelopmental outcomes. In chapter 5.1, we examine the association of maternal thyroid function with child IQ and autistic traits. In chapter 5.2, the association of maternal thyroid function with child ADHD is assessed. In chapter 6, the main findings are summarized, and the clinical implications and direction for future research are described. Finally, a summary of this thesis is included in chapter 7.

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Chapter 2

Similarities and differences of dietary and other determinants of iodine status in pregnant women from three European birth cohorts.

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Abstract

Purpose: As a component of thyroid hormones, adequate iodine intake is essential during pregnancy for fetal neurodevelopment. Across Europe, iodine deficiency is common in pregnancy, but data are lacking on the predictors of iodine status at this life stage. We, therefore, aimed to explore determinants of iodine status during pregnancy in three European populations of differing iodine status.

Methods: Data were from 6566 pregnant women from three prospective population-based birth cohorts from the United Kingdom (ALSPAC, n=2852), Spain (INMA, n=1460), and the Netherlands (Generation R, n=2254). Urinary iodine-to-creatinine ratio (UI/Creat, µg/g) was measured in spot-urine samples in pregnancy (≤18-weeks gestation). Maternal dietary intake, categorised by food groups (g/day), was estimated from food-frequency questionnaires (FFQs). Multivariable regression models used dietary variables (energy-adjusted) and maternal characteristics as predictors of iodine status.

Results: Median UI/Creat in pregnant women of ALSPAC, INMA, and Generation R was 121, 151, and 210 µg/g, respectively. Maternal age was positively associated with UI/Creat in all cohorts ($P<0.001$), while UI/Creat varied by ethnicity only in Generation R ($P<0.05$). Of the dietary predictors, intake of milk and dairy products (per 100 g/day) was positively associated with UI/Creat in all cohorts [ALSPAC ($B=3.73$, $P<0.0001$); INMA ($B=6.92$, $P=0.002$); Generation R ($B=2.34$, $P=0.001$)]. Cohort-specific dietary determinants positively associated with UI/Creat included fish and shellfish in ALSPAC and INMA, and eggs and cereal/cereal products in Generation R.

Conclusions: The cohort-specific dietary determinants probably reflect not only dietary habits but iodine-fortification policies; hence public-health interventions to improve iodine intake in pregnancy need to be country-specific.

Introduction

Iodine is an essential component of the thyroid hormones which are important for optimal fetal and early postnatal neurodevelopment [1,2]. Mild-to-moderate maternal iodine deficiency in early pregnancy has been associated with suboptimal offspring cognitive outcomes [3–7]. The early stages of pregnancy mark the beginning of crucial fetal brain development processes such as neuron proliferation, migration, and differentiation [8]. Though these early processes are thyroid hormone-dependent, the fetal thyroid gland is not fully functional until 18-20 weeks, highlighting the importance of thyroid hormone supply from the mother. In this critical early period, the mother, therefore, needs sufficient iodine intake to maintain optimal thyroid function [1]. As a result of the increased demand for thyroid hormones and other physiological changes associated with pregnancy, pregnant women have a higher iodine requirement than the general population, putting them at greater risk of deficiency [9,10].

As more than 90% of the dietary iodine absorbed is excreted by the kidneys, urinary iodine concentration (UIC) is considered to be a good estimate of recent iodine intake at the population level [11]. In pregnant populations, iodine sufficiency is defined by a median UIC in the range of 150-249 µg/L, corresponding to the iodine intake of 250 µg/day recommended by the World Health Organisation (WHO) [12].

Determining the main food sources of iodine in pregnancy is essential, so that pregnant women can be given information on how to achieve adequate iodine nutrition. Although good food sources of iodine, such as milk, eggs, fish, and, in some countries, iodised salt, are well-known, the consumption patterns of these foods vary between and within populations, as does their iodine content (i.e., as a result of seasonal variation, agricultural practices, and differences in iodine content of soil and water) [13,14]. Consequently, some variation in the importance of different iodine food sources to population iodine status is expected between countries; hence universal dietary recommendations to increase iodine intake are unlikely to be appropriate.

Considering the negative consequences of iodine deficiency in pregnancy and the fact that many pregnant women worldwide are still iodine-deficient [15], achieving adequate iodine status in the pregnant population is of public-health importance. Data are, however, lacking on the determinants of iodine status in pregnancy in both deficient and sufficient areas; knowledge of such factors would help to identify women likely to have low iodine status.

This study aimed to explore the determinants of iodine status during early pregnancy in three European populations of differing iodine status. The objectives of the study were: (i) to establish whether iodine status during early pregnancy is associated with maternal socio-demographic, anthropometric, lifestyle factors, and pregnancy characteristics; (ii) to determine how maternal iodine status is influenced by dietary intake during pregnancy; (iii) to identify similarities and differences in the main determinants of iodine status between deficient and sufficient pregnant populations.

Methods

Study population

Data from three prospective population-based birth cohorts were used: (i) the Avon Longitudinal Study of Parents and Children (ALSPAC) in the United Kingdom (UK) [16,17]; (ii) Generation R in the Netherlands [18]; and (iii) INfancia y Medio Ambiente (INMA) in Spain [19]. In ALSPAC, 14541 pregnant women living in the former Avon area in the South West of England, with an expected delivery date between 1st April 1991 and 31st December 1992, were recruited. The study website contains details of all the data that are available through a fully searchable data dictionary and variable search tool [20]. In Generation R, 9778 mothers residing in Rotterdam with an expected delivery date from April 2002 to January 2006 were enrolled in the study. In total, 2150 pregnant women were recruited as part of the INMA Project from three regions in Spain (Valencia, Sabadell, and Gipuzkoa), in the period of November 2003 to January 2008.

Ethics

Ethical approval was obtained prior to recruitment from a number of bodies: the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees (ALSPAC), the Medical Ethical Committee of the Erasmus Medical Centre (Generation R), the Ethical Committee of the Municipal Institute of Medical Investigation and the ethical committees of the hospitals involved in the studies (INMA). All participating women provided informed consent.

Selection criteria for the current study

Women were selected for the current study if they had at least one pre-existing measure of urinary iodine concentration in pregnancy [4,21,22] or urine samples available for iodine measurement, provided that the child had a measure of intelligence quotient (IQ) for ALSPAC and Generation R. In INMA, iodine was measured in all women with additional urine samples available, irrespective of child-IQ data.

Women with multiple pregnancies, in-vitro fertilisation, known thyroid disease, and/or use of thyroid-related medication were excluded (Fig. 1). We restricted analyses to measures from early pregnancy, the most critical time for iodine-dependent brain development [2,23]; hence, for this study, only samples collected ≤ 18 weeks' gestation were included.

Iodine measurements

Urinary iodine concentration (UIC, μg/L) was measured in spot-urine samples collected at a median (25-75th percentile) gestational age of 11.0 (8.0-15.0) weeks in ALSPAC, 13.1 (12.1-14.6) weeks in Generation R, and 13.0 (12.4-13.9) weeks in INMA. Gestational week was established using ultrasound examination (Generation R and INMA) or the date of the last menstrual period (ALSPAC). To adjust for variation in intra- and inter-individual daily

hydration status [24–27], iodine concentration was corrected by dividing by urinary creatinine concentration to give the iodine-to-creatinine ratio (UI/Creat, µg/g). Correcting UIC by use of urinary creatinine concentration reduces intra-individual variation [25], especially in cohorts of the same sex and age-range [i.e., our cohorts were all women of childbearing age (15-44 years)]. The iodine-to-creatinine ratio has previously been used in all three cohorts [4,7,21].

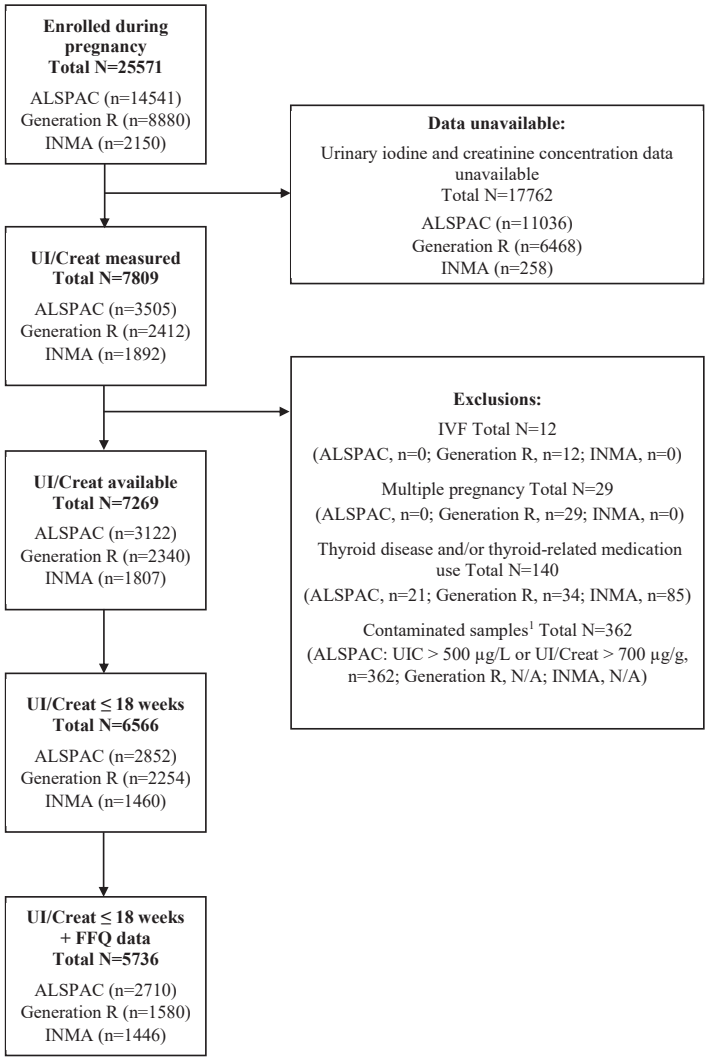

Fig. 1 Flow chart of the study population selection.

[a] Urine samples with UIC > 500 µg/L or UI/Creat > 700 µg/g were excluded only from the ALSPAC cohort, as there was a concern about contamination by the use of iodine-containing test strips (see methods). There was no such concern in Generation R and INMA; therefore, the exclusion criteria were not applicable to urine samples from these two cohorts. FFQ, food frequency questionnaire; IVF, in-vitro fertilisation; N/A, not applicable; UI/Creat, urinary iodine-to-creatinine ratio; UIC, urinary iodine concentration.

In each cohort, we reported both the median (25-75th percentile) UIC (μg/L) and UI/Creat (μg/g). The percentage with UI/Creat <150 μg/g was also reported; this cut-off was informed by the WHO threshold for adequacy in pregnancy (a median UIC ≥150 μg/L) [12] and, when corrected for creatinine concentration, has been used in previous research [4,6,7].

Laboratory analysis

Urinary creatinine concentration was determined by the Jaffe rate method in all cohorts. Urinary iodine concentration was determined as previously described in detail [4,22]; a brief description follows.

ALSPAC: urinary iodine concentration was measured as ^{127}I at the Trace Element Unit, Southampton General Hospital, on a dynamic reaction cell inductively coupled plasma mass spectrometer (NexION 300D Perkin-Elmer, Beaconsfield). The accuracy of the results was verified using the certified reference material (CRM) Seronorm urine Levels 1 and 2 (Nycomed, Norway), and accuracy was also monitored by measurement of EQUIP samples at regular intervals throughout the analysis. Within-run precision gave a relative standard deviation (RSD) of 0.8% at 42 μg/L, 2.5% at 84 μg/L, 0.6% at 149 μg/L, and 2.0% at 297 μg/L. Between-run precision was 8.7% RSD at 42 μg/L, 6.5% at 84 μg/L, 7.2% at 149 μg/L, and 6.8% at 297 μg/L.

Generation R: urinary iodine concentration was measured in Radboud University Medical Centre, Nijmegen, the Netherlands, by the Sandell-Kolthoff method. Iodine calibration was performed using the CRM Seronorm urine Levels 1 and 2 (Nycomed, Norway) and four EQUIP samples that were certified for urinary iodine concentration (Centers for Disease Control and Prevention, USA). At a level of 216 μg/L, the within-assay precision was 5.1% RSD and the between-assay precision was 14.3% RSD (n= 30).

INMA: urinary iodine concentration was measured at the Public Health Laboratory Standards, Basque Government Department of Health, Spain (*Acreditation LE/1108 with ISO 15189 for Clinical Laboratories, National Acreditation Entity*), using a paired-ion reversed-phase, high-performance liquid chromatography with electrochemical detection at a silver-working electrode (Waters Chromatography, Milford, MA). The accuracy of the results was verified using the CRM Seronorm urine Levels 1 and 2 (Nycomed, Norway) and internal quality control samples. Within-run precision was 4.5% RSD at 50 μg/L, 3.2% at 100 μg/L, and 2.0% at 300 μg/L. Between-run precision was 7.9% RSD at 50 μg/L, 3.5% at 100 μg/L, and 2.5 % at 300 μg/L.

In ALSPAC, there was concern that some urine samples had been contaminated by the use of iodine-containing test strips [28], and therefore, samples with UIC >500 μg/L and/or UI/Creat >700 μg/g were excluded (n=412, 11.8%). These cut-offs were based on previous information from ALSPAC and from other studies of UK pregnant women [4,29,30]. Some ALSPAC women had multiple urine samples and in cases, where one sample was contaminated, the results from the next available uncontaminated sample were used (n=50).

Dietary assessment

Maternal diet in all cohorts was assessed using a food-frequency questionnaire (FFQ). This was administered in late pregnancy in ALSPAC (at 32 weeks) and in early pregnancy in Generation R and INMA (at the same time as urine sampling). The FFQ was unquantified in ALSPAC, but was semi-quantitative (SFFQ) in Generation R and INMA. The FFQ was self-administered in ALSPAC and Generation R, and administered by trained interviewers in INMA.

ALSPAC: Detailed information about the design of the questionnaire can be found elsewhere [31,32]. Briefly, women were asked to indicate how frequently "nowadays" they consumed 43 food groups and individual foods with five predefined frequency categories, ranging from "never or rarely" to "more than once a day". Portion sizes were not included in the questionnaire.

Generation R: SFFQ in Generation R represented an adapted version of a validated SFFQ in elderly subjects [33]. In summary, it contained a list of 293 foods and asked about frequency of consumption in the past three months, mostly, therefore, reflecting the first trimester. Questions about portion size, estimated using food photographs or Dutch household measures, methods of preparation, and additions to foods were also included [3,34]. The food list had previously been reduced to 17 main food groups [35], based on the European Prospective Investigation into Cancer and Nutrition SOFT classification (EPIC-SOFT) [36].

INMA: SFFQ was based on an expanded adaptation of a 61-item SFFQ by Willet and colleagues [37] that was developed and validated [38,39]. To summarise, women were asked how often, on average, they had consumed a specified standard portion of 100 food items in early pregnancy (since their last menstrual period until the time of the interview at ~12 weeks), using nine frequency categories, ranging from "never or less than once a month" to "six or more times per day".

For the current study, in each cohort, food intake (g/day) was calculated by multiplying the frequency of consumption by an average standard portion (for the ALSPAC FFQ) or a specified portion (for the SFFQ in Generation R and INMA) of that food. Foods were then classified into food groups and the amounts of individual food items consumed were summed accordingly. To facilitate comparison between cohorts, the classification of food groups used in Generation [36] was used as a template. This required the formation of new food groups in ALSPAC, while the pre-existing food groups were used in Generation R and INMA, with some minor modifications to make them comparable. The definitions of food groups in the individual cohorts are shown in Supplemental Table 1. Separate information about the consumption of table salt and use of iodine-fortified table salt was collected only in the INMA cohort.

Iodine supplement use

Detailed data on the use of potassium iodide and/or iodine-containing multivitamin/mineral supplements during pregnancy were collected only in INMA. For this analysis, we used data on iodine supplements from pre-pregnancy until the end of the first trimester, expressed as mean iodine intake from supplements. In our ALSPAC sample, only two women took a kelp supplement and one took a potassium-iodide supplement; they were excluded from the analysis. Data on the use of iodine-containing multivitamin supplements from preconception until enrolment (at time of urine sample collection) were available only for a sub-set of our Generation R sample (n=381); women who took an iodine-containing supplement (n=345) were excluded in sensitivity analyses.

Maternal and pregnancy characteristics

Information on pregnancy characteristics, anthropometrics, socio-demographic, and environmental exposures was collected by means of questionnaires or extracted from obstetric medical records. Discrete variables were re-categorised, where possible, to facilitate comparisons between cohorts. Exposure factors in these analyses can be classified into three groups: (i) maternal factors: maternal age (ALSPAC: at last menstrual period; Generation R, INMA: at urine collection); pre-pregnancy body mass index (BMI, kg/m^2); ethnicity (ALSPAC: White/non-white; Generation R: Dutch/non-Dutch, where non-Dutch = Indonesian, Cape Verdean, Moroccan, Dutch Antilles, Surinamese, Turkish, other non-Western, Asian, other Western; INMA: Spanish/non-Spanish, where non-Spanish = Latin American, European, others); parity (zero, one, two or more); smoking status (never smoked, smoked initially or until pregnancy was known, continued smoking); and alcohol consumption during pregnancy (yes/no); (ii) markers of socio-economic status*:* education level (ALSPAC: low = no qualification, certificate of secondary education, or vocational; medium = O level or A level; high = a degree; Generation R: low = no education or primary; medium = secondary phase 1 and 2; high = higher phase 1 and 2; INMA: low = no education, unfinished primary, or primary; medium = secondary; high = university degree); monthly net household income (Generation R: low <€1200, medium = €1200-2200, high >€2200); home ownership (ALSPAC: owned/mortgaged, private/other rented, council rented); crowding index (ALSPAC: ≤1 person per room, +1 person per room); family adversity index [ALSPAC: 18-item measure of hardships during pregnancy [40], categorised into: no adversities, mild (0-2), severe (≥3) [41]]; life event score (ALSPAC: exposure to stressful situations during pregnancy); marital status (ALSPAC: never married, married, other; Generation R: unmarried/married); and living with father's child/partner (ALSPAC, INMA: yes/no); and (iii) child factors: child's sex (male/female).

Statistical analyses

To study the associations of maternal characteristics and diet with iodine status, we used UI/Creat as a measure of individual iodine status. UI/Creat was not normally distributed

but right-skewed. To meet parametric-test assumptions, UI/Creat was transformed using the natural logarithm. Outliers were assessed by visual inspection of box-plots. We assessed non-linearity of the associations of each continuous independent variable with UI/Creat by adding their squared term to the regression models, and also by plotting each potential determinant variable against UI/Creat and comparing the fit (R^2) of a linear *vs.* quadratic function through the data points.

Multiple linear regression models with log-transformed UI/Creat as the dependent variable were performed for each cohort using two models: Model 1 included maternal and pregnancy factors, markers of socio-economic status and child's sex as independent variables; Model 2 included variables from Model 1 plus dietary intake of food groups.

Analyses of the dietary influences on UI/Creat (Model 2) were also adjusted for estimated energy intake (kcal/day). Effect estimates (unstandardised B coefficients) for food groups were expressed per 100 g and also per portion (g). The increase in the geometric mean of UI/Creat per 100 g and per portion increase in intake and its 95% confidence interval (CI) were calculated by back-transformation [exponentiation (EXP)] of the effect estimates and 95% CIs from logarithmic scale. The following formulae were used for the back-transformations: effect estimate per 100 g = EXP (intercept + *B* * 100) – EXP (intercept); lower 95% CI = EXP [intercept + (*B* * 100) – (1.96 * standard error of estimate (SEE) *100)] – EXP (intercept); and upper 95% CI = EXP [intercept + (*B* * 100) + (1.96 * SEE * 100)] – EXP (intercept). For calculations per portion size, the multiplication by 100 in the formulae is replaced with the portion size (g) for each food group accordingly. When calculating the effect estimates from Model 2, all categorical covariates were set to their reference group and the continuous covariates gestational week, maternal age, BMI, and energy intake were centered to their means.

We conducted four types of sensitivity analyses: (i) under-reporting: to account for potential under-reporting of energy intake in Model 2, in all cohorts, we excluded women with energy intakes below the 5th percentile; (ii) iodine supplements: we adjusted Model 2 for iodine-supplement use in INMA (as the only cohort with complete iodine-supplement data) and excluded iodine-supplement-users in Generation R (as data were available only for a sub-set of the sample); (iii) gestational age: as the median gestational week at urine sampling was later in INMA and Generation R than in ALSPAC, we performed sensitivity analyses to test the associations with iodine status in the first trimester, using samples collected up to 13 weeks; and (iv) covariate creatinine-adjustment method: since age, BMI, and ethnicity are known predictors of urinary creatinine concentration [42] and urinary creatinine can also vary during gestation [43], this could result in spurious associations of these variables with the ratio of UI/Creat; we, therefore, performed sensitivity analyses for these variables (BMI, age, ethnicity, and gestational age) using Model 1 and 2 with the (natural) log-transformed UIC (μg/L) unadjusted for creatinine as our dependent variable and added creatinine concentration as a separate independent variable to the models. This method has been recommended previ-

ously [42] and ensures that UIC is adjusted for dilution by creatinine concentration, while the associations of the other variables with UIC are independent of urinary creatinine.

All analyses were conducted using multiply-imputed data to account for missing data on socio-demographic variables. Multiple imputation was performed using the automatic method in SPSS. A total of 20 datasets were generated and analysed using standard multiple imputation procedures [44]. Detailed information about the imputed variables is provided in the Electronic Supplementary Material. Missing FFQ data were not imputed, as diet has a wide inter-person variability hence imputation of dietary data would not be sufficiently accurate. All statistical analyses were performed with IBM SPSS Statistics version 24.0 (IBM Corp., Armonk, NY, USA). Values were considered statistically significant at $P<0.05$.

Results

Sample characteristics

After exclusions, the final study population comprised 6566 pregnant women: 2852 from ALSPAC, 2254 from Generation R, and 1460 from INMA (Fig. 1). Descriptive statistics of mothers by cohort are shown in Table 1. Maternal age varied across cohorts, with women in ALSPAC having a lower mean ± standard deviation (SD) age than women in INMA [28.7 (±4.5) vs. 31.4 (±4.1) years, respectively]. Median BMI was within the healthy range in all cohorts (Table 1). The majority of mothers defined themselves as White in ALSPAC (98.2%), Spanish in INMA (91.8%), while slightly more than half of the women in Generation R said they were non-Dutch (51.4%). Most women were nulliparous and non-smokers, with similar proportions between cohorts.

Iodine status

The median UI/Creat (25-75th percentile) was 121 (81-193) µg/g in women from the UK (62.8% <150 µg/g), 151 (96-255) µg/g in women from Spain (49.5% <150 µg/g), and 210 (140-303) µg/g in women from the Netherlands (28.8% <150 µg/g) (Table 2).

Association with socio-demographic and lifestyle factors

In multivariable models (adjusted Models 1 and 2), gestational week of urine sample, maternal age, and BMI were associated with UI/Creat in all three cohorts. Gestational week at urine sampling (≤18 weeks) was positively associated with UI/Creat in ALSPAC (B=0.051, $P<0.0001$) but not in the other two cohorts (Table 3). However, in sensitivity analyses restricted to samples collected up to 13 weeks, there was an association of gestational week with UI/Creat in all three cohorts (ALSPAC: B=0.029, $P<0.001$, n=1951; Generation R: B=0.031, P=0.049, n=1094; INMA: B=0.079, P=0.045, n=747) with a larger effect size in Generation R and INMA than in the analyses up to 18 weeks. In the sensitivity analysis

using covariate creatinine-adjustment, the results for the association of gestational week up to 18 weeks with UIC did not substantially differ from those with UI/Creat in ALSPAC and INMA, while in Generation R, the effect size was higher, reaching statistical significance (Supplemental Table 3).

There was a positive association of maternal age with UI/Creat (ALSPAC: *B*=0.014, *P*<0.0001; Generation R: *B*=0.018, *P*<0.0001; INMA: *B*=0.020, *P*=0.0001). After further adjusting for maternal diet and energy intake estimated from the FFQs (Model 2), the effect size of age was attenuated by 16-30% across cohorts, but remained statistically significant (Table 3). The positive association with age remained in all cohorts, except in Model 2 for ALSPAC, when using the covariate creatinine-adjustment method of UIC (Supplemental Table 3).

There was a negative association of BMI with UI/Creat (ALSPAC: *B*= -0.013, *P*=0.0001; Generation R: *B*= -0.011, *P*<0.0001; INMA: *B*= -0.013, *P*=0.005), which, after adjustment for maternal diet and energy intake remained statistically significant (Table 3, Model 2). However, BMI was not associated with UIC (μg/L) with the covariate creatinine-adjustment method in Generation R and INMA but remained negatively associated with UIC in ALSPAC, though with a lower effect size (Supplemental Table 3).

Cohort-specific socio-demographic and lifestyle factors were identified as determinants of iodine status. In Generation R, maternal ethnicity and smoking were associated with UI/Creat (Table 3). Compared to the Dutch women, Moroccan, Turkish and other non-Western women had a higher UI/Creat, whereas Surinamese and those from the Dutch Antilles had a lower UI/Creat. Some of these effects were attenuated after accounting for maternal diet in Model 2 (Table 3). Similarly to UI/Creat, UIC (with covariate creatinine-adjustment) also differed by ethnicity; Moroccan, Turkish and other non-Western women still had a significantly higher UIC than the Dutch, while the UICs of Surinamese and Dutch Antilles women did not significantly differ from those of the Dutch women (Supplemental Table 3). Generation R women who reported smoking vs. those who never smoked had a lower UI/Creat, which remained statistically significant after adjustment for maternal diet (Table 3, Model 1 and 2). In ALSPAC, family adversity index (severe *vs.* none; *B*= -0.100, *P*=0.016), and marital status (married vs. never-married; B=0.095, *P*=0.015) were associated with UI/Creat, even after adjusting for maternal diet (Table 3). Results for all predictors included in the multivariable models are presented in Supplemental Table 2.

Dietary influences on iodine status

As not all women with urinary iodine measurements before 18 weeks had also completed an FFQ, numbers for these analyses were lower for all cohorts (Fig. 1): ALSPAC (n=2710), Generation R (n=1580), INMA (n=1446). Descriptive statistics of dietary intakes of food groups for pregnant women in each cohort are presented in Supplemental Table 4.

Table 1 Descriptive statistics [a] of the study population by cohort.

Sample characteristics	ALSPAC (n=2852)	Generation R (n=2254)	INMA (n=1460)
Maternal factors			
Age [b,c] (years), mean (±SD)	28.7 (±4.5)	29.9 (±5.0)	31.4 (±4.1)
Pre-pregnancy BMI (kg/m^2), median (IQRs) [c]	22.3 (20.5 - 24.6)	23.5 (21.5 - 26.4)	22.5 (20.8 - 25.0)
Ethnicity [d], n (%)			
Reference group	2800 (98.2)	1095 (48.6)	1340 (91.8)
Non-white	52 (1.8)	N/A	N/A
Non-Dutch	N/A	1159 (51.4) [e]	N/A
Non-Spanish	N/A	N/A	120 (8.2)
Parity [c], n (%)			
0	1354 (47.5)	1279 (56.7)	806 (55.2)
1	965 (33.8)	665 (29.5)	544 (37.3)
≥ 2	533 (18.7)	310 (13.8)	110 (7.5)
Smoking status, n (%)			
Never smoked	2169 (76.1)	1671 (74.2)	1020 (69.9)
Stopped smoking	333 (11.7)	211 (9.3)	187 (12.8)
Continued smoking	350 (12.2)	372 (16.5)	253 (17.3)
Alcohol consumption, n (%)			
No	1350 (47.4)	1458 (64.7)	1330 (91.1)
Yes	1502 (52.6)	796 (35.3)	130 (8.9)
Markers of socio-economic status			
Education level, n (%)			
Low	576 (20.2)	247 (11.0)	337 (23.1)
Medium	1780 (62.4)	995 (44.1)	581(39.8)
High	496 (17.4)	1012 (44.9)	542 (37.1)
Net household income (€ per month), n (%)			
Low < €1200	N/A	492 (21.8)	N/A
Medium €1200-2200	N/A	597 (26.5)	N/A
High > €2200	N/A	1165 (51.7)	N/A
Home ownership, n (%)			
Owned/mortgaged	2425 (85.0)	N/A	N/A
Private/other rented	236 (8.3)	N/A	N/A
Council rented	191 (6.7)	N/A	N/A
Crowding index, n (%)			
≤ 1 person per room	2747 (96.3)	N/A	N/A
+ 1 person per room	105 (3.7)	N/A	N/A
Family adversity index, n (%)			
None 0	1395 (48.9)	N/A	N/A
Mild 1-2	1124 (39.4)	N/A	N/A
Severe > 3	333 (11.7)	N/A	N/A

Table 1 Descriptive statistics [a] of the study population by cohort. (continued)

Sample characteristics	ALSPAC (n=2852)	Generation R (n=2254)	INMA (n=1460)
Life event score, median (IQRs)	3.0 (2.0 - 5.0)	N/A	N/A
Marital status, n (%)			
Never-married	355 (12.5)	1136 (50.4)	N/A
Married	2357 (82.6)	1118 (49.6)	N/A
Other [f]	140 (4.9)	N/A	N/A
Living with a partner, n (%)			
Yes	2720 (95.4)	N/A	1445 (99.0)
No	132 (4.6)	N/A	15 (1.0)
Child factors			
Child's sex [c], n (%)			
Male	1405 (49.3)	1147 (50.9)	737 (50.5)
Female	1447 (50.7)	1107 (49.1)	723 (49.5)

[a] Data presented as mean (±SD) for continuous normally distributed variables, median (IQRs) for continuous non-normally distributed variables and n (%) for categorical variables.

[b] Maternal age at urine sample collection, except in ALSPAC (age at last menstrual period).

[c] Data were not imputed, due to no missing values for age (in ALSPAC, Generation R), pre-pregnancy BMI (INMA), parity (Generation R) and child's sex (ALSPAC). The rest of the data are shown after imputation of the missing values (see methods).

[d] ALSPAC (Reference group=White); Generation R (Reference group=Dutch, Non-Dutch=Indonesian, Cape Verdean, Moroccan, Dutch Antilles, Surinamese, Turkish, Other non-Western, Asian, or other Western, see Table 3); INMA (Reference group=Spanish, Non-Spanish=Latin American, European, or Others).

[e] Non-Dutch group in Generation R presented in detail in Table 3.

[f] ALSPAC (Other=widowed, divorced, or separated).

Abbreviations: BMI, body mass index; IQRs, interquartile ranges; N/A, data not available or not applicable; SD, standard deviation.

Table 2 Urinary iodine status in early pregnancy (≤ 18 gestational weeks) expressed as UIC, UI/Creat and proportion of mothers with UI/Creat below 150 µg/g.

	ALSPAC (n=2852)	Generation R (n=2254)	INMA (n=1460)
Gestational age at urine sampling, weeks [a]	11.0 (8.0 - 15.0)	13.1 (12.1 - 14.6)	13.0 (12.4 - 13.9)
Urinary iodine concentration (UIC), µg/L [a]	95 (56 - 151)	165 (94 - 277)	130 (76 - 219)
Iodine-to-creatinine ratio (UI/Creat), µg/g [a]	121 (81 - 193)	210 (140 - 303)	151 (96 - 255)
UI/Creat < 150 µg/g, n (%)	1792 (62.8)	650 (28.8)	723 (49.5)

Abbreviations: UI/Creat, urinary iodine-to-creatinine ratio; UIC, urinary iodine concentration.

[a] Data presented as median (25^{th} - 75^{th} percentiles).

Table 3 Determinants [a] of urinary iodine-to-creatinine ratio measured at ≤ 18 gestational weeks, statistically significant in at least one cohort.

Determinants	ALSPAC (n=2852)						Generation R (n=2254)						INMA (n=1460)					
	Adjusted Model 1 [b]			Adjusted Model 2 [c]			Adjusted Model 1 [b]			Adjusted Model 2 [c]			Adjusted Model 1 [b]			Adjusted Model 2 [c]		
	n	*B*	*P*	*n*	*B*	*P*	*n*	*B*	*P*	*n*	*B*	*P*	*n*	*B*	*P*	*n*	*B*	*P*
Gestational age at urine sampling, weeks	2852	0.051	<0.001	2710	0.052	<0.001	2254	0.007	0.300	1580	0.011	0.133	1460	0.004	0.755	1446	-0.009	0.513
Age [d], years	2852	0.014	<0.001	2710	0.010	0.002	2254	0.018	<0.001	1580	0.015	<0.001	1460	0.020	<0.001	1446	0.014	0.008
Pre-pregnancy BMI, kg/m^2	2852	-0.013	<0.001	2710	-0.012	<0.001	2254	-0.011	<0.001	1580	-0.013	<0.001	1460	-0.013	0.005	1446	-0.010	0.033
Family adversity index																		
None 0	1395	Ref.		1334	Ref.		N/A	.	.	.	.	.	N/A	.	.	.	.	.
Mild 1-2	1124	-0.007	0.765	1069	-0.003	0.906	N/A	.	.	.	.	.	N/A	.	.	.	.	.
Severe > 3	333	-0.100	0.016	307	-0.086	0.046	N/A	.	.	.	.	.	N/A	.	.	.	.	.
Marital status																		
Never-married	355	Ref.		320	Ref.		1136	Ref.		825	Ref.		N/A	.	.	.	.	.
Married	2357	0.095	0.015	2269	0.119	0.003	1118	0.030	0.285	755	0.044	0.168	N/A	.	.	.	.	.
Other [e]	140	0.028	0.666	121	0.051	0.447	N/A	.	.	.	.	.	N/A	.	.	.	.	.
Ethnicity [f]																		
Reference group	2800	Ref.		2674	Ref.		1095	Ref.		930	Ref.		1340	Ref.		1328	Ref.	
Non-white	52	-0.018	0.851	36	0.043	0.676	N/A	.	.	.	.	.	N/A	.	.	.	.	.
Non-Dutch:	N/A	.	.	.	.	.							N/A	.	.	.	.	.
- *Indonesian*	N/A	.	.	.	.	.	72	-0.047	0.494	58	-0.081	0.286	N/A	.	.	.	.	.
- *Cape Verdean*	N/A	.	.	.	.	.	87	-0.013	0.848	41	0.065	0.502	N/A	.	.	.	.	.
- *Moroccan*	N/A	.	.	.	.	.	165	0.255	<0.001	72	0.182	0.028	N/A	.	.	.	.	.
- *Dutch Antilles*	N/A	.	.	.	.	.	65	-0.174	0.025	37	-0.225	0.031	N/A	.	.	.	.	.
- *Surinamese*	N/A	.	.	.	.	.	196	-0.115	0.017	111	-0.160	0.014	N/A	.	.	.	.	.
- *Turkish*	N/A	.	.	.	.	.	226	0.391	<0.001	103	0.360	<0.001	N/A	.	.	.	.	.
- *Other, non-Western*	N/A	.	.	.	.	.	107	0.157	0.011	53	0.125	0.137	N/A	.	.	.	.	.
- *Asian*	N/A	.	.	.	.	.	32	0.091	0.383	19	0.028	0.835	N/A	.	.	.	.	.

- *Other, Western*	N/A	.	.	.	.	.	209	0.052	0.228	156	0.062	0.213	N/A	.	.	.	.	.
Non-Spanish	N/A	.	.	.	.	.	N/A	.	.	.	.	.	120	-0.016	0.810	118	-0.003	0.961
Smoking status																		
Never smoked	2169	Ref.		2077	Ref.		1671	Ref.		1202	Ref.		1020	Ref.		1011	Ref.	
Stopped smoking	333	-0.003	0.943	312	-0.010	0.797	211	-0.093	0.035	147	-0.121	0.020	187	-0.060	0.296	186	-0.060	0.287
Continued smoking	350	0.018	0.620	321	0.060	0.116	372	-0.008	0.831	231	0.049	0.296	253	-0.001	0.990	249	0.020	0.707

[a] Effect estimates (*B*=unstandardised regression coefficient) and *P*-values from multiple linear regression models performed for each cohort with (natural) log-transformed iodine-to-creatinine ratio (UI/Creat) as the dependent variable and maternal characteristics and dietary intakes as independent variables (for full models, see footnotes b and c). Reported *B* coefficients represent the change in the mean (natural) log of UI/Creat per unit increase in the continuous independent variables and for each category compared to the reference for the categorical independent variables.

[b] Adjusted Model 1 (adjusted for maternal and pregnancy characteristics); ALSPAC (R^2=0.123, $P < 0.0001$): gestational age (weeks), age (years), pre-pregnancy BMI (kg/m^2), ethnicity, parity, smoking status, alcohol consumption, education, home ownership, crowding index, family adversity index, life event score, marital status and child's sex; Generation R (R^2=0.086, $P < 0.0001$): gestational age (weeks), age (years), pre-pregnancy BMI (kg/m^2), ethnicity, parity, smoking status, alcohol consumption, education, net household income, marital status and child's sex; INMA (R^2=0.021, $P < 0.0001$): gestational age (weeks), age (years), pre-pregnancy BMI (kg/m^2), ethnicity, parity, smoking status, alcohol consumption, education, living with a partner and child's sex .

[c] Adjusted Model 2 (adjusted for maternal and pregnancy characteristics + dietary intakes); ALSPAC (R^2=0.147, $P < 0.0001$): Model 1 + energy intake (kcal/day) + intake of vegetables (g/day), fruit (g/day), nuts and seeds (g/day), potatoes (g/day), legumes (g/day), cereals and cereal products (g/day), cakes, confectionery and added sugar (g/day), added fats (g/day), milk and dairy products (g/day), meat and meat products (g/day), eggs (g/day), fish and shellfish (g/day), processed and fried foods (g/day), non-alcoholic beverages (g/day), miscellaneous (g/day); Generation R (R^2=0.091, $P < 0.0001$): Model 1 + energy intake (kcal/day) + intake of vegetables (g/day), fruit (g/day), nuts and seeds (g/day), potatoes (g/day), legumes (g/day), cereals and cereal products (g/day), cakes, confectionery and added sugar (g/day), added fats (g/day), milk and dairy products (g/day), meat and meat products (g/day), eggs (g/day), fish and shellfish (g/day), condiments and seasoning (g/day), processed and fried foods (g/day), non-alcoholic beverages (g/day), alcoholic beverages (g/day), miscellaneous (g/day); INMA (R^2=0.060, $P < 0.0001$): Model 1 + energy intake (kcal/day) + intake of vegetables (g/day), fruit (g/day), nuts and seeds (g/day), potatoes (g/day), legumes (g/day), cereals and cereal products (g/day), cakes, confectionery and added sugar (g/day), added fats (g/day), milk and dairy products (g/day), meat and meat products (g/day), eggs (g/day), fish and shellfish (g/day), condiments and seasoning (e.g., salt) (g/day), processed and fried foods (g/day), non-alcoholic beverages (g/day), alcoholic beverages (g/day).

[d] Maternal age at urine sample collection, except in ALSPAC (age at last menstrual period).

[e] ALSPAC (Other=widowed, divorced, or separated).

[f] ALSPAC (Reference group=White); Generation R (Reference group=Dutch, Non-Dutch=Indonesian, Cape Verdean, Moroccan, Dutch Antilles, Surinamese, Turkish, Other non-western, Asian, or Other western); INMA (Reference group=Spanish, Non-Spanish=Latin American, European, or Others).

Abbreviations: BMI, body mass index; N/A, data not available or not applicable; Ref, reference category; UI/Creat, urinary iodine-to-creatinine ratio.

Table 4 Multivariable associations of food group intakes estimated from FFQ (per portion size, g) [a] with urinary iodine-to-creatinine ratio measured at ≤ 18 gestational weeks, statistically significant in at least one cohort.

Food group	Standard portion (g) [a]	Description [a]	ALSPAC (n=2710)		Generation R (n=1580)		INMA (n=1446)	
			B (95% CI) [b]	*P* [c]	*B* (95% CI) [b]	*P* [d]	*B* (95% CI) [b]	*P* [e]
Fruit	80 g	*a portion '5-a-day'*	2.22 (0.47, 4.03)	0.012	0.90 (-0.90, 2.75)	0.328	-3.51 (-7.16, 0.20)	0.064
Nuts and seeds	30 g	*a handful*	5.08 (-0.63, 11.53)	0.083	12.92 (4.83, 21.73)	0.001	18.63 (-10.61, 52.17)	0.223
Cereals and cereal products	36 g	*a medium slice of bread*	0.51 (-0.33, 1.36)	0.238	4.25 (2.23, 6.31)	<0.001	0.07 (-5.12, 5.40)	0.978
Cakes, confectionery and added sugar	50 g	*a chocolate bar*	2.39 (0.25, 4.63)	0.028	2.64 (-1.13, 6.57)	0.172	-9.19 (-22.95, 5.59)	0.217
Added fats	10 g	*spread on a slice of bread*	1.12 (-0.14, 2.41)	0.082	2.70 (0.39, 5.08)	0.022	-4.19 (-11.03, 2.89)	0.243
Milk and dairy products	200 g	*a glass of milk*	7.77 (5.65, 9.99)	<0.001	4.74 (2.01, 7.56)	0.001	14.07 (5.07, 23.45)	0.002
Meat and meat products	130 g	*a medium portion chicken breast*	1.65 (-2.96, 6.78)	0.497	3.75 (-5.35, 13.87)	0.433	-41.48 (-63.44, -16.20)	0.002
Eggs	50 g	*an average egg*	2.64 (-0.60, 6.12)	0.112	28.10 (12.27, 46.47)	<0.001	39.69 (-6.67, 96.68)	0.099
Fish and shellfish	120 g	*a medium cod fillet*	6.18 (1.07, 11.87)	0.016	2.42 (-19.04, 30.69)	0.845	34.08 (3.28, 69.37)	0.029
Condiments and seasoning (e.g., salt) [f]	5 g	*a level teaspoon (WHO)* [g]	N/A	.	1.34 (-1.50, 4.29)	0.359	214.41 (56.88, 465.35)	0.003

[a] Portion sizes are based on Food Standard Agency (1998) Food Portion Sizes (3ed.) London: TSO.

[b] Effect estimates (*B*=unstandardised regression coefficient) represent the actual change in the geometric mean of UI/Creat (μg/g) associated with a portion size increase in intake of a food group. *B* coefficients and their 95% CIs are calculated by back-transformation from logarithmic scale (see methods). Values are adjusted for dietary intake of other food groups, energy intake and other potential confounders (for full models, see Table 3, footnote c). When calculating the *B* coefficients all categorical covariates were set to their reference group and the continuous covariates gestational age, maternal age, pre-pregnancy BMI and energy intake were centred to their means.

[c] *P*-value adjusted for energy (kcal/day) + gestational age (weeks), age (years), pre-pregnancy BMI (kg/m^2), ethnicity, parity, smoking status, alcohol consumption, education, home ownership, crowding index, family adversity index, life event score, marital status and child's sex (R^2=0.147, $P < 0.0001$).

[d] *P*-values adjusted for energy (kcal/day) + gestational age (weeks), age (years), pre-pregnancy BMI (kg/m^2), ethnicity, parity, smoking status, alcohol consumption, education, net household income, marital status and child's sex (R^2=0.091, $P < 0.0001$).

[e] *P*-values adjusted for energy (kcal/day) + gestational age (weeks), age (years), pre-pregnancy BMI (kg/m^2), ethnicity, parity, smoking status, alcohol consumption, education, living with a partner and child's sex (R^2=0.060, $P < 0.0001$).

[f] In INMA this food group also includes iodised salt. Separate information about the consumption of table salt and use of iodine-fortified table salt was collected only in the INMA cohort.

[g] Maximum daily salt intake, as recommended by WHO (5 g/day salt, or 2 g/day Sodium).

Abbreviations: BMI, body mass index; 95% CI, confidence interval; FFQ, food frequency questionnaire; N/A, data not available; UI/Creat, urinary iodine-to-creatinine; WHO, World Health Organisation.

"Milk and dairy products" was the only food group positively associated with UI/Creat in all three cohorts: ALSPAC (*B*=3.73, *P*<0.0001); Generation R (*B*=2.34, *P*=0.001); INMA (*B*=6.92, *P*=0.002) (Supplemental Table 5). Based on the fully-adjusted models, one-portion increase in consumption of milk and dairy products (e.g., a glass of milk, 200 g) [45] was associated with a 5-14 μg/g increase in the geometric mean of UI/Creat (Table 4). Intake of "fish and shellfish" was positively associated with UI/Creat in pregnant women in Spain (INMA) and the UK (ALSPAC), though the effect size per 100 g was more than five times larger in INMA women (*B*=28.04, *P*=0.029 vs. *B*=5.10, *P*=0.017).

Cohort-specific dietary determinants were also identified (Table 4). In ALSPAC, consumption of fruit (*P*=0.012), cakes and confectionery (*P*=0.028) were positively associated with UI/Creat. Intake of cereals and cereal products (*P*<0.0001), eggs (*P*=0.0001), nuts and seeds (*P*=0.001), and added fat (*P*=0.022) were all positively associated with UI/Creat in Generation R. Higher salt intake (including iodised salt) (per 1 g) was associated with higher UI/Creat (*P*=0.003), while increasing meat intake was associated with lower UI/Creat (*P*=0.002) in INMA.

Excluding women with extremely low energy intakes (< 5th percentile) did not considerably change our results, except for small changes in INMA where the association between fish intake and UI/Creat was borderline significant but the effect size remained relatively unchanged (*data not shown*). In INMA, mean daily iodine-supplement intake was an important determinant of UI/Creat (Standardised *B*=0.235, *P*<0.0001). Adjusting for iodine-supplement use in INMA attenuated the result for maternal age (*B*=0.012, *P*=0.018), but did not change the rest of the results substantially (*data not shown*). In Generation R, the exclusion of iodine-supplement users resulted in higher effect estimates for some ethnic groups (e.g., Moroccan, Turkish and other Western); the associations between intake of non-alcoholic and alcoholic beverages and UI/Creat reached statistical significance but the effect sizes remained unchanged; the rest of the results remained relatively unchanged (*data not shown*).

Discussion

In this study of pregnant women, several dietary (milk and dairy products) and maternal factors (maternal age, BMI, gestational week) were associated with UI/Creat across all cohorts in adjusted models. Furthermore, important cohort-specific dietary determinants were identified, such as fish intake in ALSPAC (UK), egg and cereal/cereal product intake in Generation R (Netherlands), and fish, salt, and meat intake in INMA (Spain).

The population of pregnant women from the Netherlands was iodine-sufficient, while populations from the UK and Spain were mildly-to-moderately deficient. It should be noted that the ALSPAC median-UIC value, although from samples that are nearly 25 years old,

is almost identical to the UK value from the 2017 Global Scorecard for Iodine Nutrition in pregnant women, i.e., 95 µg/L vs. 99 µg/L [15].

The differences in iodine status between the countries may be partly explained by differing use of iodised salt. The iodised salt penetration rate in households and the food industry (e.g., bread-making) in the Netherlands has been estimated as 60% and 70%, respectively, while a 16% penetration rate has been reported in Spanish households [46]. By contrast, iodisation of salt was never common in the UK [47], and even nowadays, its availability is very limited (21.5%); furthermore, the iodine concentration of the major UK brand is low [48].

Association with socio-demographic and lifestyle factors

Gestational week of urine samples was positively associated with a UI/Creat in ALSPAC but not in the other cohorts. This may be because ALSPAC samples were collected at an earlier gestational age than in the other cohorts; if the greatest increase in urinary iodine excretion is in the first weeks of pregnancy, the effect of gestational age may be attenuated in later samples (i.e., up to 18 weeks as in our study). Indeed, in sensitivity analyses, when we restricted analysis to samples collected up to 13 weeks, the effect size was higher in the other two cohorts. The positive association between gestational week of urine sample with UI/Creat in early pregnancy could be attributed to an increase in glomerular filtration rate and subsequent renal iodine loss in early pregnancy [49,50], though data are conflicting as studies report both an increase and a decrease in the UI/Creat [51–54]. As urinary iodine excretion in early vs. late pregnancy stages might be higher as a result of increased renal clearance of iodine, using early measurement to assess status might overestimate true intake [55]. Creatinine clearance has also been shown to vary during gestation [43], which could have biased our results when using UI/Creat. However, gestational week was also positively associated with UIC (µg/L) alone when including creatinine separately in the model.

In all three cohorts, BMI was negatively associated with UI/Creat. In a study in non-pregnant adults [56], creatinine clearance was positively associated with BMI, independent of adiposity, and a positive association with lean thigh-tissue area was reported; this suggests that the association of creatinine with BMI might be explained by lean body mass. The negative association between BMI and UI/Creat that we report may therefore be partly explained by the use of creatinine adjustment and may highlight potential issues with the use of this measure of iodine status [42,57]. Indeed, in sensitivity analysis, we did not find an association of BMI with UIC alone in two of the cohorts when we adjusted for creatinine by including it as a covariate in the model instead of using the ratio of UI/Creat.

There was a positive association of maternal age with UI/Creat in all cohorts. Age was still significantly associated with UI/Creat after adjustment for dietary intake though the effect size was attenuated; it was further reduced when iodine-supplement use in INMA was accounted for, suggesting that some of the effect of age could be explained by diet and supplement use in older women. Similarly to BMI, age is a known predictor of urinary creatinine [42]. However,

in sensitivity analysis when adjusting for creatinine separately in the model, unlike BMI, age remained positively associated with UIC but with a lower effect size.

The iodine status of pregnant women from the Netherlands varied by ethnic origin, with higher iodine status in Moroccan, Turkish and other non-Western women, and lower iodine status in Surinamese and Dutch Antilles women, even after adjusting for socio-demographic factors. Variation in diet may partially explain these differences as some of the effect estimates were attenuated when adjusting for dietary intake. Alternatively, there may be genetic variability in iodine or creatinine clearance [42,58]. Indeed, we found that when using UIC and adjusting for urinary creatinine separately in the model, UIC varied similarly to UI/Creat between the ethnic groups though some of the associations were attenuated and were no longer significant (e.g., for Surinamese and Dutch Antilles women). It should be noted that numbers in some of these ethnic-group categories were relatively small (n<100). Ethnicity was not significantly associated with iodine status in ALSPAC or INMA, but this may reflect the small sample sizes of other ethnic groups in these cohorts. Ethnic differences in iodine status could help to identify subgroups at high-risk for iodine insufficiency; in countries with a large proportion of diverse ethnic groups, culturally-specific approaches to improve dietary adequacy may be more suitable than a single solution for the whole population.

Dietary influences on iodine status

The only food group that was positively associated with UI/Creat in all three cohorts was "milk and dairy products", demonstrating their significant role as an important dietary determinant of iodine status in pregnancy. This finding is consistent with the results of other studies in European pregnant women, i.e., Norway [59,60], Iceland [61], Italy [62], Spain [63], and the UK [52,64], as well as studies of pregnant women in Australia [65].

Based on our model, a portion of "milk and dairy products" equivalent to a glass of milk (200 g) was associated with 5 to 14 µg/g increase in UI/Creat across cohorts. The effect sizes for milk differed between cohorts; the highest effect size was in Spain, while the lowest was in the Netherlands. This is in line with the milk-iodine concentration in each country (i.e., ~26 µg in Spain [66], ~15 µg in the Netherlands [67], and 15 µg in the UK in 1990/1991 [68]). The results might be different if repeated now as the iodine concentration in UK milk is higher than estimated when ALSPAC women were recruited in 1990/1991, i.e., 427 vs. 150 µg/kg [68,69].

Consumption of eggs and fish were positively associated with UI/Creat, though not consistently across the cohorts; the association with egg intake was statistically significant only in the Netherlands, while intake of fish and shellfish was associated with UI/Creat only in Spain and the UK. The effect size for eggs was higher than that expected, given their iodine content, but similar to values reported previously [60]. These higher values may reflect the consumption of eggs with salt in the Netherlands, which is likely to be iodised. An average portion of fish (120 g) was associated with some 6 to 34 µg/g increase in UI/Creat, across cohorts. Variation in the effect size could partly reflect the variability in average fish consumption, particularly

of white fish, which is a good iodine source [70] (e.g., pregnant women in Spain consumed a higher amount of white fish daily than did women in the UK). The wide CIs around the estimates probably relate to the variability in fish-iodine concentration [14] (i.e., the proportion of oily fish in the food group has a much lower iodine concentration [70]) and to the irregular nature of fish consumption which may not be captured by a spot-UI/Creat.

Consumption of cereals and cereal products was a statistically significant determinant of UI/Creat only in the Netherlands; this association is probably driven by consumption of bread which is made with iodised salt in the Netherlands [71], whereas without iodised salt, bread has a low iodine concentration and was not found to be a predictor of iodine status in the UK or Spanish cohorts. Intake of iodised table salt was measured only in pregnant women from Spain; 1 g of salt was associated with around 32 μg/g increase in UI/Creat. Both of these results suggest that iodised salt consumed either discretionarily (e.g., as table salt in Spain), or as part of processed foods (e.g., in bread in the Netherlands) is an important dietary determinant of iodine status. We observed the highest UI/Creat in the Netherlands, followed by Spain, and the lowest in the UK, suggesting that iodised-salt use might be a key dietary factor with large influence on iodine status.

Surprisingly, meat intake was negatively associated with UI/Creat in the Spanish cohort, even after controlling for socio-demographic variables. Higher urinary creatinine concentrations have been reported in individuals with a high-meat diet, which may account for the lower ratio [72,73].

Some of our other food-group associations are difficult to explain and may be chance findings, e.g., the positive association with fruit, and cakes/confectionary in the UK. However, fruit intake was also associated with urinary iodine excretion in Norwegian pregnant women [59] and the finding might warrant further investigation.

Although we have observed significant increases in UI/Creat, the effect sizes were relatively small and the total explained variance in UI/Creat (R^2) was low. This is probably explained by the large day-to-day variability in iodine intake which cannot be captured in a single spot-urine sample and the measurement errors of dietary assessment methods in capturing habitual iodine intake. Moreover, as a result of the physiological changes occurring during gestation (e.g., increased renal iodine clearance), pregnancy may not represent a steady state of iodine metabolism (intake vs. excretion) [10].

Strengths and limitations

Strengths of our study include the large sample size, the inclusion of pregnant women from three geographically and culturally different populations. Furthermore, we explored associations with the entire range of food groups, rather than focusing on a few groups or isolated foods as in previous studies [62,63]. However, our study also has a number of limitations. Firstly, the use of an FFQ for measuring diet and the use of spot-urine samples for estimating individual iodine intake have their methodological disadvantages [24–26,74]. Although we only had

spot-urine samples which might not reflect individual iodine intake or status, we used UI/Creat which has been shown as a valid alternative to the 24-hour urinary iodine excretion when used in homogenous population groups [75]. Although urinary iodine concentration was measured in three different laboratories using different assays, each laboratory ensured accuracy by use of certified reference materials. Exploring associations between data from an FFQ and spot-UI/Creat can also be problematic, as an FFQ is designed to measure habitual diet [76], whereas a spot-urine sample reflects iodine intake in the last 24-48 hours [11]; this may be reflected in the more consistent association between UI/Creat and intake of daily food items (e.g., milk) than those infrequently consumed (e.g., fish). However, our large cohort sample size would have helped to overcome this limitation to some extent. Second, it should be noted that in ALSPAC there was a time difference in the administration of the FFQ (at 32 weeks) and urine-sample collection (≤18 weeks); hence the FFQ might not reflect diet during early pregnancy. Third, although we harmonised the classification of foods into food groups, there were some differences in the foods included in each group which may explain variation in effect sizes between cohorts (i.e., in ALSPAC the dairy food group included milk and cheese, while in INMA and Generation R, ice cream, yoghurt, cream were also included). Fourth, we had incomplete, or no, data on iodine supplement use in two of the cohorts (Generation R and ALSPAC), which could be an important determinant that we were unable to evaluate; however this is unlikely to be a limitation in ALSPAC as it is unlikely that women would have taken an iodine-containing supplement in the early 1990s. Finally, we measured urinary iodine only in women with available child IQ data in two of the cohorts which might have created bias.

Conclusion

Various maternal characteristics and dietary habits were associated with UI/Creat in pregnancy, some of which were population-specific. For that reason, universal interventions and dietary recommendations to improve the iodine intake of pregnant women might not be appropriate; a country-specific approach needs to be adopted. Between countries, but also within countries with a large proportion of different ethnic groups, culturally-specific recommendations are probably necessary. Achieving and maintaining iodine sufficiency in populations require monitoring dietary determinants of iodine status so that appropriate action can be taken, where necessary.

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Supplementary material

ALSPAC

For the women in the analysis (n=2852), there were 2.6% missing values in total (n=1263) and 19.3% of the cases (n=550) had at least one missing value. Data were imputed for 12 out of the 15 variables used in the analysis, including pre-pregnancy BMI (n=250), ethnicity (n=96), parity (n=134), smoking status (n=51, smoking status variable was made from two imputed smoking variables with n=102 missing values in total), alcohol consumption (n=101, alcohol consumption variable was made from two imputed variables with n=159 missing values in total), education level (n=86), home ownership (n=72), crowding index (n=94), family adversity index (n=30), life event score (n=96), marital status (n=47), and living with a partner (n=97). Data were not imputed for gestational age, maternal age and child's sex due to no missing values.

Generation R

For the women in the analysis (n=2254), there were 5.5% missing values in total (n=1369) and 31.7% of the cases (n=715) had at least one missing value. Data were imputed for nine out of 11 variables, including gestational age (n=1), pre-pregnancy BMI (n=12), ethnicity (n=71), smoking status (n=248), alcohol consumption (n=254), education level (n=149), net household income (n=479), marital status (n=154), and child's sex (n=1). Data were not imputed for maternal age and parity due to no missing values.

INMA

For the women in the analysis (n=1460), there were 1.1% missing values in total (n=154) and 5.8% of the cases (n=84) had at least one missing value. Data were imputed for nine out of ten variables, including gestational age (n=4), maternal age (n=4), ethnicity (n=3), parity (n=2), smoking status (n=44), alcohol consumption (n=56), education level (n=5), living with a partner (n=1), and child's sex (n=35). Data were not imputed for pre-pregnancy BMI due to no missing values.

Supplemental Table 1 Overview of the classification of foods into food groups by cohort.

Food group	ALSPAC	Generation R	INMA
Vegetables	green leafy vegetables (e.g., cabbage), other green vegetables (e.g., leeks), carrots, other root vegetables (e.g., turnip), salad vegetables (e.g., tomatoes)	leafy (e.g., spinach), root (e.g., carrots), cabbage, mixed salad vegetables, mushrooms, allium (e.g., onion, garlic), stems and sprouts (e.g., asparagus), fruiting (e.g., tomatoes)	leafy (e.g., spinach), root (e.g., carrots), cruciferous (e.g., broccoli), salad vegetables (e.g., lettuce), allium (e.g., onions, leek, garlic), fruiting (e.g., tomatoes, aubergines, peppers)
Fruit	fresh fruit (e.g., apple, grapes, banana)	fresh fruit (e.g., apple, grapes, banana), olives	fresh fruit (e.g., apple, grapes, banana)
Nuts and seeds	nuts, tahini	nuts, seeds, nut spread	almonds, peanuts, hazelnuts, pine nuts
Potatoes	chips, roast potatoes, boiled/ mashed/ jacket potatoes	potatoes and other tubers (does not include potato crisps)	fried, boiled/roasted potatoes, potato crisps
Legumes	pulses (e.g., lentils, chickpeas), baked beans, peas, sweetcorn, broad beans	dried lentils, beans and peas	lentils, chickpeas, beans
Cereals and cereal products	breakfast cereals (e.g., oats, cornflakes, bran cereals), pasta, rice, bread, crispbreads, pizza	breakfast cereals, pasta, rice, bread, pretzels, crispbreads, pizza, flour and thickeners	breakfast cereals, pasta, rice, bread, boiled corn
Cakes, confectionery and added sugar	cakes or buns, biscuits, chocolate bars (e.g., Mars), chocolate (e.g., dairy milk), sweets (e.g., toffees), pudding (e.g., cheesecake, mousse), sugar	cakes, pastries, biscuits, candy bars, chocolate, non- chocolate sweets (e.g., toffees) jam, honey, sugar, ice cream, syrups, water ice	cakes, pastries, biscuits, chocolate, jams, honey, sugar
Added fats	animal fats (e.g., butter, ghee), vegetable oils and spreads (e.g., olive oil)	animal fats (e.g., butter),vegetable oils (e.g., olive oil), spreads and margarines	animal fats (e.g., butter), vegetable oils and spreads (e.g., olive oil)
Milk and dairy products	milk, cheese	milk, milk drinks, evaporated milk, yoghurt, fresh cheese (e.g., cottage cheese), cheese, milk puddings (e.g., mouse, cream base), cream	fresh milk, condensed milk, yoghurt, cheese, custard, ice cream, cream
Meat and meat products	red meat, poultry, offal (e.g., liver), processed meat (e.g., sausages, burgers, bacon), pies and pasties (e.g., meat pies)	red meat, poultry, offal (e.g., liver), processed meat (e.g., sausage, pate)	red meat, white meat (e.g., chicken), offal (e.g., liver), processed meat (e.g., sausages, pate, bacon)
Eggs	eggs, quiche	eggs	eggs

Supplemental Table 1 Overview of the classification of foods into food groups by cohort. (continued)

Food group	ALSPAC	Generation R	INMA
Fish and shellfish	white fish (e.g., cod), oily fish (e.g., salmon), shellfish (e.g., prawns)	white fish (e.g., cod), oily fish (e.g., salmon), shellfish (e.g., prawns), processed fish (e.g., fish fingers)	boiled/fried/ grilled white fish (e.g., cod), boiled/ fried/ grilled oily fish (e.g., salmon), seafood and shellfish (e.g., oysters, clams, lobster)
Condiments and seasoning	N/A	seasonings (e.g., salt, herbs, spices)	salt, including iodised salt
Processed and fried foods *(e.g., sauces, soups, fried foods, crisps)*	fried food (e.g., bacon, eggs, egg fried fish), crisps	sauces (e.g., tomato, dressings, mayonnaise), soups, bouillons	vegetable soups, tomato sauce, chicken croquettes, pizza
Non-alcoholic beverages *(excluding coffee and tea)*	tinned juice (e.g., tomato juice), pure fruit juice, soft drinks	fruit and vegetable juices, soft drinks, isotonic drinks, water	fruit juice, soft drinks, non-alcoholic beer, tap water, bottled water
Alcoholic beverages	N/A	wine, beer, liquors and spirits	wine, beer, liquors and spirits
Miscellaneous *(e.g., soy products, diet foods and sweeteners)*	soy products (e.g., TVP, vegeburgers), bean curd (e.g., tofu)	diet products, soy products, artificial sweeteners	N/A

Abbreviations: N/A, data not available; TVP, texturised vegetable protein.

Supplemental Table 2 Determinants [a] of urinary iodine-to-creatinine ratio measured at ≤ 18 gestational weeks by cohort.

	ALSPAC (n=2852)			Generation R (n=2254)			INMA (n=1460)		
Determinants	*n*	*B* (95% CI)	*P* [b]	*n*	*B* (95% CI)	*P* [b]	*n*	*B* (95% CI)	*P* [b]
Maternal factors									
Gestational age at urine sampling, weeks	2852	0.051 (0.046, 0.057)	<0.001	2254	0.007 (-0.006, 0.019)	0.300	1460	0.004 (-0.023, 0.032)	0.755
Age [c], years	2852	0.014 (0.008, 0.020)	<0.001	2254	0.018 (0.012, 0.024)	<0.001	1460	0.020 (0.010, 0.030)	<0.001
Pre-pregnancy BMI, kg/m^2	2852	-0.013 (-0.019, -0.007)	<0.001	2254	-0.011 (-0.017, -0.006)	<0.001	1460	-0.013 (-0.022, -0.004)	0.005
Ethnicity [d]									
Reference group	2800	Ref.		1095	Ref.		1340	Ref.	
Non-white	52	-0.018 (-0.203, 0.168)	0.851	N/A	.	.	N/A	.	.
Non-Dutch	N/A	.	.	1159	(see separate table) e		N/A	.	.
Non-Spanish	N/A	.	.	N/A	.	.	120	-0.016 (-0.150, 0.118)	0.810
Parity									
0	1354	Ref.		1279	Ref.		806	Ref.	
1	965	0.032 (-0.021, 0.085)	0.232	665	-0.001 (-0.057, 0.056)	0.982	544	-0.029 (-0.110, 0.052)	0.481
≥ 2	533	0.040 (-0.029, 0.108)	0.254	310	-0.041 (-0.123, 0.041)	0.327	110	-0.128 (-0.276, 0.020)	0.090
Smoking status									
Never smoked	2169	Ref.		1671	Ref.		1020	Ref.	
Stopped smoking	333	-0.003 (-0.075, 0.070)	0.943	211	-0.093 (-0.179, -0.007)	0.035	187	-0.060 (-0.172, 0.052)	0.296
Continued smoking	350	0.018 (-0.055, 0.092)	0.620	372	-0.008 (-0.083, 0.066)	0.831	253	-0.001 (-0.105, 0.104)	0.990
Alcohol consumption									
No	1350	Ref.		1458	Ref.		1330	Ref.	
Yes	1502	-0.006 (-0.052, 0.040)	0.795	796	-0.002 (-0.059, 0.055)	0.954	130	-0.051 (-0.181, 0.079)	0.442

Supplemental Table 2 Determinants [a] of urinary iodine-to-creatinine ratio measured at ≤ 18 gestational weeks by cohort. (continued)

	ALSPAC (n=2852)			Generation R (n=2254)			INMA (n=1460)		
Determinants	*n*	*B* (95% CI)	*P* [b]	*n*	*B* (95% CI)	*P* [b]	*n*	*B* (95% CI)	*P* [b]
Markers of socio-economic status									
Education level									
Low	576	Ref.		247	Ref.		337	Ref.	
Medium	1780	0.005 (-0.056, 0.066)	0.869	995	-0.077 (-0.165, 0.012)	0.089	581	-0.016 (-0.112, 0.079)	0.736
High	496	0.026 (-0.055, 0.107)	0.531	1012	-0.057 (-0.159, 0.045)	0.272	542	0.089 (-0.013, 0.190)	0.088
Net household income (€ per month)									
Low < €1200	N/A	.	.	492	Ref.		N/A	.	.
Medium €1200-2200	N/A	.	.	597	0.022 (-0.063, 0.108)	0.606	N/A	.	.
High > €2200	N/A	.	.	1165	0.089 (-0.001, 0.179)	0.054	N/A	.	.
Home ownership									
Owned/mortgaged	2425	Ref.		N/A	.	.	N/A	.	.
Private/other rented	236	-0.037 (-0.126, 0.053)	0.422	N/A	.	.	N/A	.	.
Council rented	191	-0.032 (-0.136, 0.072)	0.547	N/A	.	.	N/A	.	.
Crowding index									
≤ 1 person per room	2747	Ref.		N/A	.	.	N/A	.	.
+ 1 person per room	105	0.134 (-0.006, 0.274)	0.060	N/A	.	.	N/A	.	.
Family adversity index									
None 0	1395	Ref.		N/A	.	.	N/A	.	.
Mild 1-2	1124	-0.007 (-0.056, 0.041)	0.765	N/A	.	.	N/A	.	.
Severe > 3	333	-0.100 (-0.182, -0.018)	0.016	N/A	.	.	N/A	.	.
Life event score	2852	0.007 (-0.001, 0.015)	0.097	N/A	.	.	N/A	.	.

Supplemental Table 2 Determinants [a] of urinary iodine-to-creatinine ratio measured at ≤ 18 gestational weeks by cohort. (continued)

	ALSPAC (n=2852)			Generation R (n=2254)			INMA (n=1460)		
Determinants	*n*	*B* (95% CI)	*P* [b]	*n*	*B* (95% CI)	*P* [b]	*n*	*B* (95% CI)	*P* [b]
Marital status									
Never-married	355	Ref.		1136	Ref.		N/A	.	.
Married	2357	0.095 (0.018, 0.171)	0.015	1118	0.030 (-0.025, 0.086)	0.285	N/A	.	.
Other [f]	140	0.028 (-0.097, 0.153)	0.666	N/A	.	.	N/A	.	.
Living with a partner									
Yes	N/A	.	.	N/A	.	.	1445	Ref.	
No	N/A	.	.	N/A	.	.	15	-0.233 (-0.598, 0.131)	0.209
Child factors									
Child's sex									
Male	1405	Ref.		1147	Ref.		737	Ref.	
Female	1447	-0.025 (-0.070, 0.019)	0.261	1107	-0.026 (-0.073, 0.021)	0.278	723	-0.014 (-0.087, 0.058)	0.700

[a] Effect estimates (*B*=unstandardized regression coefficient), their 95% CIs and *P*-values from multiple linear regression models performed for each cohort with (natural) log-transformed iodine-to-creatinine ratio (UI/Creat) as the dependent variable and maternal characteristics as independent variables (for full models, see footnote b). Reported *B* coefficients represent the change in the mean (natural) log of UI/Creat per unit increase in the continuous independent variables and for each category compared to the reference for the categorical independent variables.

[b] *P*-values from adjusted Model 1 (adjusted for maternal and pregnancy characteristics); ALSPAC (R^2=0.123, $P < 0.0001$): gestational age (weeks), age (years), pre- pregnancy BMI (kg/m^2), ethnicity, parity, smoking status, alcohol consumption, education, home ownership, crowding index, family adversity index, life event score, marital status and child's sex; Generation R (R^2=0.086, $P < 0.0001$): gestational age (weeks), age (years), pre-pregnancy BMI (kg/m^2), ethnicity, parity, smoking status, alcohol consumption, education, net household income, marital status and child's sex; INMA (R^2=0.021, $P < 0.0001$): gestational age (weeks), age (years), pre- pregnancy BMI (kg/m^2), ethnicity, parity, smoking status, alcohol consumption, education, living with a partner and child's sex.

[c] Maternal age at urine sample collection, except in ALSPAC (age at last menstrual period).

[d] ALSPAC (Reference group=White); Generation R (Reference group=Dutch, Non-Dutch=Indonesian, Cape Verdean, Moroccan, Dutch Antilles, Surinamese, Turkish, Other non-western, Asian, or Other western); INMA (Reference group=Spanish, Non-Spanish=Latin American, European, or Others).

[e] Non-Dutch group in Generation R presented in detail in Table 3.

[f] ALSPAC (Other=widowed, divorced, or separated).

Abbreviations: BMI, body mass index; 95% CI, confidence interval; N/A, data not available or not applicable; Ref, reference category; UI/Creat, urinary iodine-to-creatinine ratio.

Supplemental Table 3 Determinants [a] of urinary iodine concentration measured at ≤ 18 gestational weeks by cohort.

	ALSPAC (n=2852)						Generation R (n=2254)						INMA (n=1460)					
	Adjusted Model 1 [b]			Adjusted Model 2 [c]			Adjusted Model 1 [b]			Adjusted Model 2 [c]			Adjusted Model 1 [b]			Adjusted Model 2 [c]		
Determinants	*n*	*B*	*P*	*n*	*B*	*P*	*n*	*B*	*P*	*n*	*B*	*P*	*n*	*B*	*P*	*n*	*B*	*P*
Gestational age at urine sampling, weeks	2852	0.033	<0.001	2710	0.034	<0.001	2254	0.014	0.020	1580	0.021	0.004	1460	0.009	0.516	1446	-0.007	0.605
Age [d], years	2852	0.009	0.002	2710	0.006	0.063	2254	0.011	<0.001	1580	0.010	0.009	1460	0.019	<0.001	1446	0.013	0.008
Pre-pregnancy BMI, kg/m^2	2852	-0.009	0.004	2710	-0.009	0.008	2254	-0.003	0.279	1580	-0.004	0.192	1460	-0.007	0.113	1446	-0.002	0.650
Family adversity index																		
None 0	1395	Ref.		1334	Ref.		N/A	.	.	.	.	.	N/A	.	.	.	.	.
Mild 1-2	1124	-0.025	0.309	1069	-0.020	0.423	N/A	.	.	.	.	.	N/A	.	.	.	.	.
Severe > 3	333	-0.112	0.008	307	-0.101	0.019	N/A	.	.	.	.	.	N/A	.	.	.	.	.
Marital status																		
Never-married	355	Ref.		320	Ref.		1136	Ref.		825	Ref.		N/A	.	.	.	.	.
Married	2357	0.044	0.257	2269	0.078	0.054	1118	0.034	0.208	755	0.042	0.179	N/A	.	.	.	.	.
Other [e]	140	-0.015	0.815	121	0.009	0.889	N/A	.	.	.	.	.	N/A	.	.	.	.	.
Ethnicity [f]																		
Reference group	2800	Ref.		2674	Ref.		1095	Ref.		930	Ref.		1340	Ref.		1328	Ref.	
Non-white	52	0.048	0.611	36	0.109	0.284	N/A	.	.	.	.	.	N/A	.	.	.	.	.
Non-Dutch:	N/A	.	.	.	.	.							N/A	.	.	.	.	.
- *Indonesian*	N/A	.	.	.	.	.	72	-0.012	0.861	58	-0.026	0.725	N/A	.	.	.	.	.
- *Cape Verdean*	N/A	.	.	.	.	.	87	0.061	0.370	41	0.150	0.112	N/A	.	.	.	.	.
- *Moroccan*	N/A	.	.	.	.	.	165	0.286	<0.001	72	0.242	0.003	N/A	.	.	.	.	.
- *Dutch Antilles*	N/A	.	.	.	.	.	65	-0.064	0.393	37	-0.117	0.245	N/A	.	.	.	.	.
- *Surinamese*	N/A	.	.	.	.	.	196	-0.046	0.327	111	-0.024	0.698	N/A	.	.	.	.	.
- *Turkish*	N/A	.	.	.	.	.	226	0.323	<0.001	103	0.308	<0.001	N/A	.	.	.	.	.
- *Other, non-Western*	N/A	.	.	.	.	.	107	0.125	0.034	53	0.054	0.502	N/A	.	.	.	.	.

Supplemental Table 3 Determinants [a] of urinary iodine concentration measured at ≤ 18 gestational weeks by cohort. (continued)

	ALSPAC (n=2852)						Generation R (n=2254)						INMA (n=1460)					
	Adjusted Model 1 [b]			Adjusted Model 2 [c]			Adjusted Model 1 [b]			Adjusted Model 2 [c]			Adjusted Model 1 [b]			Adjusted Model 2 [c]		
Determinants	*n*	*B*	*P*	*n*	*B*	*P*	*n*	*B*	*P*	*n*	*B*	*P*	*n*	*B*	*P*	*n*	*B*	*P*
-*Asian*	N/A	.	.	.	.	.	32	0.062	0.544	19	0.022	0.863	N/A	.	.	.	.	.
-*Other, Western*	N/A	.	.	.	.	.	209	0.023	0.575	156	0.050	0.297	N/A	.	.	.	.	.
Non-Spanish	N/A	.	.	.	.	.	N/A	.	.	.	.	.	120	0.007	0.909	118	0.032	0.627
Smoking status																		
Never smoked	2169	Ref.		2077	Ref.		1671	Ref.		1202	Ref.		1020	Ref.		1011	Ref.	
Stopped smoking	333	-0.008	0.825	312	-0.015	0.686	211	-0.078	0.065	147	-0.120	0.016	187	-0.097	0.077	186	-0.096	0.076
Continued smoking	350	0.005	0.896	321	0.034	0.380	372	0.021	0.557	231	0.039	0.385	253	-0.015	0.765	249	0.010	0.838

[a] Effect estimates (*B*=unstandardised regression coefficient) and *P*-values from multiple linear regression models performed for each cohort with (natural) log-transformed urinary iodine concentration (UIC) as the dependent variable and maternal characteristics and dietary intakes as independent variables (for full models, see footnotes b and c). All models were additionally adjusted for urinary creatinine concentration (UCreat, g/L). Reported *B* coefficients represent the change in the mean (natural) log of UIC per unit increase in the continuous independent variables and for each category compared to the reference for the categorical independent variables.

[b] Adjusted Model 1 (adjusted for maternal and pregnancy characteristics); ALSPAC (R^2=0.424, $P < 0.0001$): gestational age (weeks), age (years), pre-pregnancy BMI (kg/m2), ethnicity, parity, smoking status, alcohol consumption, education, home ownership, crowding index, family adversity index, life event score, marital status, child's sex, UCreat (g/L) and UCreat^2; Generation R (R^2=0.525, $P < 0.0001$): gestational age (weeks), age (years), pre-pregnancy BMI (kg/m^2), ethnicity, parity, smoking status, alcohol consumption, education, net household income, marital status, child's sex, UCreat (g/L) and UCreat^2; INMA (R^2=0.193, $P < 0.0001$): gestational age (weeks), age (years), pre-pregnancy BMI (kg/m^2), ethnicity, parity, smoking status, alcohol consumption, education, living with a partner, child's sex, UCreat (g/L) and UCreat^2.

[c] Adjusted Model 2 (adjusted for maternal and pregnancy characteristics + dietary intakes); ALSPAC (R^2=0.443, $P < 0.0001$): Model 1 + energy intake (kcal/day) + intake of vegetables (g/day), fruit (g/day), nuts and seeds (g/day), potatoes (g/day), legumes (g/day), cereals and cereal products (g/day), cakes, confectionery and added sugar (g/day), added fats (g/day), milk and dairy products (g/day), meat and meat products (g/day), eggs (g/day), fish and shellfish (g/day), processed and fried foods (g/day), non-alcoholic beverages (g/day), miscellaneous (g/day); Generation R (R^2=0.548, $P < 0.0001$): Model 1 + energy intake (kcal/day) + intake of vegetables (g/day), fruit (g/day), nuts and seeds (g/day), potatoes (g/day), legumes (g/day), cereals and cereal products (g/day), cakes, confectionery and added sugar (g/day), added fats (g/day), milk and dairy products (g/day), meat and meat products (g/day), eggs (g/day), fish and shellfish (g/day), condiments and seasoning (g/day), processed and fried foods (g/day), non-alcoholic beverages (g/day), alcoholic beverages (g/day), miscellaneous (g/day); INMA (R^2=0.227, $P < 0.0001$): Model 1 + energy intake (kcal/day) + intake of vegetables (g/day), fruit (g/day), nuts and seeds (g/day), potatoes (g/day), legumes (g/day), cereals and cereal products (g/day), cakes, confectionery and added sugar (g/

day), added fats (g/day), milk and dairy products (g/day), meat and meat products (g/day), eggs (g/day), fish and shellfish (g/day), condiments and seasoning (e.g., salt) (g/day), processed and fried foods (g/day), non-alcoholic beverages (g/day), alcoholic beverages (g/day).

[d] Maternal age at urine sample collection, except in ALSPAC (age at last menstrual period).

[e] ALSPAC (Other=widowed, divorced, or separated).

[f] ALSPAC (Reference group=White); Generation R (Reference group=Dutch, Non-Dutch=Indonesian, Cape Verdean, Moroccan, Dutch Antilles, Surinamese, Turkish, Other non- western, Asian, or Other western); INMA (Reference group=Spanish, Non-Spanish=Latin American, European, or Others).

Abbreviations: BMI, body mass index; N/A, data not available or not applicable; Ref, reference category; UCreat, urinary creatinine concentration; UCreat^2, squared urinary creatinine concentration; UIC, urinary iodine concentration.

Supplemental Table 4 Descriptives of dietary intakes of food groups (g/day) and energy intake (kcal/day) estimated from FFQ by cohort.

	ALSPAC (n=2710)	**Generation R (n=1580)**	**INMA (n=1446)**
Food group (grams/day) [a]	*Mean (±SD)*	*Mean (±SD)*	*Mean (±SD)*
Vegetables	117 (±64)	144 (±64)	220 (±114)
Fruit	100 (±56)	171 (±116)	320 (±200)
Nuts and seeds	3 (±7)	17 (±12)	6 (±9)
Potatoes	98 (±49)	52 (±43)	63 (±37)
Legumes	54 (±32)	4 (±7)	38 (±25)
Cereals and cereal products	195 (±79)	195 (±71)	184 (±77)
Cakes, confectionery and added sugar	80 (±55)	98 (±56)	44 (±36)
Added fats	19 (±11)	25 (±14)	23 (±15)
Milk and dairy products	391 (±155)	412 (±251)	444 (±233)
Meat and meat products	71 (±40)	78 (±43)	116 (±50)
Eggs	21 (±18)	11 (±10)	20 (±9)
Fish and shellfish	35 (±30)	14 (±13)	69 (±36)
Condiments and seasoning (e.g., salt, iodised salt)	N/A	6 (±5)	0.3 (±0.4)
Processed and fried foods (e.g., sauces, soups, fried foods, crisps)	8 (±8)	100 (±90)	101 (±66)
Non-alcoholic beverages (excluding coffee and tea) [b]	175 (±83)	940 (±550)	1488 (±494)
Alcoholic beverages	N/A	6 (±17)	3 (±13)
Miscellaneous (e.g., soy products, diet foods and sweeteners)	2 (±9)	6 (±20)	N/A
Energy, kcal/day	1743 (±459)	2076 (±520)	2083 (±532)

[a] Dietary intake of food groups presented as mean (±SD) grams per day, energy intake presented as mean (±SD) kcal per day.

[b] Food group also includes water in INMA and Generation R (but not in ALSPAC).

Abbreviations**:** FFQ, food frequency questionnaire; N/A, data not available; SD, standard deviation.

Supplemental Table 5 Multivariable associations of food group intakes estimated from FFQ (per 100 g/day) [a] with urinary iodine-to-creatinine ratio measured at ≤ 18 gestational weeks by cohort.

	ALSPAC (n=2710)		Generation R (n=1580)		INMA (n=1446)	
Food group intakes (per 100 g/day) [a]	*B* (95% CI) [b]	*P* [c]	*B* (95% CI) [b]	*P* [d]	*B* (95% CI) [b]	*P* [e]
Vegetables	0.24 (-1.51, 2.06)	0.791	-0.76 (-4.78, 3.46)	0.719	0.48 (-7.07, 8.31)	0.903
Fruit	2.79 (0.59, 5.09)	0.012	1.13 (-1.12, 3.45)	0.328	-4.38 (-8.91, 0.26)	0.064
Nuts and seeds	19.30 (-2.05, 51.25)	0.083	51.07 (17.18, 96.03)	0.001	68.80 (-33.30, 230.33)	0.223
Potatoes	2.66 (-0.20, 5.71)	0.069	5.92 (-1.17, 13.61)	0.104	-16.46 (-36.05, 5.36)	0.134
Legumes	2.33 (-1.33, 6.29)	0.219	16.98 (-17.38, 68.39)	0.384	28.00 (-5.39, 66.86)	0.105
Cereals and cereal products	1.42 (-0.92, 3.88)	0.238	12.32 (6.34, 18.69)	<0.001	0.21 (-13.90, 15.34)	0.978
Cakes, confectionery and added sugar	4.90 (0.50, 9.73)	0.028	5.36 (-2.24, 13.64)	0.172	-17.98 (-43.38, 11.33)	0.217
Added fats [a]	0.11 (-0.01, 0.24)	0.082	0.27 (0.04, 0.50)	0.022	-0.42 (-1.13, 0.29)	0.243
Milk and dairy products	3.73 (2.74, 4.74)	<0.001	2.34 (1.00, 3.70)	0.001	6.92 (2.52, 11.41)	0.002
Meat and meat products	1.27 (-2.30, 5.13)	0.497	2.87 (-4.14, 10.49)	0.433	-32.71 (-50.77, -12.58)	0.002
Eggs	5.44 (-1.18, 13.06)	0.112	65.32 (26.28, 117.87)	<0.001	86.92 (-13.13, 238.12)	0.099
Fish and shellfish	5.10 (0.89, 9.70)	0.017	2.01 (-16.18, 24.91)	0.845	28.04 (2.73, 56.38)	0.029
Condiments and seasoning (e.g., salt) [a,f]	N/A	.	0.27 (-0.30, 0.84)	0.359	31.69 (10.31, 55.16)	0.003
Processed and fried foods (e.g., sauces, soups, fried foods, crisps)	0.85 (-12.40, 19.47)	0.915	1.88 (-0.98, 4.83)	0.199	0.00 (-11.26, 11.91)	0.999
Non-alcoholic beverages (excluding coffee and tea)	0.62 (-0.71, 1.99)	0.367	0.40 (-0.07, 0.87)	0.099	-1.46 (-3.05, 0.13)	0.072
Alcoholic beverages	N/A	.	13.86 (-1.99, 32.66)	0.090	-34.72 (-85.12, 36.20)	0.297
Miscellaneous (e.g., soy products, diet foods and sweeteners)	14.61 (-0.70, 35.22)	0.063	8.13 (-4.77, 23.06)	0.230	N/A	.

[a] Added fats and salt intakes expressed per 1 g/day.

[b] Effect estimates (*B*=unstandardized regression coefficient) represent the actual change in the geometric mean of UI/Creat (µg/g) associated with 100 g increase in intake of a food group. *B* coefficients and their 95% CIs are calculated by back-transformation from logarithmic scale (see methods). Values are adjusted for dietary intake of other food groups, energy intake and other potential confounders (for full models, see Table 3, footnote c). When calculating the *B* coefficients, all categorical covariates were set to their reference group and the continuous covariates gestational age, maternal age, pre-pregnancy BMI and energy intake were centred to their means.

[c] *P*-value adjusted for energy (kcal/day) + gestational age (weeks), age (years), pre-pregnancy BMI (kg/m^2), ethnicity, parity, smoking status, alcohol consumption, education, home ownership, crowding index, family adversity index, life event score, marital status and child's sex (R^2=0.147, $P < 0.0001$).

[d] *P*-values adjusted for energy (kcal/day) + gestational age (weeks), age (years), pre-pregnancy BMI (kg/m^2), ethnicity, parity, smoking status, alcohol consumption, education, net household income, marital status and child's sex (R^2=0.091, $P < 0.0001$).

[e] *P*-values adjusted for energy (kcal/day) + gestational age (weeks), age (years), pre-pregnancy BMI (kg/m^2), ethnicity, parity, smoking status, alcohol consumption, education, living with a partner and child's sex (R^2=0.060, $P < 0.0001$).

[f] In INMA this food group also includes iodised salt. Separate information about the consumption of table salt and use of iodine-fortified table salt was collected only in the INMA cohort.

Abbreviations: BMI, body mass index; 95% CI, confidence interval; FFQ, food frequency questionnaire; N/A, data not available; UI/Creat, urinary iodine-to- creatinine ratio.

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Chapter 3

The association of maternal iodine status in early pregnancy with thyroid function in the Swedish Environmental Longitudinal, Mother and Child, Asthma and Allergy study.

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Abstract

Background: Severe maternal iodine deficiency can impact fetal brain development through effects on maternal and/or fetal thyroid hormone availability. The effects of mild-to-moderate iodine deficiency on thyroid function are less clear. The aim was to investigate the association of maternal urinary iodine concentration corrected for creatinine (UI/Creat) with thyroid function and auto-antibodies in a mild-to-moderate iodine-deficient pregnant population.

Methods: This study was embedded within the Swedish Environmental Longitudinal, Mother and child, Asthma and allergy (SELMA) study. Clinical reference ranges were determined by the 2.5th and 97.5th population-based percentile cut-offs. The associations of UI/Creat with thyrotropin (TSH), free T4 (FT4), free triiodothyronine (FT3), total T4 (TT4), and total triiodothyronine (TT3) were studied using multivariable linear regression in thyroid peroxidase antibody (TPOAb)-negative women. The association of UI/Creat with TPOAb and thyroglobulin antibody (TgAb) positivity was analyzed using multivariable logistic regression.

Results: Urinary iodine and thyroid function were measured at a median (95% range) gestational age of 10 (6-14) weeks in 2009 women. The median (95% range) UI/Creat was 85 µg/g (36-386) and the UI/Creat was below 150 µg/g in 80.1% of women. Reference ranges did not differ substantially by UI/Creat. A lower UI/Creat was associated with a lower TSH (*P*=0.027), a higher TT4 (*P*=0.032), and with a corresponding trend towards slightly higher FT4 (*P*=0.081), FT3 (*P*=0.079), and TT3 (*P*=0.10). UI/Creat was not associated with the FT4/FT3 (*P*=0.94) or TT4/TT3 ratios (*P*=0.63). Women with a UI/Creat of 150-249 µg/g had the lowest prevalence of TPOAb positivity (6.1%), while women with a UI/Creat of <150 µg/g had a higher prevalence [11.0%, odds ratio (OR) confidence interval (95% CI) 1.84 (1.07–3.20), *P*=0.029]. Women with a UI/Creat ≥500 µg/g showed the highest prevalence and a higher risk of TPOAb positivity, however, only a small proportion of women had such a UI/Creat [12.5%, OR (95% CI) 2.36 (0.54–10.43), *P*=0.26].

Conclusions: We could not identify any meaningful differences in thyroid function reference ranges. Lower iodine availability was associated with a slightly lower TSH and a higher TT4. Women with adequate iodine intake had the lowest risk of TPOAb positivity.

Introduction

During pregnancy there is an increased demand for maternal thyroid hormone because of the placental transport of thyroid hormone to the fetus, an increase in thyroxine-binding globulin concentrations, and a higher degradation of thyroid hormone due to placental type III iodothyronine deiodinase [1]. Iodine is an essential component of thyroid hormone and both low and high iodine intakes may adversely affect thyroid function [2]. A considerable increase in preconceptional or gestational daily iodine intake is required to meet the higher demand for thyroid hormone, to compensate for greater iodine losses during pregnancy, and to transfer a sufficient amount of iodine to the fetus [3–5]. The current guidelines of the World Health Organization therefore recommend an iodine intake of 250 µg per day for pregnant and lactating women as opposed to 150 µg per day in an adult non-pregnant state [3]. However, data on the effects of iodine availability on maternal thyroid function during pregnancy are inconsistent.

A large Chinese study indicated that in pregnant women, the optimal urinary iodine concentration (UIC) ranged from 150 to 250 µg/L [6]. Outside this range there was a higher prevalence of women with thyroid autoimmunity and women with a UIC above this range had a higher risk of subclinical hypothyroidism and isolated hypothyroxinemia [6]. A few studies show that a lower UIC during pregnancy is associated with a higher free thyroxine (FT4) [7,8] and free triiodothyronine (FT3) [7–9], while a higher UIC has been associated with higher thyrotropin (TSH) [8,10] and higher FT3 [11]. On the contrary, other studies, of which several are small in size (N<700) [11–14], do not report any association of UIC with TSH or FT4 [9,11–15] and data on (F)T3 are often lacking. While it is recommended that pregnancy TSH and FT4 reference ranges should be calculated in an iodine sufficient population [4], there is insufficient evidence to determine whether reference ranges are affected by iodine status.

Therefore, the aim of our study was to analyze the cross-sectional association of maternal iodine status with maternal TSH, FT4, FT3, total T4 (TT4), total T3 (TT3), the FT4/FT3 and TT4/TT3 ratios, thyroid peroxidase antibodies (TPOAbs), and thyroglobulin antibodies (TgAbs), and to determine variation in thyroid function reference ranges according to iodine status.

Materials and Methods

Study population

This study was embedded in the Swedish Environmental Longitudinal, Mother and child, Asthma and allergy (SELMA) study, which is a population-based prospective birth cohort study originating in the county of Värmland, Sweden [16]. Sweden is a country with a voluntary fortification program and iodized salt since 1936 [17]. Pregnant women who visited an antenatal care center for the first time around week 10, but before week 22 of pregnancy, between

September 2007 and March 2010 were invited to participate in the SELMA study. Pregnant women provided written informed consent, and the SELMA study is approved by the regional ethical committee, Uppsala, Sweden (2007-05-02, Dnr: 2007/062). For the current study, women were included if a measure of urinary iodine and creatinine concentration, and thyroid function during pregnancy were available. Exclusion criteria were twin pregnancy, thyroid interfering medication usage, and pre-existing thyroid disease.

Laboratory measurements

Early morning void urine samples were collected at the day for enrollment and stored at -20°C. Urinary iodine was measured by the Sandell-Kolthoff method. Iodine calibration was performed using certified reference materials (CRM), Seronorm urine Levels one and two (Nycomed, Norway), and four EQUIP samples certified for UIC (Centers for Disease Control and Prevention, USA). At a level of 1.7 µmol/l iodine the within-assay coefficients of variation (CV) was 5.1% and the between-assay CV was 14.3% (N=30). The urinary creatinine concentration was determined by the Jaffe rate method. The UIC corrected for creatinine (UI/Creat) was used as a marker of iodine status.

Non-fasting blood samples were collected at enrollment and were stored frozen at -80°C in a bio-bank at the Central Hospital in Karlstad, Sweden. Serum TSH, FT4, FT3, TT4, TT3, TPOAbs, and TgAbs were measured using electrochemoluminescence assay (Cobas e601 platform; Roche Diagnostics, Mannheim, Germany) at the Department of Clinical Chemistry, Máxima Medical Center in Veldhoven, the Netherlands. Human chorionic gonadotropin (hCG) was determined in lithium-heparin plasma using electrochemoluminescence assays (Cobas® e601; Roche Diagnostics, Mannheim, Germany). Within-laboratory CVs were 2.1%, 3.5%, 3.8%, 3.8%, and 7.7% for TSH, FT4, FT3, TT4, and TT3, respectively. For hCG, the CV was 2.5% at 4.3 U/L and 2.1% at 171 U/L. The manufacturer's cut-offs for TPOAb and TgAb positivity were at the concentrations of >34 IU/mL or >115 IU/mL, respectively.

Serum cotinine was used as a marker for smoking. Briefly, aliquots of 100 µL serum were added with labelled internal standard and proteins were precipitated by acetonitrile and analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Participants were categorized as non-smokers if their cotinine concentrations were <0.2 ng/mL and as active smokers if their cotinine concentrations were ≥15 ng/mL. If their concentrations were in between, participants were considered to be passive smokers.

Covariates

Information on gestational age at blood and urine sampling, maternal age at enrollment, maternal ethnicity (Western, non-Western), and maternal educational level (low = none or primary education; medium = secondary education; high = higher education) was collected through questionnaires. The Swedish National Birth Register was used to collect data on a diagnosis of thyroid disease, parity, and child sex. Information on pregnancy weight and

height, which was used to calculate body mass index (BMI), was collected either through a prenatal questionnaire or the Swedish National Birth Register.

Statistical analyses

Reference ranges were defined based on the 2.5th and 97.5th percentiles after exclusion of TPOAb-positive women. To check whether reference ranges differed according to iodine status, we defined reference ranges in women subdivided by quartiles of UI/Creat – to have equal numbers of women per category – and by alternative categorization, which broadly relates to cut-off values based on the epidemiological criteria for assessing iodine nutrition in pregnant women (UI/Creat <50, 50-99, 100-149, 150-249, 250-499, and ≥500 µg/g) [3]. As a sensitivity analysis, we also defined the reference ranges in women using the alternative categorization based on the UIC.

We studied the association of UI/Creat across the full range with TSH, FT4, FT3, TT4, TT3, and the FT4/FT3 and TT4/TT3 ratios using multivariable linear regression analyses in TPOAb-negative women. Non-linearity was tested using restricted cubic splines with three knots and we evaluated normality of residuals using histograms. We studied the association of UI/Creat across the full range with TPOAb and TgAb positivity using multivariable logistic regression. Similar models were used for exploring the association of a UI/Creat of <150 (<50, 50-99, 100-149), 250-499, and ≥500 µg/g with thyroid antibody positivity compared with the reference group of women with a UI/Creat of 150-249 µg/g. This analysis was repeated using the same categorization based on the UIC. UI/Creat, TSH, and TPOAb concentrations were log-transformed. Back-transformed values are shown in plots for better interpretation. Values in the lowest and highest centile of UI/Creat and thyroid function tests were considered as outliers and excluded from the regression analyses when analyzed across the full range.

All analyses were adjusted for hCG, gestational age at blood and urine sampling, maternal age, maternal ethnicity, maternal educational level, parity, maternal BMI, smoking status based on the serum cotinine concentration, and child sex. We additionally adjusted for TPOAbs in the analysis of the association between UI/Creat and thyroid function tests. With ANOVA, we tested the null hypothesis that the thyroid hormone concentrations were similar across the full range of the natural logarithm of UI/Creat. Missing data of covariates were imputed using multiple imputation by chained equations, generating 25 datasets. The statistical analyses were performed using R statistical software version 3.4.3 (R Core team, Vienna, Austria; packages *mice* and *rms*).

Results

The final study population comprised 2009 pregnant women, in whom data on both UI/Creat and thyroid function was collected at a median (95% range) gestational age of 10 (6-14) weeks (Fig. 1). The median UI/Creat (95% range) was 85 (36-386) µg/g and the UI/Creat was below 150 µg/g in 80.1% of women. The majority of women were of Western descent (97.3%), non-smokers (84.9%), and had a secondary ("middle") or higher ("high") educational level (33.6% and 54.4%, respectively). Further study population characteristics are shown in Table 1.

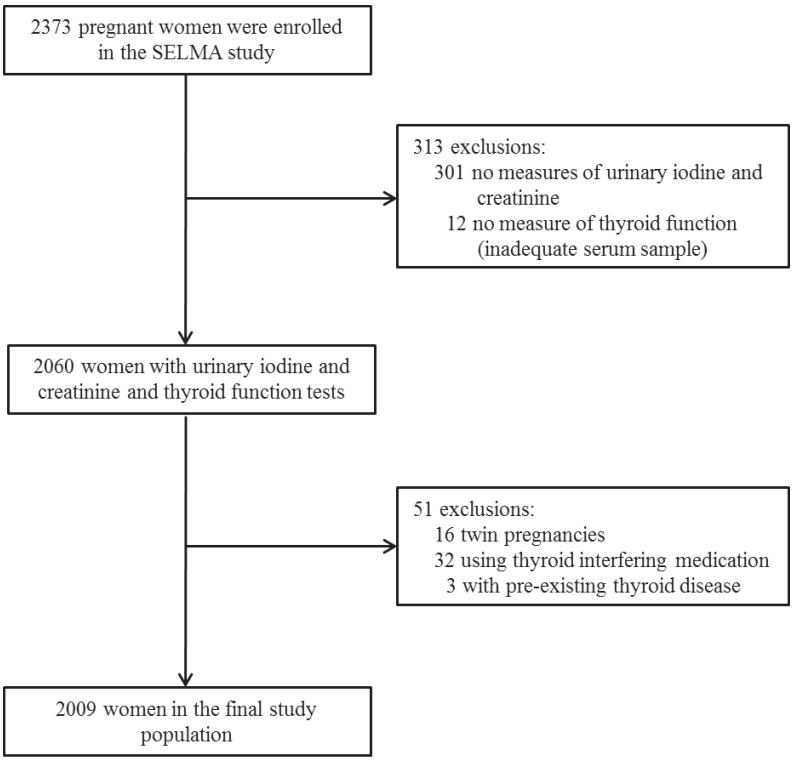

Fig. 1 Flowchart of the selection of the study population.

Thyroid function reference ranges

Thyroid function reference ranges did not differ substantially according to UI/Creat when analyzed per quartile (Table 2). The relative largest difference in cut-offs for thyroid function reference ranges was for the upper limit of TSH. However, these differences were small in absolute values and there was no clear trend across iodine status. In the lowest quartile of UI/Creat, the upper limit for TSH was 3.24, which is 0.16 mU/L (4.7%) lower than that in the total population. In the highest quartile, the upper limit was 3.26, which was 0.14 mU/L

(4.1%) lower than that calculated in the total population. For other cut-offs of UI/Creat or UIC, the number of women with more-than-adequate and excessive values was too small for reliable calculations (Supplementary Table 1 and 2).

Table 1 Characteristics of the study population (N=2009).

Characteristic	Values
UIC, median (95% range), µg/L	90 (38-439)
UI/Creat, median (95% range), µg/g	85 (36-386)
UIC <150 µg/L, %	78.6
UI/Creat < 150 µg/g, %	80.1
TSH, median (95% range), mU/L	1.31 (0.13-4.13)
FT4, median (95% range), pmol/L	15.0 (11.4-19.4)
FT3, median (95% range), pmol/L	4.67 (3.72-5.94)
TT4, median (95% range), nmol/L	118.0 (81.1-165.5)
TT3, median (95% range), nmol/L	1.93 (1.27-2.89)
hCG, median (95% range), U/L	69937.0 (9973.2-167580.0)
TPOAb positivity, %	10.2
TgAb positivity, %	7.9
Gestational age at sampling, median (95% range), weeks	10 (4-16)
Maternal age, mean (SD), years	30 (5.2)
Maternal ethnicity, %	
Western	97.3
Non-Western	2.7
Maternal educational level, %	
Low	12.0
Middle	33.6
High	54.4
Parity, %	
0	44.3
1	36.5
≥ 2	19.2
Maternal BMI, median (95% range), kg/m^2	23.6 (18.6-35.9)
Cotinine, ng/mL, %	
<0.2: non-smoker	84.9
0.2-15: passive smoker	6.2
>15: active smoker	9.0
Female sex, %	47.7

TPOAb positivity and TgAb positivity were defined as > 34 IU/ml and >115 IU/ml, respectively. Numbers of covariates are based on imputed data. BMI, body mass index; FT3, free triiodothyronine; FT4, free thyroxine; hCG: human chorionic gonadotropin; T3, triiodothyronine; T4, thyroxine; TT3, total triiodothyronine; TT4, total thyroxine; TgAb, thyroglobulin antibody; TPOAb, thyroid peroxidase antibody; TSH, thyrotropin; UIC, urinary iodine concentration; UI/Creat, UIC corrected for creatinine.

Table 2 reference ranges (2.5th and 97.5th percentile values) of thyroid function tests in TPOAb negative women, stratified by quartiles of UI/Creat.

	Total	Quartile 1	Quartile 2	Quartile 3	Quartile 4	Range of difference compared to total group	
	N=1802	N=443	N=446	N=449	N=464	lower limit	upper limit
UI/Creat in µg/g	86 (20-1368)	50 (20-61)	72 (61-85)	103 (86-132)	191 (133-1368)	NA	NA
TSH in mU/L	0.11-3.40	0.11-3.24	0.15-3.39	0.17-3.77	0.07-3.26	-0.04 to +0.06	-0.16 to +0.37
FT4 in pmol/L	11.6-19.3	12.0-19.8	11.5-18.8	11.5-18.8	11.7-19.6	-0.1 to +0.4	-0.5 to +0.5
FT3 in pmol/L	3.73-5.89	3.71-5.83	3.85-5.88	3.73-5.92	3.69-6.00	-0.04 to +0.12	-0.06 to +0.17
TT4 in nmol/L	82.4-165.6	85.4-170.1	81.3-163.8	80.9-163.7	82.4-161.1	-1.5 to +3.0	-4.6 to +4.5
TT3 in nmol/L	1.29-2.88	1.35-2.84	1.29-2.88	1.28-2.92	1.27-2.88	-0.02 to +0.06	-0.04 to +0.04

TPOAb positivity defined as >34 IU/mL. The range of difference of the lower limit is calculated by comparing the 2.5th percentile value of each thyroid function test in the whole study population ("Total") to the most deviating 2.5th percentile value calculated in women subdivided into the four quartiles of UI/Creat. The range of difference of the upper limit is calculated by comparing the 97.5th percentile value of each thyroid function test in the whole study population ("Total") to the most deviating 97.5th percentile value calculated in women subdivided into the four quartiles of UI/Creat. NA, not applicable.

Iodine status and thyroid function

A lower UI/Creat was associated with a lower TSH and a higher TT4 (Fig. 2; Supplementary Table 3). There was a trend towards an association of a lower UI/Creat with a higher FT4 (*P*=0.081), FT3 (*P*=0.079), and TT3 (*P*=0.10). There was no association of UI/Creat with the TT4/TT3 and FT4/FT3 ratios (Fig. 2; Supplementary Table 3).

In the whole study population, an increase of one unit in the natural logarithm of UI/Creat was associated with a 27% lower risk of TPOAb positivity [odds ratio (OR) 0.73, 95% confidence interval (CI) 0.56–0.98, *P*=0.034)], but not with TgAb positivity (OR 0.83, 95% CI 0.61–1.14, *P*=0.25, analyses per quartile in Supplementary Table 4). While TPOAb positivity was present in 6.1% of women with a UI/Creat of 150-249 µg/g, this was 11.0 % in women with a UI/Creat of <150 µg/g (OR 1.84, 95% CI 1.07–3.20, *P*=0.029,Table 3). Women with a UI/Creat of ≥500 µg/g had a higher risk of TPOAb positivity with 12.5% being TPOAb positive. However, only a small proportion of women entered in this category (N=24; OR 2.36, 95% CI 0.54–10.43, *P*=0.26). Analyses based on the UIC showed similar results (Supplementary Table 5). For TgAb positivity, there was a similar pattern as for TPOAb positivity but with considerably smaller percentage differences (Table 3).

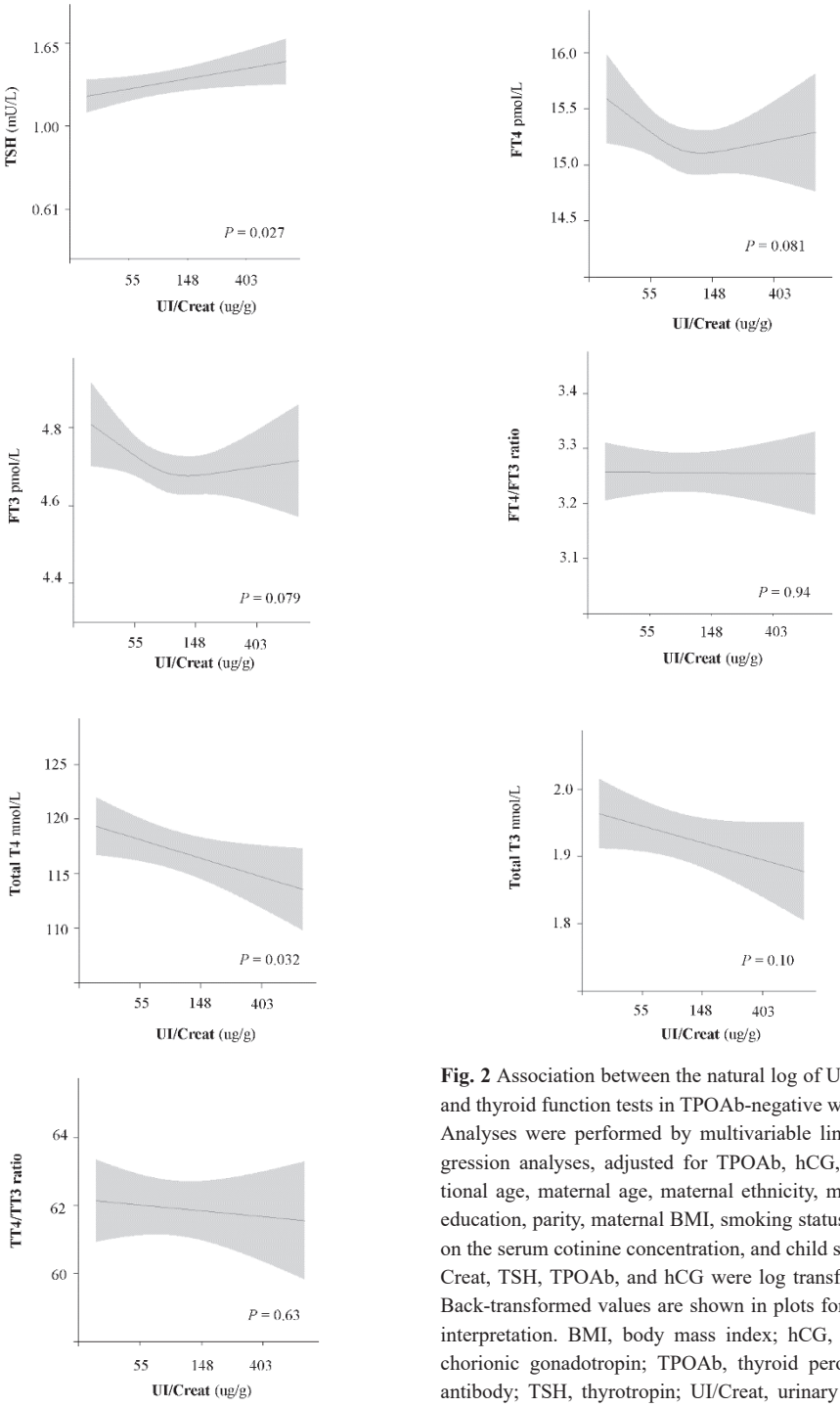

Fig. 2 Association between the natural log of UI/Creat and thyroid function tests in TPOAb-negative women. Analyses were performed by multivariable linear regression analyses, adjusted for TPOAb, hCG, gestational age, maternal age, maternal ethnicity, maternal education, parity, maternal BMI, smoking status based on the serum cotinine concentration, and child sex. UI/Creat, TSH, TPOAb, and hCG were log transformed. Back-transformed values are shown in plots for better interpretation. BMI, body mass index; hCG, human chorionic gonadotropin; TPOAb, thyroid peroxidase antibody; TSH, thyrotropin; UI/Creat, urinary iodine concentration corrected for creatinine.

Table 3 Association between UI/Creat and thyroid auto-antibodies.

	TPOAb positivity		TgAb positivity	
UI/Creat, µg/g[a]	**positive/negative (%)**	**OR (95% CI), *P***	**positive/negative (%)**	**OR (95% CI), *P***
<150[b]	176/1430 (11.0)	1.84 (1.07–3.20), 0.029	134/1467 (8.4)	1.60 (0.88-2.88), 0.12
150-249	15/232 (6.1)	1.00 (ref)	13/233 (5.3)	1.00 (ref)
250-499	10/119 (7.8)	1.26 (0.52–3.09), 0.61	9/120 (7.0)	1.45 (0.53–3.93), 0.47
≥ 500	3/21 (12.5)	2.36 (0.54–10.43), 0.26	2/22 (8.3)	1.61 (0.27–9.65), 0.60

Analyses were performed by multivariable logistic regression analyses, adjusted for hCG, gestational age, maternal age, maternal ethnicity, maternal education, parity, maternal BMI, cotinine, and child sex. TPOAb positivity and TgAb positivity were defined as
>34 IU/mL and >115 IU/mL, respectively.

[a] The total number (%) of women with a UI/Creat <150, 150-249, 250-499, and ≥ 500 µg/g in the total study population is 1609 (80.1%), 247 (12.3%), 129 (6.4%), and 24 (1.2%), respectively.

[b] Stratified sub-analysis TPOAb positivity: UI/Creat <50 µg/g, OR 1.70 (95% CI 0.86-3.37), *P*=0.13; 50-99 µg/g, OR 1.92 (1.09-3.40), *P*=0.025; 100-149 µg/g, OR 1.74 (0.93-3.25), *P*=0.084. Stratified sub-analysis TgAb positivity: UI/Creat <50 µg/g, OR 1.79 (95% CI 0.85-3.78), *P*=0.13; 50-99 µg/g, OR 1.62 (0.87-3.01), *P*=0.13; 100-149 µg/g, OR 1.66 (0.84-3.26), *P*=0.14.

CI, confidence interval; OR, odds ratio.

Discussion

In this study of pregnant women from a mild-to-moderately iodine-deficient population, we studied the association of iodine status with a detailed profile of thyroid function and auto-antibodies. Gestational reference ranges for thyroid function tests did not differ substantially by iodine status. We show that a lower UI/Creat was associated with significantly lower TSH, higher TT4, and a higher risk of TPOAb positivity. A lower UI/Creat was also associated with a higher FT4, FT3, and TT3, but these associations did not reach statistical significance ($P \leq 0.10$). The FT4/FT3 and TT4/TT3 ratios were stable across the full range of UI/Creat.

Low iodine intakes may trigger mechanisms to counteract any potential thyroidal consequences. These include an increase in the thyroidal uptake of iodine, mainly via stimulation of the sodium-iodide symporter by increasing TSH concentrations [18]. This typically occurs from a daily iodine intake of <100 µg/day and results in a lower renal iodine clearance, thus likely reflected by a lower UI/Creat. In the current study, a higher UI/Creat was associated with a higher TSH. This finding has been reported in other pregnant populations as well [6,8]. A possible explanation would be that a lower UI/Creat also increases the risk of autonomous thyroid hormone production by nodules/multinodular goiters, which is associated with a lower TSH [19,20]. Alternatively, a higher TSH at a higher range of UI/Creat values may also partially reflect the Wolff-Chaikoff effect caused by higher iodine intakes, especially when this increase occurs rapidly [15,21]. In rats, a higher TSH was explained by reduced deiodinase type II activity due to excessive iodine intakes [22,23]. The TSH reference ranges in the total population, however, did not deviate much from the reference ranges among groups of UI/

Creat, ranging from iodine deficiency to iodine sufficiency, which is in line with a previous study from Norway [7]. While it is known that reference ranges differ between populations [24], and that those differences may be partially explained by differences in ethnicity [25–27], there has been insufficient evidence that iodine status may also affect thyroid function within its normal range. There is a concern that reference ranges may be affected when calculated in populations with (mild-to-moderate) iodine deficiency. This concern could be ascribed to the association with thyroid autoimmunity, as there may be a higher risk of TPOAb positivity at lower iodine intakes. However, it has already been recommended that the calculation of thyroid function reference ranges should be performed in TPOAb-negative pregnant populations [4]. This study therefore calls into question the relevance of the selection criterion of iodine sufficiency for calculating thyroid reference ranges during pregnancy in addition to the criterion of excluding TPOAb-positive women.

Low iodine intake can cause a shift in thyroid hormone availability from T4 to the more biologically active T3 via up-regulating peripheral type II deiodinase and by increasing the thyroidal secretion of T3 [28,29]. Given this knowledge, we hypothesized that a lower UI/Creat would be associated with lower TT4 and FT4. In contrast, our analyses show that lower UI/Creat was associated with a higher TT4 and also a trend towards a higher FT4 with UI/Creat lower than roughly 100 μg/g. The results of other studies have also been contra-intuitive with regards to the underlying physiology. Two of those, of which one was performed in an iodine-replete population and the other in a mild-to-moderate iodine deficient population, also found a negative association between UI/Creat and FT4 [7,8]. Others investigated the potential effect of iodine supplementation on FT4 [7,30]. One of these found that women who started iodine supplementation between week 13 and 20 of gestation had lower FT4 than non-users or women who started supplementation preconceptionally [7]. Another study showed that although the majority of women using iodine supplements had a FT4 within the normal range, those who had received iodine supplements in early pregnancy exhibited a faster decline in FT4 than those who had consumed iodized salt for at least two years before pregnancy [30]. This could indicate that a more acute increase in iodine intake during pregnancy, rather than prolonged exposure to higher iodine availability, affects the association of UI/Creat with thyroid function, leading to a transient decrease in FT4, for example, via the Wolff-Chaikoff effect [21]. Future studies are needed to identify thresholds from which iodine supplementation could affect thyroid function.

Based on the physiological adaptations in thyroid hormone physiology, we expected *a priori* that a lower UI/Creat would be associated with a higher FT3 and TT3, and possibly would decrease the FT4/FT3 and TT4/TT3 ratios. Our study shows a trend toward an association of UI/Creat - lower than roughly 100 μg/g - with an increase in FT3, but this association did not reach statistical significance. A similar, but statistically significant, association was identified in a study including a population with a slightly lower median UIC than in our population (68 μg/L vs. 90 μg/L in SELMA) and with a larger proportion of women below the recommended

cut-off for defining iodine deficiency during pregnancy, i.e., UIC<150 μg/L (86% vs. 78.5% in SELMA) [7]. Other studies show a weak but statistically significant negative correlation between UIC and FT4 conducted in similarly iodine-insufficient area (median UIC 89 μg/L) [9] and a sufficient area (median UIC 328 μg/L) [8], or oppositely, a positive correlation in a population with a large proportion of women with excessive iodine intakes [11]. However, in our study, there was no association of UI/Creat with the FT4/FT3 and TT4/TT3 ratios, which could suggest that iodine availability was not low enough to evoke preferential production of T3 in this population. Possibly in more chronic severe iodine-deficient populations, women are at a higher risk of deplete intra-thyroidal iodine storage already at the beginning or during the course of pregnancy, which may cause shifts in these ratios [1].

Thyroid autoantibody positivity occurs in 2% to 17% of pregnant women [4]. TPOAb positivity is associated with a higher risk of miscarriage [31], preterm birth [31,32], child behavioral problems [33], and lower child IQ [34]. In the current study, TPOAb positivity occurred more often in women with low or high UI/Creat. Interestingly, thyroid antibody concentrations tend to decline after the first trimester due to immune tolerance, and this could potentially affect the definition of TPOAb positivity [35–37]. However, in our study, blood samples were collected at a median gestational age of 10 weeks, before such a decrease in TPOAb titres would likely occur. Women with a UI/Creat of 150-249 μg/g had the lowest prevalence of TPOAb positivity (6.1% vs. 11.0% and 12.5% in women with a UI/Creat of <50 and ≥ 500 μg/g, respectively). This finding is in line with a large study from an iodine-sufficient area in China, showing similar differences in the prevalence of TPOAb positivity among different UIC groups [6]. Also, a study from Norway showed a U-shaped association of iodine intake measured by a food frequency questionnaire with TPOAb positivity in mild-to-moderately iodine-deficient pregnant women [7]. That association, however, did not reach statistical significance and no association of urinary iodine with TPOAb positivity was identified [7]. We speculate that owing to the few women in each cohort with excessive iodine intakes, both our study and the Norwegian study did not have sufficient statistical power to show that high iodine intake is also associated with a higher risk of thyroid autoimmunity. Excessive iodine intakes may cause a shift toward pro-inflammatory cells and a higher generation of radical oxygen species due to increased iodination of thyroglobulin, activating pathways promoting thyrocyte apoptosis and thyroiditis [38,39].

We were able to study the association of iodine status with thyroid function using an extensive dataset with multiple thyroid function tests and thyroid auto-antibodies, and with a large number of potential confounding variables. An important limitation for interpreting the results of the current study is that early morning urine void samples tend to underestimate daily iodine excretion [40]. Furthermore, it is known that urinary iodine is highly variable from day-to-day and therefore not a good indicator of individual iodine status [41,42]. It is important to note that the UI/Creat is a valid measure of iodine status of a population with the same sex and age [43], or large groups of individuals as those that were analyzed in the current study with a

standardized sampling of morning void urine, because this reduces the consequences of measurement error. Another limitation is that the use of a single void urine and blood sample to measure iodine status and thyroid function, respectively, did not allow to study iodine status trajectories during pregnancy and subsequent changes in thyroid function. There was also a lack of data on iodine containing supplement use. It would have been relevant to investigate whether the associations we observed in our study would differ based on supplement use and timing of intake as this could subdivide more acute from chronic changes in iodine intake [7]. There are currently no national recommendations in Sweden on iodine supplementation during pregnancy and no data on the use of iodine containing supplements among pregnant women [44]. However, a considerable percentage of pregnant women in Nordic countries, such as Norway and Denmark, use iodine containing supplements, which possibly suggests that the use of these supplements may also be substantial in Sweden [7,17]. Lastly, the cross-sectional design of this study limits causal inference, and therefore, it is important to interpret these results while taking into account data from interventional and experimental studies.

To conclude, this study did not find any indication that differences in iodine status within the population had a meaningful effect on the reference ranges of thyroid function tests. Furthermore, we found that suboptimal gestational iodine status is associated with lower TSH, and higher TT4, but we found no clear indication of preferential T3 production due to iodine deficiency. Both low and high gestational iodine status may be associated with a higher risk of TPOAb positivity.

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Supplemental material

Supplementary Table 1 Reference ranges (2.5th and 97.5th percentile values) of the various thyroid function tests in TPOAb-negative women according to groups of UI/Creat (µg/g).

	UI/Creat (µg/g)					
	Total	**<50**	**50-99**	**100-149**	**150-249**	**250-499**
	N=1802	N=219 (12.2%)	N=859 (47.7%)	N=352 (19.5%)	N=232 (12.9%)	N=119 (6.6%)
TSH, mU/L	0.11-3.40	0.15-3.08	0.10-3.47	0.17-3.40	0.09-2.92	0.04-3.71
FT4, pmol/L	11.6-19.3	11.9-19.3	11.7-19.1	11.4-18.7	11.9-19.8	12.2-18.4
FT3, pmol/L	3.73-5.89	3.65-5.77	3.79-5.89	3.67-5.86	3.70-5.94	3.74-5.83
TT4, nmol/L	82.4-165.6	87.2-169.8	83.3-167.1	79.7-156.9	85.5-156.4	77.4-160.5
TT3, nmol/L	1.29-2.88	1.39-2.87	1.29-2.88	1.27-2.89	1.31-2.78	1.26-2.90

The subgroup of women with UI/Creat ≥ 500 µg/g (N=21, 1.2%) was too small for reliable calculations and therefore omitted. FT3, free triiodothyronine; FT4, free thyroxine; TT3, total triiodothyronine; TT4, total thyroxine; TSH, thyrotropin; UI/Creat, urinary iodine concentration corrected for creatinine.

Supplementary Table 2 Reference ranges (2.5th and 97.5th percentile values) of the various thyroid function tests in TPOAb-negative women according to groups of UIC (µg/L).

	UIC (µg/L)				
	<50	**50-99**	**100-149**	**150-249**	**250-499**
	N=176 (8.8%)	N=965 (48.0%)	N=421 (21.0%)	N=264 (13.1%)	N=128 (6.4%)
TSH, mU/L	0.11-3.06	0.12-3.36	0.16-3.49	0.16-3.67	0.05-3.49
FT4, pmol/L	11.7-20.4	11.5-19.2	11.6-18.9	12.0-19.3	12.0-20.0
FT3, pmol/L	3.87-6.21	3.75-5.87	3.70-5.80	3.69-5.98	3.89-6.03
TT4, nmol/L	87.8-166.7	82.5-164.3	85.0-164.7	79.0-158.5	81.0-174.3
TT3, nmol/L	1.35-2.87	1.29-2.81	1.28-2.93	1.27-2.96	1.30-2.86

The subgroup of women with UIC ≥ 500 µg/L (N=38, 1.9%) was too small for reliable calculations and therefore omitted. UIC, urinary iodine concentration.

Supplementary Table 3 Association between the natural log of UI/Creat and thyroid functions test in TPOAb-negative women.

	N	**β ± SE**	***P* value**
TSH	1747	0.065 ± 0.029	0.025
FT4	1739	-0.086 ± 0.073	0.24
FT3	1736	-0.033 ± 0.021	0.11
FT4/FT3 ratio	1738	-0.002 ± 0.016	0.91
TT4	1733	-1.713 ± 0.794	0.031
TT3	1730	-0.025 ± 0.016	0.11
TT4/TT3 ratio	1733	-0.134 ± 0.370	0.72

Analyses were performed by multivariable linear regression analyses, adjusted for TPOAb, hCG, gestational age, maternal age, maternal ethnicity, maternal education, parity, maternal BMI, smoking status based on the serum cotinine concentration, and child sex. UI/Creat, TSH, TPOAb were log transformed. BMI, body mass index; hCG, human chorionic gonadotropin.

Supplementary Table 4 Association between UI/Creat and thyroid auto-antibodies according to quartiles of UI/Creat.

UI/Creat, quartiles	N	median UI/Creat (range), µg/g	TPOAb positivity, N (%)	TgAb positivity, N (%)
quartile 1	503	50 (20-61)	58 (11.6)	46 (9.2)
quartile 2	502	72 (61-85)	55 (11.0)	36 (7.2)
quartile 3	501	103 (85-132)	52 (10.4)	42 (8.4)
quartile 4	503	191 (132-1368)	39 (7.8)	34 (6.8)

TPOAb positivity and TgAb positivity were defined as > 34 IU/mL and >115 IU/mL, respectively.

Supplementary Table 5 Association between urinary iodine concentration and thyroid auto-antibodies

UIC, µg/L[a]	TPOAb positivity		TgAb positivity	
	positive/negative (%)	OR (95% CI), *P*	positive/negative (%)	OR (95% CI), *P*
<150[b]	172/1404 (10.9)	1.78 (1.06–3.00), 0.030	129/1442 (8.2)	1.22 (0.72-2.04), 0.45
150-249	17/247 (6.4)	1.00 (ref)	18/245 (6.8)	1.00 (ref)
250-499	11/117 (8.6)	1.29 (0.55–3.02), 0.56	6/122 (4.7)	0.58 (0.22–1.56), 0.28
≥ 500	4/34 (10.5)	2.20 (0.60–7.98), 0.23	5/33 (13.2)	2.27 (0.72–7.12), 0.16

Analyses were performed by multivariable logistic regression analyses, adjusted for hCG, gestational age, maternal age, maternal ethnicity, maternal education, parity, maternal BMI, smoking status based on the serum cotinine concentration, and child sex. TPOAb positivity and TgAb positivity were defined as > 34 IU/mL and >115 IU/mL, respectively.

[a] The total number (%) of women with a UIC <150, 150-249, 250-499, and ≥500 µg/L in the total study population is 1579 (78.6), 264 (13.1), 128 (6.4), and 38 (1.9%), respectively.

[b] Stratified sub-analysis TPOAb positivity: UIC <50 µg/L, OR 1.20 (95% CI 0.56-2.58), *P*=0.64; 50-99 µg/L, OR 2.0 (1.18-3.44), *P*=0.010; 100-149 µg/L, OR 1.55 (0.84-2.86), *P*=0.16. Stratified sub-analysis TgAb positivity: UIC <50 µg/L, OR 1.37 (95% CI 0.67-2.80), *P*=0.38; 50-99 µg/L, OR 1.32 (0.77-2.24), *P*=0.31; 100-149 µg/L, OR 0.83 (0.44-1.58), *P*=0.58.

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Chapter 4

Maternal iodine status and child neurodevelopment

Chapter 4.1

Association of maternal iodine status with child IQ: a meta-analysis of individual-participant data

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Abstract

Context: Although the consequences of severe iodine deficiency are beyond doubt, the effects of mild to moderate iodine deficiency in pregnancy on child neurodevelopment are less well established.

Objective: To study the association between maternal iodine status during pregnancy and child IQ and identify vulnerable time windows of exposure to suboptimal iodine availability.

Design: Meta-analysis of individual participant data from three prospective population-based birth cohorts: Generation R (Netherlands), INMA (Spain), and ALSPAC (United Kingdom); pregnant women were enrolled between 2002 and 2006, 2003 and2008, and 1990 and1992, respectively.

Setting: General community.

Participants: A total of 6180 mother-child pairs with measures of urinary iodine and creatinine concentrations in pregnancy and child IQ. Exclusion criteria were multiple pregnancies, fertility treatment, medication affecting the thyroid, and preexisting thyroid disease.

Intervention(s): None.

Main Outcome Measure: Child non-verbal and verbal IQ assessed at 1.5 to 8 years of age.

Results: There was a positive curvilinear association of urinary iodine/creatinine ratio (UI/Creat) with mean verbal IQ only. UI/Creat <150 μg/g was not associated with lower non-verbal IQ (-0.6 point; 95% CI: -1.7 to 0.4 points; *P*=0.246) or lower verbal IQ (-0.6 point; 95% CI: -1.3 to 0.1 points; *P*=0.082). Stratified analyses showed that the association of UI/Creat with verbal IQ was only present up to 14 weeks of gestation.

Conclusions: Fetal brain development is vulnerable to mild to moderate iodine deficiency, particularly in the first trimester. Our results show that in future randomized controlled trials investigating the effect of iodine supplementation on child neurodevelopment in women with mild to moderate iodine deficiency, such supplementation should begin no later than the first trimester.

Introduction

Iodine is an essential trace element required for the production of thyroid hormones; optimal thyroid hormone availability is important for normal fetal brain development [1,2]. During pregnancy, there is a higher demand for maternal iodine intake [3,4]. This is due to i) the increased maternal thyroid hormone synthesis required to ensure adequate thyroid hormone availability to the fetus, ii) greater urinary iodine loss due to an increased glomerular filtration rate, and iii) placental transfer of iodine to the fetus to facilitate fetal thyroid hormone production. Although severe iodine deficiency is no longer common in Europe, mild to moderate iodine deficiency is still common, especially in pregnant women [5]. Severe iodine deficiency in pregnancy results in a higher risk of goiter, hypothyroidism, and mental retardation in the offspring [6]. However, the consequences of mild to moderate iodine deficiency in pregnancy on child neurodevelopment are less well established [4].

Mild to moderate iodine deficiency or low iodine intake during pregnancy has been associated with adverse child neurodevelopmental outcomes observed in some [7–13] but not all studies [14–16]. Differences in results between studies may be related to methodological differences (e.g., measurement of iodine status, selected reference group, and available data on confounders), the age at assessment of the neurodevelopmental outcome of interest, the timing of the iodine measurements, and the relative severity of iodine deficiency in the population. Although the main focus in the literature has been on the effects of iodine deficiency, some studies have suggested adverse effects of supplementary intake or excess iodine on either maternal thyroid function [17,18], fetal thyroid function [19,20], or child neurodevelopment [10,15,16].

International health authorities have similar recommendations to ensure optimal iodine status in pregnancy [21–23]. It is universally recognized that any necessary iodine supplementation should be commenced before or as early as possible in pregnancy to achieve adequate iodine intake, owing to the susceptibility of the fetal brain to iodine deficiency [23]. However, whether the effect of iodine on child cognition varies during different stages of pregnancy is unknown. We therefore assessed the association between maternal iodine status in pregnancy and child IQ across three cohorts of differing iodine status and investigated potential effect modification by gestational age.

Material and Methods

Study design and populations

This study was embedded in three cohort studies: Generation R (the Netherlands), the INfancia y Medio Ambiente Project (INMA; Spain, three regions), and the Avon Longitudinal Study of Parents and Children (ALSPAC; United Kingdom). The study designs have been described elsewhere [24–27]; the ALSPAC study website contains details of all the data that

are available through a fully searchable data dictionary and variable search tool [28]. For the current study, mother-child pairs were included if a measure of urinary iodine and creatinine concentration during pregnancy and child IQ scores were available. Exclusion criteria were multiple pregnancies, fertility treatment, medication affecting the thyroid, and preexisting thyroid disease. Ethical approval was obtained from the Medical Ethical Committee of the Erasmus Medical Center (Generation R); the Ethical Committee of the Municipal Institute of Medical Investigation and the Ethical Committee of the hospitals involved in the study (INMA); and the ALSPAC Ethics and Law Committee and local research ethics committees; approval was given by participants and/or parents or guardians of the children by signed informed consent form.

Maternal iodine status

Urinary iodine concentration (UIC) and creatinine concentration were measured in spot urine samples stored at -20°C after collection. As part of this study, additional urine samples were analyzed for iodine and creatinine concentrations, and existing measurements from each cohort [7,19,29] were also included. The additional measurements were performed in the same laboratories where the existing measurements were performed. The laboratories were registered with EQUIP and used certified reference materials (Seronorm Urine Levels one and two; Nycomed, Norway) for the verification of results. In Generation R, UIC was measured by the Sandell-Kolthoff method. In INMA, UIC was measured using paired-ion reversed-phase, high-performance liquid chromatography with electrochemical detection at a silver working electrode (Waters Chromatography, Milford, MA). In ALSPAC, UIC was measured on a dynamic reaction cell inductively coupled plasma mass spectrometer. Urinary creatinine concentration was determined by the Jaffe rate method in all cohorts. More information on the measurement methods and the variability between assays can be found in an online repository [30].

In a subset of women, repeated measures of urinary iodine and creatinine were available; we used the earliest available sample as an indicator of iodine status. The urinary iodine/creatinine ratio (UI/Creat) was used as a measure of iodine status. Because of possible contamination of UIC by the use of iodine-containing test strips in ALSPAC [31], UIC >500 µg/L and/or UI/Creat >700 µg/g was excluded from the analyses in this cohort (N=363). These cutoffs were based on previous work in ALSPAC and from other studies of pregnant women in the United Kingdom [7,32,33]. We grouped women's results by UI/Creat as follows: (i) <150 µg/g, (ii) 150 to < 500 µg/g, and (iii) ≥500 µg/g; according to the World Health Organization classification, these groups broadly relate to iodine deficiency, sufficiency, and excess, respectively.

Maternal thyroid function

TSH and free thyroxine (FT4) were measured according to different methodologies between cohorts, which are described in detail elsewhere [34–36]. For the analysis, FT4 and TSH concentrations were logarithmically transformed, and cohort-specific SD scores were calculated with a mean of 0 and an SD of 1 based on the data of thyroid peroxidase antibody (TPOAb)-negative women (TPOAb measurements were available in Generation R and ALSPAC). TPOAb titres ≥ 60 IU/mL and ≥ 6 IU/mL were considered positive in Generation R and ALSPAC, respectively. These cutoffs were determined by the assay manufactures.

Non-verbal and verbal IQ

In Generation R, non-verbal IQ was assessed at a median age of 5.9 years using a subset of the Snijders Oomen Nonverbal Intelligence Test (2.5-7-Revised) [37], and verbal IQ was estimated by the short form of the McArthur Communicative Development Inventory [38] at a median age of 1.5 years. In INMA, non-verbal and verbal IQ scores were assessed at a median age of 4.6 years using McCarthy Scales of Children´s Abilities [39]. In ALSPAC, non-verbal and verbal IQ scores were assessed at a median age of 8.6 years using the Wechsler Intelligence Scale for Children, third UK edition [40]. Except for verbal IQ ascertainment in Generation R, which involved a parental questionnaire, all other measurements were performed by psychologists or trained staff. To homogenize the different scores, raw cohort-specific scores were standardized to a mean of 100 and an SD of 15. Children with IQ scores <50 or >150 (n=3) were considered as outliers and excluded from analyses. Suboptimal IQ was defined as an IQ score <85.

Potential confounding variables

Information on maternal age, educational level (low, middle, high), ethnicity/country of birth (cohort-specific categories), parity (zero, one, two or more), pre-pregnancy body mass index, and smoking during pregnancy (never smoked, smoked in the beginning or until pregnancy confirmed, continued smoking) was collected by questionnaires administered during pregnancy. Gestational age at urine sampling was defined using ultrasonography or last menstrual period. Child sex and age at time of the IQ assessment was obtained during the study visits.

Statistical analyses

UI/Creat was not normally distributed and was therefore transformed using the natural logarithm; back-transformed values are shown in plots for better interpretation. We studied the associations of UI/Creat with child non-verbal and verbal IQ by using one-step and two-step approaches. In the one-step approach, data from the cohorts were pooled, and we performed standard multivariable linear regression models with and without a quadratic term to investigate the possible non-linear nature of the associations. Non-linearity was also investigated by using ordinary least squares linear regression models with restricted cubic splines with three knots. With ANOVA, we tested the null hypothesis that child mean IQ was similar across

the full range of the natural logarithm of UI/Creat. The decision for linear regression models instead of multilevel models for the one-step analyses was made because we found no difference between multilevel models with random intercepts and/or slope per cohort vs standard linear regression correcting for cohort (e.g., cohort-specific variable ethnicity/country of birth) when assessed using the Akaike information criterion and log-likelihood tests. In the two-step approach we first studied the associations of UI/Creat < 150 µg/g and UI/Creat ≥ 500 µg/g with child IQ by using linear regression models in each cohort separately. In these analyses, the reference group consisted of women with UI/Creat of 150 to 500 µg/g. We then combined the cohort-specific effect estimates using random-effects meta-analyses.

Potential effect modification according to gestational age was analyzed by adding a product interaction term between UI/Creat and gestational age to the one-step approach models. Because of the known constraints of statistical power for interaction analyses, a *P* value <0.15 for interaction terms was used to screen for potential relevant modification [41]. We further quantified potential relevant differences by performing stratified analyses by tertiles of gestational age (≤12 weeks, >12 to ≤14 weeks, and >14 weeks). We also studied associations with suboptimal IQ (score <85) by combining cohort-specific estimates from logistic regression models into random-effects meta-analyses.

Sensitivity analyses were designed to study i) the associations of UI/Creat with verbal IQ score in mother-child pairs from INMA and ALSPAC only, as verbal IQ was assessed at a preschool age in Generation R; ii) the association between UI/Creat and maternal TSH and FT4 SD scores within the ± 4 SD range around the mean, as TSH and FT4 values outside this range were considered outliers (n=19 for TSH; n=5 for FT4); and iii) whether the association between UI/Creat and IQ score could potentially be explained by maternal thyroid function by adjusting for FT4 and TSH in the models.

Heterogeneity between cohorts was assessed using the Cochran Q test and the I^2 statistic [42]. All models were adjusted for potential confounding variables. However, because of collinearity between maternal ethnicity/country of birth, child age at IQ ascertainment, and cohort, we only adjusted for maternal ethnicity/country of birth in the one-step approach models.

We applied inverse probability weighting to take into account the potential differential loss to follow-up (Supplemental Table 1) [30] (*i.e.,* to account for selection bias that potentially arises when only the population with available data on iodine status and child IQ is included compared with a full initial cohort recruited at pregnancy [43]). Briefly, we used information available for all participants at recruitment to predict the probability of participation in the study and used the inverse of these probabilities as weights in the analyses so that results would be representative of the initial populations of the cohorts. In addition, missing values in potential confounding variables were imputed using chained equations [44]. A total of 25 datasets were generated. A *P* value <0.05 was defined as statistically significant. Statistical analyses were performed in STATA (version 14.0; StataCorp, College Station, TX) and R statistical software (version 3.3.2, package rms).

Results

The final study population consisted of 6180 mother child-pairs (Fig. 1). The median UIC (UI/Creat) was 159 µg/L (214 µg/g) in Generation R (adequate intake), 128 µg/L (152 µg/g) in INMA (mild deficiency), and 96 µg/L (124 µg/g) in ALSPAC (moderate deficiency; Table 1). Iodine status was determined at a median [interquartile range (IQR)] gestational age of 13.1 (12.1,14.8) weeks, 13.0 (12.4,14.1) weeks, and 12.0 (8.0-16.0) weeks in Generation R, INMA, and ALSPAC, respectively.

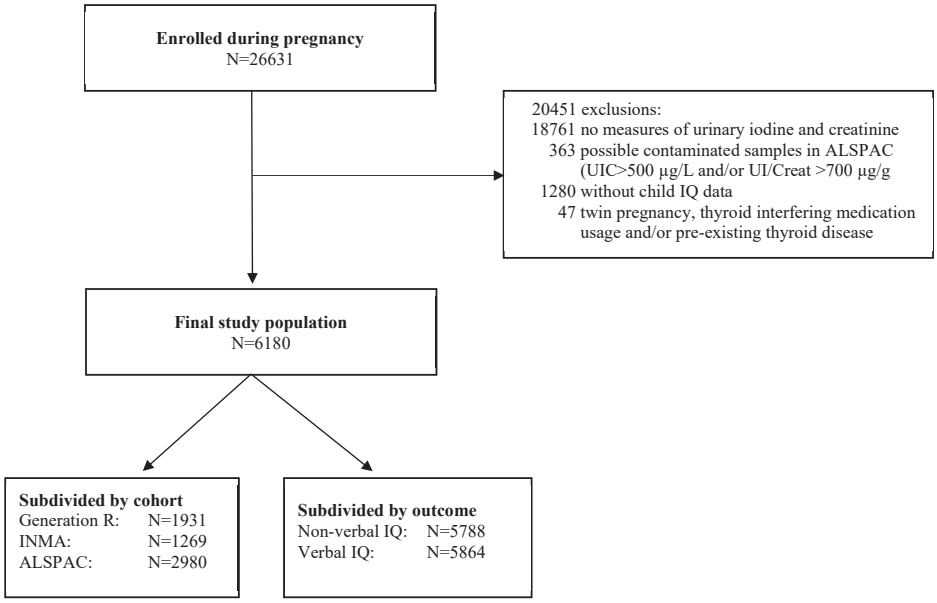

Figure 1 Flowchart of selection of the study population.

Non-verbal IQ

Using pooled data in the one-step approach, we observed a positive linear association between the UI/Creat and mean non-verbal IQ [Fig. 2(a) and Supplemental Table 2 [30]], although this association was not statistically significant. Using the two-step approach in which we combined cohort-specific effect estimates using random-effects meta-analysis, neither UI/Creat <150 µg/g nor UI/Creat ≥500 µg/g was associated with non-verbal IQ (-0.6 point; 95% CI: -1.7 to 0.4 points; *P*=0.246 and -1.1 points, 95% CI: -4.2 to 2.0 points; *P*=0.478) [(Fig. 2(b) and 2(c)]. UI/Creat was not associated with suboptimal non-verbal IQ (Supplemental Figure 1 [30]).

Verbal IQ

Using the one-step approach, we observed a positive curvilinear association between UI/Creat and verbal IQ [Fig. 3(a) and Supplemental Table 2 [30]]. There was a positive linear association when measures in preschool children from Generation R were excluded. Using the two-step approach, neither UI/Creat <150 µg/g nor UI/Creat ≥500 µg/g was associated with verbal IQ (-0.6 point, 95% CI: -1.3 to 0.1 points; *P*=0.082 and -0.6 point, 95% CI: -2.6 to 1.4 points; *P*=0.552, respectively) [Fig. 3(b) and 3(c) or suboptimal verbal IQ; Supplemental Figure 1 [30]].

Effect modification according to gestational age

The continuous association of UI/Creat with non-verbal IQ did not differ according to gestational age at measurement *(P* for interaction term=0.306*)*. By contrast, we identified possible effect modification by gestational age in the association with verbal IQ (*P* for interaction term=0.078). Stratification by tertile of gestational age showed a positive curvilinear association of UI/Creat with mean child verbal IQ, with an overall effect of ~5 IQ points during the first 12 weeks of pregnancy [Fig. 4(a) and Supplemental Table 3 [30]]. Furthermore, there was a positive linear association between UI/Creat and mean child verbal IQ during the 12th to 14th weeks of pregnancy, with an overall effect of ~3 IQ points [Fig. 4(b)]. This association was no longer present after the 14th week of pregnancy [Fig. 4(c)].

Table 1 Population characteristics.

	Generation R (n=1931)		INMA (n=1269)		ALSPAC (n=2980)	
Variable	**n**	**values**	**n**	**values**	**n**	**values**
Offspring neurodevelopment, no. (%)						
Suboptimal non-verbal IQ[a]	1540	175 (11.4)	1269	216 (17.0)	2979	479 (16.1)
Suboptimal verbal IQ[a]	1618	279 (17.2)	1269	211 (16.6)	2977	480 (16.1)
Female sex, no. (%)	1931	963 (49.9)	1268	632 (49.8)	2980	1514 (50.8)
Iodine status	1931		1269		2980	
UI/Creat, µg/g, median (IQR)		214 (143-308)		152 (96-258)		124 (82-199)
UI/Creat < 150 µg/g, no. (%)		531 (27.5)		623 (49.1)		1831 (61.4)
UI/Creat > 500 µg/g, no. (%)		97 (5.0)		52 (4.1)		81 (2.7)
UIC, µg/L, median (IQR)		159 (90-275)		128 (75-213)		96 (57-153)
Gestational age at urine sampling, wk	1931		1267		2980	
Median (IQR)		13.1 (12.1-14.8)		13.0 (12.4-14.1)		12.0 (8.0-16.0)
Range (min-max)		6.1-30.5		8.6-39.4		1.0-42.0
>20th week of gestation, no. (%)		66 (3.4)		130 (10.2)		211 (7.1)
Maternal thyroid function						
TSH, mIU/L, median (IQR)	1719	1.29 (0.79-1.95)	1227	1.25 (0.85-1.80)	1102	0.97 (0.64-1.38)
FT4, pmol/L, median (IQR)	1728	14.6 (12.9-16.5)	1229	10.6 (9.7-11.6)	1108	16.2 (14.9-17.7)
TPOAb positivity, no. (%)	1737	98 (5.6)	NA	NA	1111	146 (13.1)
Gestational age, wk, mean (SD)	1733	13.3 (1.9)	1228	13.2 (1.4)	1118	10.3 (2.7)

Table 1 Population characteristics. (continued)

	Generation R (n=1931)		INMA (n=1269)		ALSPAC (n=2980)	
Variable	**n**	**values**	**n**	**values**	**n**	**values**
Educational level, no. (%)	1835		1265		2888	
Low		154 (8.4)		270 (21.3)		573 (19.8)
Middle		760 (41.4)		525 (41.5)		1810 (62.7)
High		921 (50.2)		470 (37.2)		505 (17.5)
Maternal ethnicity/country of birth, no. (%)	1803		1266		2877	
Spanish		NA		1184 (93.5)		NA
Latin-American		NA		56 (4.4)		NA
European/other		NA		26 (2.1)		NA
Dutch		1012 (53.2)		NA		NA
Indonesian		69 (3.6)		NA		NA
Cape Verdean		58 (3.1)		NA		NA
Moroccan		115 (6.0)		NA		NA
Surinamese		154 (8.1)		NA		NA
Turkish		170 (9.0)		NA		NA
Other, non-Western		150 (7.9)		NA		NA
Other, Western		174 (9.1)		NA		NA
Maternal ethnicity/country of birth, no. (%)						
White		NA		NA		2841 (98.7)
Non-White		NA		NA		36 (1.3)
Maternal age, y, mean (SD)	1931	30.5 (4.8)	1257	31.6 (3.9)	2980	28.6 (4.5)
Parity, no. (%)	1931		1267		2877	
0		1121 (58.1)		727 (57.4)		1346 (46.8)
1		564 (29.2)		458 (36.1)		992 (34.5)
≥2		246 (12.7)		82 (6.5)		539 (18.7)
Smoking during pregnancy, no. (%)	1744		1254		2926	
Never		1319 (75.6)		870 (69.4)		2434 (83.2)
In the beginning of pregnancy		168 (9.6)		168 (13.4)		125 (4.3)
Continued		257 (14.7)		216 (17.2)		367 (12.5)
Pre-pregnancy BMI, kg/m^2, median (IQR)	1694	22.6 (20.8-25.2)	1269	22.5 (20.8-25.0)	2713	22.2 (20.5-24.4)

Values are based on unimputed data.

Abbreviations: BMI, body mass index; IQR, interquartile range; NA, not applicable.

[a] Suboptimal defined as an IQ score <85.

Iodine status and thyroid function

UI/Creat was not associated with TSH (0.007, 95 CI%: -0.044 to 0.058; *P*=0.789) or with FT4 (-0.044, 95 CI%: -0.092 to 0.005; *P*=0.079). The association did not change between UI/Creat and child non-verbal or verbal IQ score after adjusting for TSH and/or FT4; there was also no sign of effect modification by TSH or FT4 (data not shown). There was no association of UI/Creat with TSH and FT4 in TPOAb-negative women only.

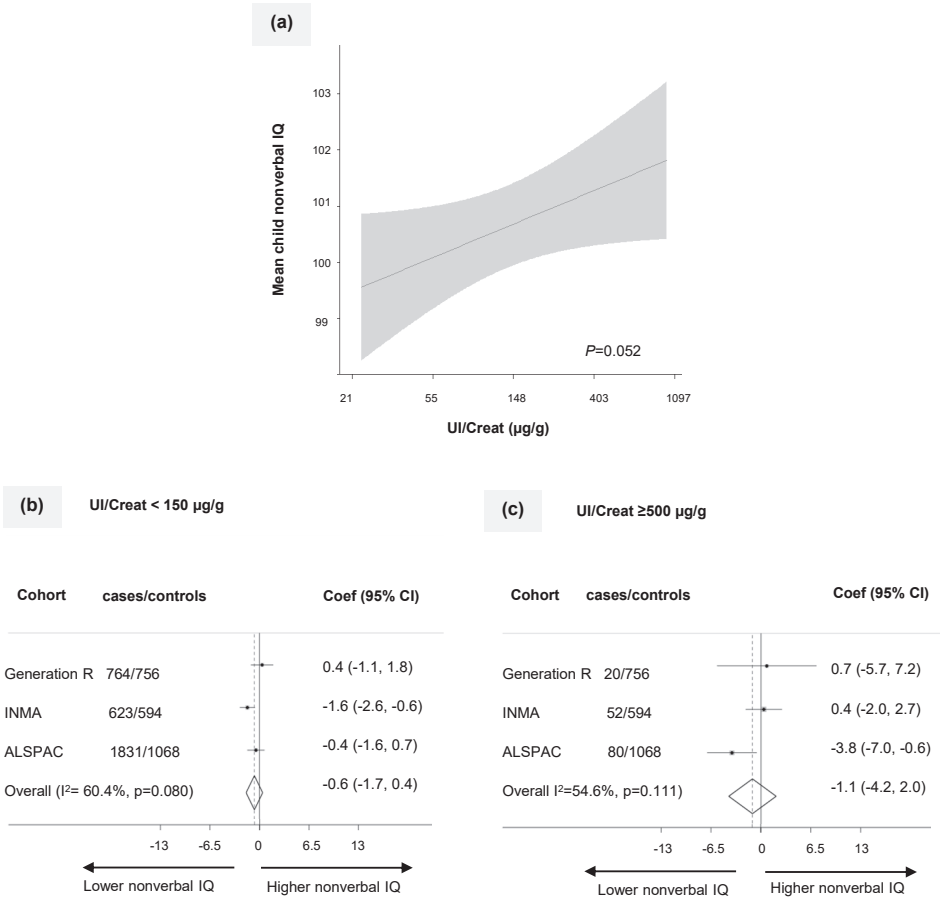

Figure 2 Association of maternal iodine status in pregnancy with child non-verbal IQ.
(a) Continuous association, depicted as the mean child non-verbal IQ (black line) with 95% confidence interval (gray area) using pooled data. Models were adjusted for gestational age, child sex, maternal ethnicity/country of birth, maternal education, parity, maternal age, pre-pregnancy body mass index, and smoking during pregnancy. The *P* value was provided by an ANOVA test of the null hypothesis that child mean non-verbal IQ was similar across the whole range of the natural logarithm of UI/Creat. Forest plots of (b) UI/Creat <150 µg/g ("deficiency") and (c) UI/Creat ≥500 µg/g ("excess") compared with the reference group of UI/Creat ≥150 to <500 µg/g ("sufficient"), depicted as effect estimate (dot) with 95% CI per cohort, and overall as estimated by random-effects meta-analysis (diamond). Abbreviation: Coef, coefficient.

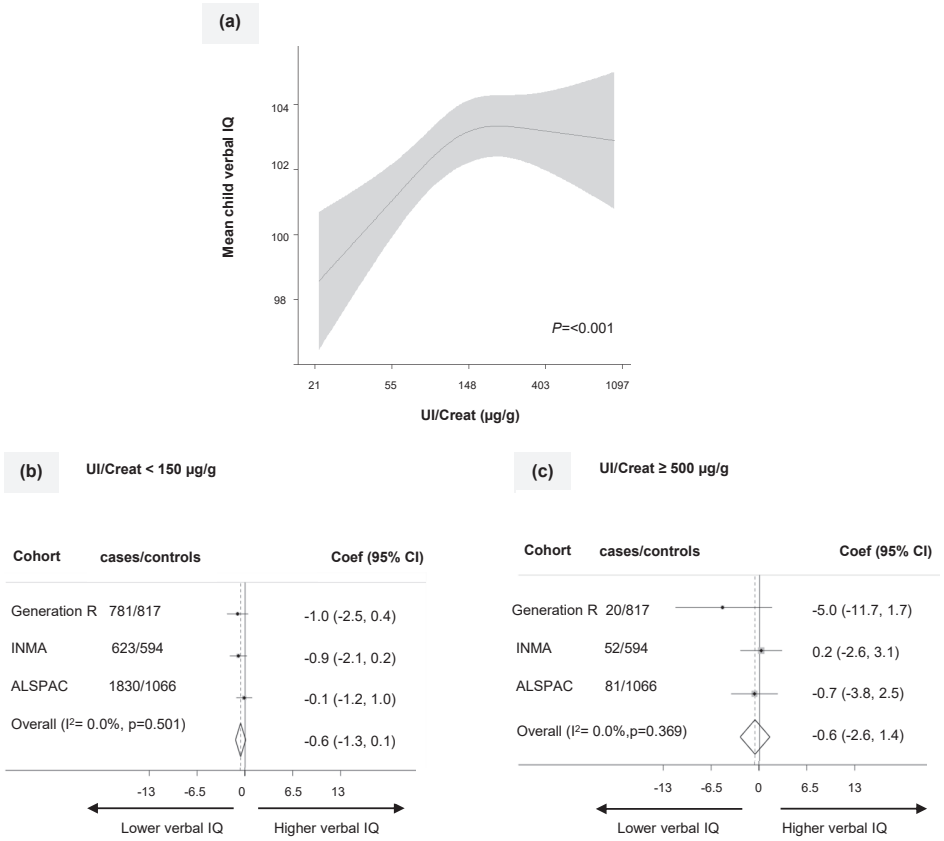

Figure 3 Association of maternal iodine status during pregnancy with child verbal IQ.
(a) Continuous association, depicted as the mean child verbal IQ (black line) with 95% CI (gray area) using pooled data. Models were adjusted for gestational age, child sex, maternal ethnicity/country of birth, maternal education, parity, maternal age, pre-pregnancy body mass index, and smoking during pregnancy. The *P* value was provided by an ANOVA test of the null-hypothesis that child mean verbal IQ was similar across the whole range of the natural logarithm of UI/Creat. Forest plots of (b) UI/Creat <150 µg/g ("deficiency") and (c) UI/Creat ≥500 µg/g ("excess") compared with the reference group of UI/Creat ≥150 to <500 µg/g ("sufficient"), depicted as effect estimate (dot) with 95% CI per cohort and overall as estimated by random-effects meta-analysis (diamond). Abbreviation: Coef, coefficient.

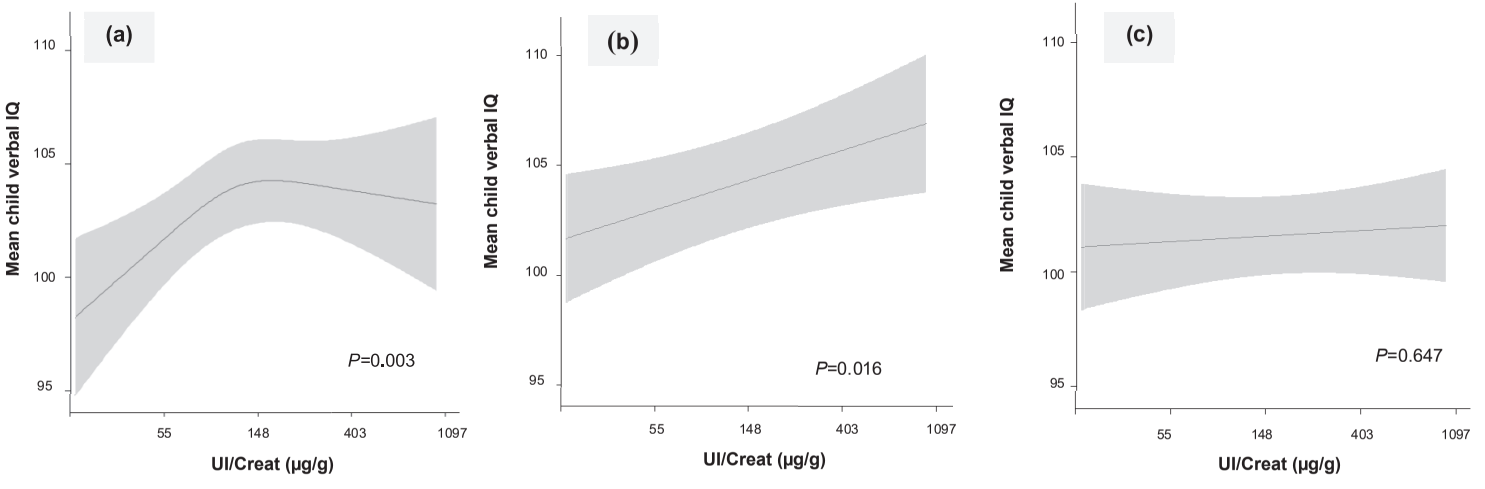

Figure 4 Association of maternal iodine status during pregnancy with child verbal IQ stratified by tertiles of gestational age.
Continuous association, depicted as the mean child verbal IQ (black line) with 95% CI (gray area) was restricted to (a) the first 12 weeks of gestation (lowest tertile, median UI/Creat 116 µg/g, n=2209); (b) from weeks 12 to 14 of gestation (middle tertile, median UI/Creat 147 µg/g; n=1776); and (c) later than week 14 of gestation (highest tertile, median UI/Creat 157 µg/g;. n=1879). Models were adjusted for gestational age, child sex, maternal ethnicity/country of birth, maternal education, parity, maternal age, pre-pregnancy body mass index, and smoking during pregnancy. The *P* value was provided by an ANOVA test of the null hypothesis that child mean verbal IQ was similar across the whole range of the natural logarithm of UI/Creat.

Discussion

This meta-analysis of individual participant data showed that a lower UI/Creat during pregnancy was associated with lower verbal IQ score. The association of UI/Creat with verbal IQ was only seen up to the start of the second trimester (up to the 14th week of gestation). In contrast, we observed no associations between IQ and UI/Creat <150 μg/g or ≥500 μg/g.

Only a few of the previous single-center studies (i.e., the Generation R and ALSPAC cohort studies) focused on child non-verbal IQ score [7,14]. They found no association between UI/Creat <150 μg/g and non-verbal IQ score. It was suggested that iodine deficiency in the Generation R cohort may not have been severe enough for an association to be identified [14]. After combining these two cohorts of contrasting iodine status with a third mildly deficient population (INMA), there was still no effect of iodine deficiency on non-verbal IQ score.

Our meta-analysis using predefined cutoffs showed that UI/Creat <150 μg/g was not associated with lower verbal IQ score. The estimates we found for ALSPAC contrasted with the strong negative association of maternal UI/Creat <150 μg/g in the first trimester (defined as ≤13 weeks gestation) with child verbal IQ score found in a previously published study from that cohort (fully adjusted: -2.9, 95% CI: -5.0 to -0.8, P=0.006) [7]. However, there are a few important differences between the studies. Compared with the previous publication, the ALSPAC data in our study included a larger number of mother-child pairs (2980 vs 958), fewer iodine-deficient women [1831 (61.4%) vs 646 (67.4%)], a study population with a higher UI/Creat [median UI/Creat (IQR): 124 (82 to 199) vs 110 (74 to 170)], and most notably, a higher number of mothers with iodine status measured after the first trimester [1135 (38%) vs 0 (0%); median gestational age (IQR): 12 (8 to 16) weeks vs 10.0 (9 to 12) weeks]. In addition, we adjusted our analysis for a more stringent selection of variables. Comparison between study populations and additional analysis of the association between UI/Creat <150 μg/g and a verbal IQ score in the bottom quartile in the whole cohort and in samples ≤13 weeks' gestation are described in Supplemental Table 4 and 5 [30].

The importance of iodine status in the preconceptional stage for child IQ has recently been shown [45]. In early pregnancy, the fetus is fully dependent on the placental transfer of thyroid hormone to support the crucial processes of brain development [2]. There is a need for optimal iodine supply from the initiation of conception, implying that sufficient intrathyroidal iodine stores at the preconception stage may well be critical. Indeed, our results suggest that the fetus is particularly sensitive to suboptimal iodine status in the early stages of pregnancy (*e.g.,* ≤14 weeks of gestation) for optimal development of verbal IQ. Effects on verbal IQ could possibly be explained by the impact of mild iodine deficiency, via thyroid hormone, on the auditory system [13,46]. In our study, we did not find evidence that the association between UI/Creat and verbal IQ was mediated via maternal thyroid function. Possible explanations could be that urinary iodine excretion is a highly volatile and crude measurement of individual iodine

status and/or a crude marker of thyroidal iodine availability. Alternatively, it is also possible that the effects are (in part) mediated via fetal thyroid function.

This study confirms that low iodine status is associated with a reduction in verbal IQ scores, putting these children at potential risk for poorer academic achievement [47]. Furthermore, our findings may have implications on a national level (*e.g.,* by negatively affecting economic growth) [48]. However, there is still inconclusive evidence that supplementation in pregnant women with mild to moderate iodine deficiency is beneficial for child neurodevelopment [11,15,16,49–53]. A recent randomized, placebo-controlled trial showed no benefit on children's non-verbal or verbal IQ score with daily supplementation with 200 μg iodine (as potassium iodide) in women with mild iodine deficiency [52]. In addition to the already mentioned limitations of that trial [54], our results provide an additional explanation for the null finding; the trial randomly assigned women at up to 14 weeks of gestation, whereas we showed that maternal iodine status is particularly important in the first trimester. Although our study needs replication, it suggests that the trial might have missed a critical period of vulnerability in women with iodine deficiency. Our results clearly suggest that additional randomized controlled trials should start with iodine supplementation early in the first trimester or preferably even before pregnancy.

The strengths of our study are as follows: A consistent approach to the analysis and harmonization of potential confounding variables across cohorts optimized comparisons; advanced statistical methods were used to overcome selection bias due to loss to follow up and missing data; and UI/Creat was used as a marker of iodine status. The latter has been shown to be a more valid measure of iodine excretion when used in the same groups of age and sex [55], though we recognize that a single measure may not be reflective of overall iodine status in an individual. A limitation of the study is that the assessment of IQ was performed with different tools at different ages. Nevertheless, the tools measured the same construct (non-verbal or verbal IQ), and the standardization of IQ scores in each cohort facilitated comparison of results across cohorts. Sensitivity analysis in older children only (*e.g.,* excluding children from Generation R, thus reducing the age range at which verbal IQ was assessed) confirmed the association between UI/Creat and verbal IQ score. Another limitation is that UIC was measured in different laboratories using different assays; it is known that urinary iodine measurements vary between laboratories [56]. We used laboratories that were registered with EQUIP, and the use of certified reference materials enabled us to ensure the accuracy of results.

In conclusion, this study confirms that iodine status in pregnancy is associated with child IQ scores, and results indicate that the development of verbal IQ of the fetus is particularly vulnerable to suboptimal iodine concentration during early pregnancy up until the start of second trimester. As such, our results suggest that iodine supplementation after the first 14 weeks of pregnancy could be outside of the critical period during which iodine availability affects fetal brain development. However, further studies should replicate these data and investigate the effects of iodine supplementation.

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Supplemental Table 1 Distribution and comparison of maternal and child characteristics in the included and excluded population.

	Generation R			INMA			ALSPAC		
	included (n=1931)	**not included (n=7818)**	***P* value**	**Included (n=1269)**	**not included (n=881)**	***P* value**	**Included (n=2980)**	**not included (n=11752)**	***P* value**
Female sex, n (%)	963 (49.9)	3846 (49.2)	0.605	632 (49.8)	348 (46.5)	0.150	1514 (50.8)	5312 (47.6)	0.002
Educational level, n (%)									
Low	154 (8.4)	791 (12.0)		270 (21.3)	293 (34.4)		573 (19.8)	3183 (33.1)	
Middle	760 (41.4)	3117 (47.2)	<0.001	525 (41.5)	337 (39.6)	<0.001	1810 (62.7)	5318 (55.4)	<0.001
High	921 (50.2)	2692 (40.8)		470 (37.2)	221 (26.0)		505 (17.5)	1104 (11.5)	
Maternal ethnicity/country of birth, n (%)									
Spanish	NA	NA		1184 (93.5)	731 (85.8)		NA	NA	
Latin-American	NA	NA		56 (4.4)	80 (9.4)		NA	NA	
European/other	NA	NA		26 (2.1)	41 (4.8)		NA	NA	
Dutch	1012 (53.2)	3490 (49.5)		NA	NA		NA	NA	
Indonesian	69 (3.6)	198 (2.8)		NA	NA		NA	NA	
Cape Verdean	58 (3.1)	314 (4.5)		NA	NA		NA	NA	
Moroccan	115 (6.0)	487 (6.9)	0.001	NA	NA	<0.001	NA	NA	<0.001
Surinamese	154 (8.1)	636 (9.0)		NA	NA		NA	NA	
Turkish	170 (9.0)	617 (8.7)		NA	NA		NA	NA	
Other, non-Western	150 (7.9)	724 (10.3)		NA	NA		NA	NA	
Other, Western	174 (9.1)	589 (8.3)		NA	NA		NA	NA	
White	NA	NA		NA	NA		2841 (98.7)	9234 (97.0)	
Non-white	NA	NA		NA	NA		36 (1.3)	290 (3.0)	
Maternal age, years, mean (SD)	30.5 (4.8)	29.8 (5.5)	<0.001	31.6 (3.9)	30.7 (4.8)	<0.001	28.6 (4.5)	26.9 (5.1)	<0.001

Parity, n (%)									
0	1121 (58.1)	3986 (51.8)		727 (57.4)	441 (51.8)		1346 (46.8)	4527 (44.2)	
1	564 (29.2)	2231 (29.0)	<0.001	458 (36.1)	335 (39.4)	0.018	992 (34.5)	3596 (35.1)	0.018
≥2	246 (12.7)	1480 (19.2)		82 (6.5)	75 (8.8)		539 (18.7)	2124 (20.7)	
Smoking during pregnancy, n (%)									
Never	1319 (75.6)	4648 (72.9)		870 (69.4)	478 (64.2)		2434 (83.2)	7446 (71.4)	
In the beginning of pregnancy	168 (9.6)	523 (8.2)	<0.001	168 (13.4)	116 (15.6)	0.055	125 (4.3)	682 (6.5)	<0.001
Continued	257 (14.7)	1208 (18.9)		216 (17.2)	151 (20.3)		367 (12.5)	2305 (22.1)	
Pre-pregnancy BMI, kg/m^2, median (IQR)	22.6 (20.8-25.2)	22.6 (20.7-25.5)	0.926	22.5 (20.8-25.0)	22.6 (20.7-25.3)	0.670	22.2 (20.5-24.4)	22.1 (20.4-24.4)	0.077

P-value for differences calculated using Chi-square test for categorical variables, Student´s t-test for continuous normal-distributed variables, and Kruskal-Wallis test for continuous non-normal distributed variables. NA: not applicable. Numbers are based on unimputed data; percentages add up to 100% without taking into account missing values.

Supplemental material

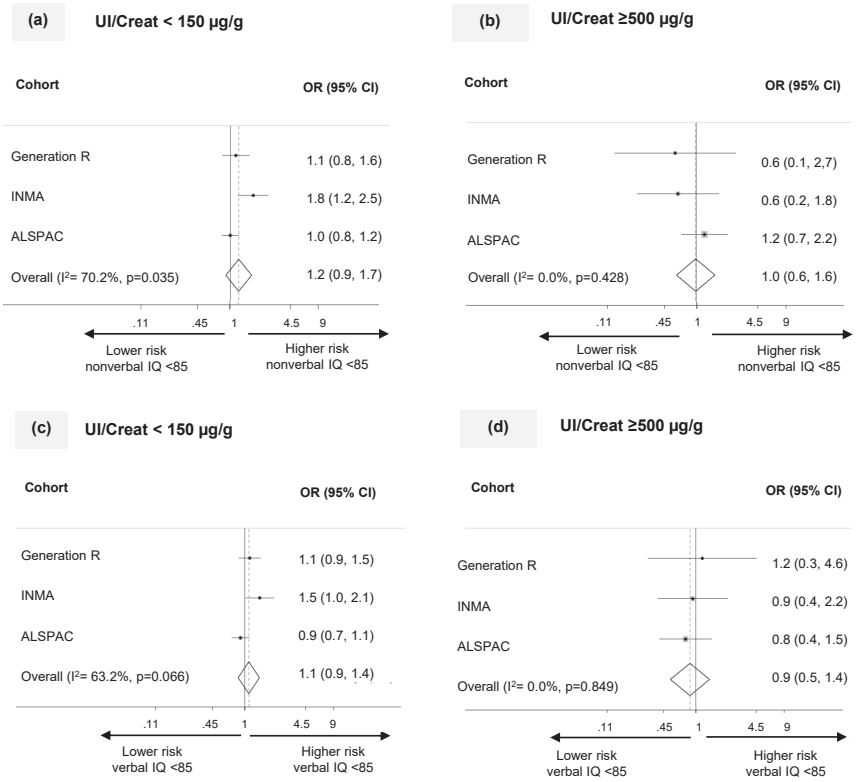
Methods of urinary iodine measurements

In Generation R, urinary iodine was measured by the Sandell-Kolthoff method. Iodine calibration was performed using certified reference materials (CRM) Seronorm urine Levels one and two (Nycomed, Norway), and four EQUIP samples certified for urinary iodine concentration (Centers for Disease Control and Prevention, USA). At a level of 1.7 µmol/l iodine the within-assay CV was 5.1% and the between-assay CV was 14.3% (n= 30). In INMA, urinary iodine was measured using paired-ion reversed-phase, high-performance liquid chromatography (HPLC) with electrochemical detection at a silver working electrode (Waters Chromatography, Milford, MA). The accuracy of the results was verified using the CRM Seronorm urine Levels one and two (Nycomed, Norway), and internal quality control samples. Within run precision was 4.5% relative standard deviation (RSD) at 50 µg/L, 3.2% at 100 µg/L and 2.0% at 300 µg/L. Between run precision was 7.9% RSD at 50 µg/L, 3.5% at 100 µg/L and 2.5 % at 300 µg/L. In ALSPAC, iodine (^{127}I) was measured on a dynamic reaction cell inductively-coupled plasma (ICP) mass spectrometer. The accuracy of the results was verified using the CRM Seronorm urine Levels one and two (Nycomed, Norway), and accuracy was also monitored by measurement of EQUIP samples at regular intervals throughout the analysis. Within run precision was 0.83% RSD at 42 µg/L, 2.47% at 84 µg/L, 0.56% at 149 µg/L and 2.01% at 297 µg/L. Between run precision was 8.69% RSD at 42 µg/L, 6.54% at 84 µg/L, 7.2% at 149 µg/L and 6.8% at 297 µg/L.

Supplementary Table 2 Continuous analysis of the association of maternal iodine status during pregnancy with child non-verbal and verbal IQ using standard linear regression models.

Type of model		Non-verbal IQ		verbal IQ	
	Variables in the model	Beta (95% CI)	*P*-value	Beta (95% CI)	*P*-value
linear model	UI/Creat	0.602 (-0.007 to 1.210)	0.053	1.061 (0.450 to 1.672)	0.001
non-linear model	UI/Creat	0.632 (-5.848 to 7.113)	0.848	10.011 (3.499 to 16.523)	0.003
	UI/Creat^2	-0.003 (-0.651 to 0.644)	0.993	-0.898 (-1.549 to -0.247)	0.007

Reported beta and 95% CI are increase in mean child IQ per natural log increase in UI/Creat. UI/Creat^2 refers to the addition of a squared logtransformed UI/Creat variable in the model. Analyses were performed using linear regression models. Analyses were adjusted for gestational age at urine sampling, child sex, maternal ethnicity/country of birth, maternal education, parity, maternal age, pre-pregnancy BMI, and smoking during pregnancy.



Supplemental Figure 1 Association of UI/Creat < 150 µg/g and UI/Creat ≥ 500 µg/g during pregnancy with child suboptimal non-verbal and verbal IQ.

Figure shows the association of UI/Creat <150 µg/g ("deficiency") with a) non-verbal IQ score <85 and c) verbal IQ score <85, and UI/Creat >=500 µg/g ("excess") with b) non-verbal IQ score <85 and d) verbal IQ score <85 as compared to the reference group (UI/Creat ≥150 to <500 µg/g). Associations are depicted as effect estimate (dot) with 95% CI per cohort and overall as estimated by random-effects meta-analysis (diamond). Models are adjusted for gestational age, child sex, maternal ethnicity/country of birth, maternal education, parity, maternal age, pre-pregnancy BMI, smoking during pregnancy, child age and region in INMA.

Supplemental Table 3 Continuous analysis of the association of maternal iodine status during pregnancy with child verbal IQ stratified by tertile of gestational age using standard linear regression models.

Type of model	Variables in the model	Gestational age (weeks)	Beta (95% CI)	*P*-value
non-linear model	UI/Creat	≤12	18.276 (6.923 to 29.628)	0.002
	UI/Creat^2		-1.786 (-2.953 to -0.620)	0.003
Linear model	UI/Creat	>12 and ≤14	1.333 (0.230 to 2.436)	0.018
Linear model	UI/Creat	>14	0.444 (-0.601 to 1.489)	0.405

Reported beta and 95% CI are increase in mean child verbal IQ per log increase in UI/Creat. UI/Creat^2 refers to the addition of a squared UI/Creat variable in the model. Analyses were performed using linear regression models. UI/Creat was transformed by the natural logarithm. Analyses were adjusted for gestational age at urine sampling, child sex, maternal ethnicity/country of birth, maternal education, parity, maternal age, pre-pregnancy BMI, and smoking during pregnancy.

Supplemental Table 4 Comparison of the ALSPAC study population included in the current study and in the previous publication of Bath et al. 2013.

	this study cohort	this study cohort restricted to ≤13 weeks gestation	Bath et al. 2013
No.	2980	1845	958
gestational age, median (IQR)	12 (8-16)	9 (7-11)	10 (9-12)
UI/Creat, µg/g, median (IQR)	124 (82-199)	108 (75-161)	110 (74-170)
UI/Creat < 150 µg/g , %	61.4	70.1	67.4
UIC, µg/L, median (IQR)	96 (57-153)	96.8 (60-149)	91.1 (53.8-143)

Supplemental Table 5 Association of maternal iodine status with a child verbal IQ score in the lowest quartile as studied in the current ALSPAC study population and as in the previous publication of Bath et al. 2013.

this study cohort	this study cohort restricted to ≤13 weeks gestation	Bath et al. 2013
OR (95% CI), *P*-value	**OR (95% CI), *P*-value**	**OR (95% CI), *P*-value**
1.13 (0.92 to 1.39), 0.259	1.42 (1.06 to 1.91), 0.019	1.58 (1.09 to 2.30), 0.02

Reported OR and 95% CI for a verbal IQ score in the bottom quartile when exposed to a maternal UI/Creat < 150 µg/g vs ≥ 150 µg/g (reference group) during pregnancy. Analyses with un-imputed data were performed using logistic regression models and were adjusted as similarly as possible as compared to the fully adjusted model (model 3) as reported in the publication of Bath et al. Analyses were adjusted for preterm birth, birthweight, child sex, maternal education, paternal education, maternal ethnicity (instead of child ethnicity), parity, age at delivery, smoking, maternal alcohol intake, breastfeeding, iron intake, and omega-3 fatty acid intake estimated from the Food Frequency Questionnaire completed at 32 weeks' of gestation. Missing information that was corrected for in model 3 of Bath et al but lacking in the analysis of this study cohort, were: mothers parenting score (seven-factor measure of cognitive stimulation at 6 months of age), home observation for measurement of environment (HOME) score (six-factor measure of the emotional and cognitive environment at 6 months of age), the family adversity index (18-factor measure of hardship during pregnancy), life-event score (exposure to 41 stressful events during pregnancy), maternal depression since birth, fish-oil supplements during pregnancy, housing status, and crowding.

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Chapter 5

Maternal thyroid function and child neurodevelopment

Chapter 5.1

Thyroid Function in Early Pregnancy, Child IQ, and Autistic Traits: A Meta-Analysis of Individual Participant Data.

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Abstract

Context: Low maternal free thyroxine (FT4) has been associated with poor child neurodevelopment in some single-center studies. Evidence remains scarce for the potential adverse effects of high FT4 and whether associations differ in countries with different iodine status.

Objective: To assess the association of maternal thyroid function in early pregnancy with child neurodevelopment in countries with a different iodine status.

Design, Setting and Participants: Meta-analysis of individual participant data from 9,036 mother-child pairs from three prospective population-based birth cohorts: INMA [INfancia y Medio Ambiente (Environment and Childhood project) (Spain)], Generation R (Netherlands) and ALSPAC (Avon Longitudinal Study of Parents and Children, United Kingdom). The exclusion criteria were multiple pregnancies, fertility treatments, thyroid interfering medication usage, and known thyroid disease.

Main outcomes: Child non-verbal IQ at 5 to 8 years of age, verbal IQ at 1.5 to 8 years of age, and autistic traits within the clinical range at 5 to 8 years of age.

Results: FT4 <2.5th percentile was associated with a 3.9-point (95% CI -5.7 to -2.2) lower non-verbal IQ and a 2.1-point (95% CI -4.0 to -0.1) lower verbal IQ. A suggestive association of hypothyroxinemia with a greater risk of autistic traits was observed. FT4 >97.5th percentile was associated with a 1.9-fold (95% CI 1.0 to 3.4) greater risk of autistic traits. No independent associations were found with TSH.

Conclusions: Low maternal FT4 was consistently associated with lower IQ across the cohorts. Further studies are needed to replicate the findings of autistic traits and investigate the potential modifying role of maternal iodine status. FT4 seems a reliable marker of fetal thyroid state in early pregnancy, regardless of the type of immunoassay.

Introduction

Thyroid hormone regulates crucial processes of brain development, including the proliferation, migration, and differentiation of neuronal cells, as shown in animal studies [1,2]. Because the fetal thyroid gland is not functionally mature until approximately week 18 of pregnancy [3], the fetus is dependent on placental transfer of maternal thyroid hormone during this period. Adequate maternal thyroid hormone concentrations during early pregnancy are therefore essential for optimal fetal brain development.

Previous studies focused mainly on the possible adverse effects of low maternal hormone availability on fetal brain development. In several studies, either overt hypothyroidism or low maternal free T4 (FT4) was associated with lower child IQ [4–8] and lower gray-matter volume [4], a greater risk of autistic traits [9], impaired psychomotor function [10], and schizophrenia [11]. Although the association of high maternal FT4 on child neurodevelopment has been less well studied, experimental evidence from rodents has indicated that high hormone availability may also have adverse effects [12–18]. A recent study from the Netherlands has shown that high maternal FT4 is associated with lower IQ and gray matter volumes in the child [4]. However, it is unclear whether these findings from an iodine-sufficient population in the Netherlands [19] can be extrapolated to other countries with a different iodine status and whether high maternal FT4 is also associated with other adverse neurodevelopmental outcomes other than IQ.

Neither of the two randomized controlled trials that studied the effect of levothyroxine treatment in women with subclinical hypothyroidism or hypothyroxinemia on child IQ showed any benefit of treatment [20,21]. However, these negative results could be ascribable to a relatively late start of treatment in both trials (13 weeks and 16 to 18 weeks, respectively), a relatively high dose was given that might have led to potential overtreatment [20], or a lack of power to detect the expected 3- to 4-point difference in IQ [21,22]. Therefore, further studies are required to better quantify and replicate the potential effects of both low and high maternal thyroid hormone availability on fetal neurodevelopment. These studies can help improve the design of future controlled trials.

The aim of the present study was to investigate the association of maternal thyroid function in early pregnancy across the full range of FT4 and thyrotropin (TSH) concentrations with child's IQ and autistic traits in three prospective birth cohorts.

Material and Methods

Study design and populations

For the present study, we used individual participant data from three prospective population-based birth cohorts: INfancia y Medio Ambiente [INMA (Environment and Childhood project), Spain, three regions] [23], Generation R (the Netherlands) [24], and the Avon Longitu-

dinal Study of Parents and Children (ALSPAC, United Kingdom) [25]. In INMA, the eligible study participants were pregnant women with a singleton pregnancy residing in the regions of Valencia, Sabadell, and Gipuzkoa from November 2003 to January 2008. In Generation R, the eligible study participants were pregnant women living in the Rotterdam area with an expected delivery date from April 2002 to January 2006. In ALSPAC, the eligible study participants resided in a defined area in the southwest of England, with an expected date of delivery from April 1991 to December 1992 (the study website of ALSPAC contains details of all the data available through a fully searchable data dictionary [26]). For the present study, eligible women were enrolled in the three cohorts during the first half of pregnancy (≤18th week of gestation). Women with multiple pregnancies or fertility treatment and/or using medication affecting the thyroid or having a known thyroid disease were excluded (Fig. 1). Ethical approval was obtained from the local ethical committees at the time of study enrollment; all parents or guardians of the children provided informed consent.

Thyroid function

Thyroid function was measured in serum samples stored at -80°C (INMA and Generation R) or -20°C (ALSPAC). The samples were obtained at early pregnancy [(mean ± SD) gestational age: INMA, 13.1 ± 1.3 weeks; Generation R, 13.4 ± 2.0 weeks; ALSPAC, 11.0 ± 3.2 weeks] (Table 1). Different assays were used to measure FT4 and TSH (Supplemental Table 1). Although thyroid peroxidase antibody (TPOAb) was not measured in INMA, TPOAb measurements were available from Generation R and ALSPAC. The FT4 and TSH concentrations were logarithmically transformed, and cohort-specific SD scores were calculated with a mean of 0 and a SD of 1 based on the data of TPOAb-negative women, as advocated by the guidelines when defining population-based reference ranges [27].

Hypothyroxinemia [normal (2.5th-97.5th percentile) TSH, low (<2.5th percentile) FT4], subclinical hypothyroidism [high (>97.5th percentile) TSH, normal FT4], and subclinical hyperthyroidism (low TSH, normal FT4) were defined according to the 2.5th and the 97.5th population-based percentiles of the whole study population in INMA, because TPOAb measurements were not available. Thyroid disease entities were defined using the same population-based percentiles in Generation R and ALSPAC. However, in these cohorts, the population-based percentiles were based on the results from TPOAb-negative women. The reference group consisted of euthyroid women (TSH and FT4 between the 2.5th and 97.5th percentiles). Additionally, to improve the statistical power, we identified thyroid disease entities using the 5th and the 95th population-based percentiles. The untransformed 2.5th and 97.5th population-based percentiles. The untransformed 2.5th and 97.5th population-based percentiles based on TPOAb-negative women when possible were 0.14 and 3.86, 0.05 and 4.13, and 0.07 and 2.58 mIU/L for TSH and 8.4 and 14.0, 10.4 and 22.1, and 12.6 and 22.5 pmol/L for FT4 in INMA, Generation R, and ALSPAC, respectively.

Non-verbal and verbal IQ

In INMA, non-verbal and verbal IQ were assessed by a psychologist at a median age of 4.6 years using the McCarthy Scales of Children´s Abilities [28]. In Generation R, non-verbal IQ was assessed by trained staff at a median age of 6.0 years using a subset of the Snijders Oomen Nonverbal Intelligence Test (2.5-7-Revised) [29], and verbal IQ was estimated by the parent-reported short form of the McArthur Communicative Development Inventory [30] obtained at a median age of 1.5 years. In ALSPAC, non-verbal and verbal IQ were assessed by trained staff at a median age of 8.6 years using the Wechsler Intelligence Scale For Children, third UK edition [31]. To homogenize the different scores, raw cohort-specific scores were standardized to a mean of 100 and a SD of 15 (new score = 100 + 15 x SD).

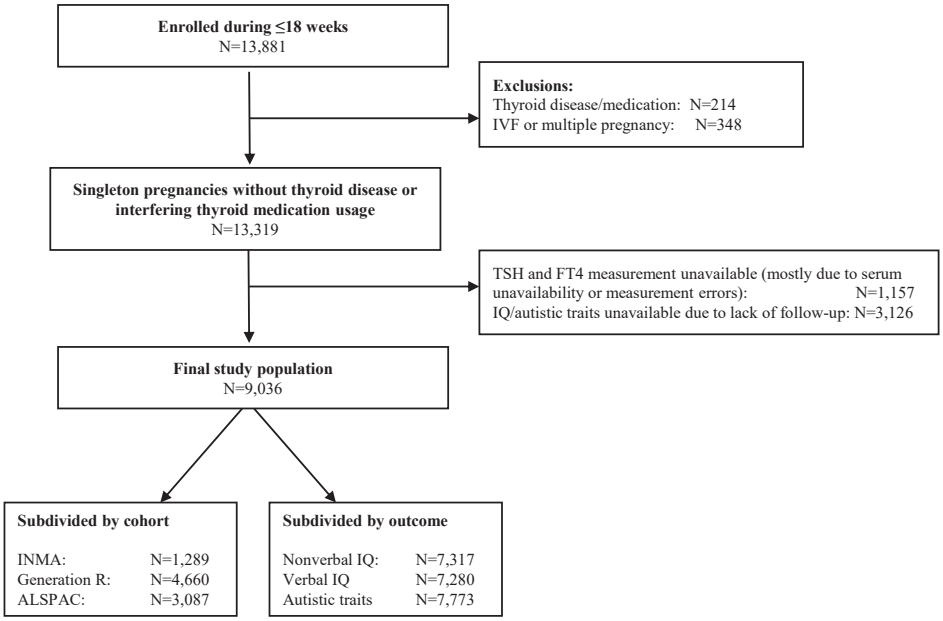

Figure 1 Flowchart for the selection of the final study population. IVF, *in vitro* fertilization.

Autistic traits within the clinical range

Autistic traits are symptoms that represent subclinical deficits in social behavior, communication, and or restricted, repetitive patterns of behavior common to ASD but that do not meet clinical ASD diagnosis [32]. Autistic traits within the clinical range were defined as the presence of an autistic traits score greater than the specific cutoff for each assessment tool, which had been previously validated in other studies to detect children at risk of ASD. In INMA, autistic traits were assessed with the Childhood Autism Spectrum Test (CAST) by a psychologist at a median age of 4.6 years, with a cutoff of ≥ 15 points to define autistic traits within the clinical range [33]. In Generation R, autistic traits were assessed using the Pervasive Developmental

Problems subscale of the Child Behavior Checklist for Toddlers (CBCL1½–5) by the parents at a median age of 5.9 years, with a cutoff of ≥98th percentile to define autistic traits within the clinical range [34]. In ALSPAC, autistic traits were assessed with the Social Communication Disorder Checklist (SCDC) by the parents at a median age of 7.6 years of age, with a cutoff of nine or more points to define autistic traits within the clinical range [35].

Potential confounding variables

A direct acyclic graph [36] facilitated decision making regarding which covariates should be adjusted for in the analysis. Information on maternal variables [age, educational level (low, medium, high), ethnicity (cohort-specific categories), parity (zero, one, two or more), pre-pregnancy body mass index (BMI), and smoking during pregnancy (never smoked, smoked in the beginning or until pregnancy confirmed, continued smoking)] was collected during pregnancy using questionnaires. Gestational age at blood sampling was defined using ultrasonography or the last menstrual period. Child sex and age at IQ or autistic trait ascertainment were obtained during the study visits.

Statistical analyses

We used linear regression models to study the association of maternal FT4, TSH, and thyroid disease entities with child non-verbal or verbal IQ. We used logistic regression models to study the association of maternal FT4, TSH, and thyroid disease entities with child autistic traits within the clinical range.

We studied these associations using a one-step and a two-step approach. In the one-step approach, we assessed non-linearity between FT4 and TSH and each outcome using restricted cubic splines with three to five knots. An ANOVA test was used to report an overall *P* value for the null hypothesis that the mean IQ or probability of autistic traits within the clinical range was similar across the whole distribution of TSH or FT4. In the two-step approach, we combined cohort-specific effect estimates of the association between FT4, TSH, and thyroid disease entities and each outcome using random-effects meta-analyses. For this analysis, FT4 and TSH concentrations were categorized as <2.5th, <5th, >95th, or >97.5th percentiles using women with values within the interquartile range (within the 25th and 75th percentile range) as the reference group. Compared with the one-step approach, the two-step approach allows for differences in participant characteristics between cohorts, and heterogeneity between cohorts can be calculated [37]. Heterogeneity was assessed using the Cochran Q test and the I^2 statistic [38]. All models were adjusted for maternal age, educational level, ethnicity, parity, pre-pregnancy BMI, smoking, gestational age at blood sampling, and child sex. Because one-step approach models could not be adjusted for age at IQ or autistic trait ascertainment, cohort, and ethnicity at the same time owing to collinearity, we adjusted them only for ethnicity. The two-step approach models could be adjusted for these variables because the effect-estimates were calculated separately by cohort.

Table 1 Distribution of maternal and child characteristics.

Variable	INMA (n=1,289)	Generation R (n=4,660)	ALSPAC (n=3,087)
Maternal TSH, median (IQR), mIU/L	1.24 (0.84-1.81)	1.36 (0.85-2.03)	1.00 (0.64-1.46)
Maternal FT4, median (IQR), pmol/L	10.6 (9.7-11.6)	14.8 (13.2-16.7)	16.2 (14.8-17.7)
Thyroid disease entities,[a] n (%)			
Hypothyroxinemia	32 (2.5)	111 (2.4)	61 (2.0)
Subclinical hypothyroidism	31 (2.4)	140 (3.0)	110 (3.6)
Subclinical hyperthyroidism	20 (1.6)	69 (1.5)	34 (1.1)
TPOAb positivity, n (%)	NA	254 (5.8)	392 (12.8)
Gestational age at blood sampling, mean ± SD, wk	13.1 (1.3)	13.4 (2.0)	11.0 (3.2)
Maternal educational level, n (%)			
Low	281 (21.9)	353 (8.0)	736 (24.7)
Medium	537 (41.8)	1,904 (42.9)	1,828 (61.3)
High	468 (36.4)	2,179 (49.1)	416 (14.0)
Maternal ethnicity, n (%)			
Spanish	1,202 (93.4)	NA	NA
Latin-American	60 (4.7)	NA	NA
European/other	25 (1.9)	NA	NA
Dutch	NA	2,606 (56.7)	NA
Indonesian	NA	150 (3.3)	NA
Cape Verdean	NA	170 (3.7)	NA
Moroccan	NA	225 (4.9)	NA
Dutch Antilles	NA	104 (2.3)	NA
Surinamese	NA	351 (7.6)	NA
Turkish	NA	356 (7.8)	NA
Asian	NA	51 (1.1)	NA
Other, non-Western	NA	162 (3.5)	NA
Other, Western	NA	418 (9.1)	NA
White	NA	NA	2,924 (98.6)
Non-white	NA	NA	42 (1.4)
Maternal age, mean ± SD, y	31.5 (4.0)	30.3 (4.8)	28.0 (4.6)
Parity, n (%)			
0	731 (56.8)	2,721 (58.4)	1,410 (47.2)
1	472 (36.7)	1,386 (29.7)	1,033 (34.6)
≥2	84 (6.5)	553 (11.9)	543 (18.2)
Maternal smoking, n (%)			
Never smoked	883 (69.4)	3,085 (73.5)	2,391 (79.2)
Smoked at the beginning of pregnancy	174 (13.7)	396 (9.4)	142 (4.7)
Continued smoking	216 (17.0)	719 (17.1)	486 (16.1)
Pre-pregnancy BMI, median (IQR), kg/m^2	22.5 (20.8-25.1)	22.6 (20.7-25.2)	22.1 (20.5-24.2)
Child female sex, n (%)	635 (49.3)	2,313 (49.6)	1,500 (48.6)
Child autistic traits within the clinical range, n (%)	16 (1.4)	117 (3.1)	206 (7.5)

Data might not sum to 100 because of rounding.

Abbreviations: BMI, body mass index; IQR, interquartile range; NA, not available.

[a] Based on the 2.5th and 97.5th population-based percentiles.

As a sensitivity analysis, we adjusted the analyses of autistic traits for non-verbal IQ, a language- and culture-free measure of cognitive ability. Additionally, when we observed associations between maternal TSH and child IQ or autistic traits, we repeated the analysis stratifying by low-, mid-, and high-normal FT4. Finally, all analyses were repeated in the TPOAb-negative women only.

We applied inverse probability weighting to correct for potential differential loss to follow-up [39]. We performed multiple imputation using chained equations to account for missing values for the potential confounding variables [40]. A total of 25 datasets were generated and analyzed using standard procedures for multiple imputation. Statistical analyses were performed in STATA, version 14.0 (StataCorp, College Station, TX) and R statistical software, version 3.3.2, package rms and lme4 (R Foundation, Vienna, Austria).

Results

After exclusions, the final study population included 9036 mother-child pairs (Fig. 1), the characteristics of which are shown in Table 1. The mean maternal age varied across cohorts: 31.5 years in INMA, 30.3 years in Generation R, and 28.0 years in ALSPAC. The percentage of mothers who continued smoking during pregnancy was similar among the cohorts (~ 16% to 17%). Autistic traits within the clinical range occurred in 1.4% of the children in INMA, 3.1% in Generation R, and 7.5% in ALSPAC. The two most prevalent thyroid disease entities were hypothyroxinemia (2.0% to 2.5% across the cohorts) and subclinical hypothyroidism (2.4% to 3.6% across the cohorts). Compared with the final study population, the women not included in the analysis had a lower level of education, were less often native or white, and were younger in all three cohorts (Supplemental Table 2).

Non-verbal IQ

We observed a statistically significant nonlinear association between maternal FT4 and mean non-verbal IQ (Fig. 2). FT4 $\leq 2.5^{th}$ percentile was associated with a 3.9-point (95% CI -5.7 to -2.3; $P<0.001$) lower non-verbal IQ. Similar results were observed when using the fifth percentile cut-off. A high FT4 was not associated with the non-verbal IQ. TSH $\geq 97.5^{th}$ and $\geq 95^{th}$ percentile was associated with a statistically non-significant slightly greater non-verbal IQ (1.5 points; 95% CI, -0.3 to 3.3; $P=0.100$; and 1.2 points, 95% CI, -0.1 to 2.5; $P=0.063$, respectively; Supplemental Fig.1). However, the sensitivity analysis showed that this association was driven by women with a FT4 concentration in the mid- or high-normal range (Supplemental Table 3). No heterogeneity was observed among the cohorts. Results remained similar after excluding TPOAb-positive women.

Verbal IQ

A statistically non-significant linear association was found between maternal FT4 and mean verbal IQ (Fig. 3). FT4 ≤2.5th percentile was associated with a 2.1-point (95% CI, -4.0 to -0.1; *P*=0.039) lower verbal IQ. In contrast, the association of FT4 at the fifth percentile or less was associated with a statistically non-significant slightly lower verbal IQ (-1.4 points; 95% CI -2.9 to 0.2; *P*=0.078). A high FT4 was not associated with verbal IQ. A low TSH was also not associated with verbal IQ (Supplemental Fig. 2). TSH ≥97.5th percentile was associated with a greater verbal IQ (1.9 points; 95% CI, 0.1 to 3.7; *P*=0.039). However, no association was found for TSH ≥95th percentile. The sensitivity analysis showed that the positive association of a high TSH ≥97.5th percentile with verbal IQ was driven by women with a FT4 concentration in the mid- or high-normal range (Supplemental Table 4). No heterogeneity was observed among the cohorts. The results remained similar after excluding TPOAb-positive women.

Autistic traits

No continuous association was found for maternal FT4 with child autistic traits (Fig.4). FT4 ≤2.5th percentile was not associated with autistic traits, but FT4 ≤5th percentile was associated with a statistically non-significant slightly higher risk of autistic traits [odds ratio (OR), 1.5; 95% CI, 1.0 to 2.3; *P*=0.080). FT4 ≥97.5th percentile was associated with a 1.9-fold (95% CI, 1.0 to 3.4; *P*=0.043) greater risk of autistic traits. A similar association was found after adjusting for non-verbal IQ (data not shown). FT4 ≥95th percentile was not associated with autistic traits. TSH was not associated with autistic traits (Supplemental Fig. 3). No heterogeneity was observed among the cohorts. The results remained similar after excluding TPOAb-positive women.

Clinical disease entities

Highly similar results were obtained when FT4 and TSH were combined into clinical disease entities. Hypothyroxinemia, based on the 2.5th and 97.5th population-based percentiles, was associated with a 3.8-point (95% CI, -5.7 to -2.0; *P*<0.001) lower non-verbal IQ and a 2.8-point (95% CI, -4.8 to -0.7; *P*=0.007) lower verbal IQ (Supplemental Fig. 4) but was not associated with autistic traits. For hypothyroxinemia, based on the 5th and 95th population-based percentiles, similar results were found with non-verbal and verbal IQ, with a 1.8-fold (95% CI, 1.1 to 2.8; *P*=0.011) greater risk was found with autistic traits (Supplemental Fig. 4), which remained after adjusting for non-verbal IQ (data not shown).

Subclinical hypothyroidism, based on the 2.5th and 97.5th population-based percentiles, was associated with a 1.9-point (95% CI, 0.1 to 3.6; *P*=0.037) greater non-verbal IQ but not with verbal IQ or autistic traits (Supplemental Fig. 5). When defining subclinical hypothyroidism using the 5th and 95th population-based percentiles, the association with non-verbal IQ became statistically non-significant (1.3 points; 95% CI -0.2 to 2.9; *P*=0.096). Subclinical hyperthyroidism was not associated with non-verbal IQ, verbal IQ or autistic traits (Supplemental Fig. 6).

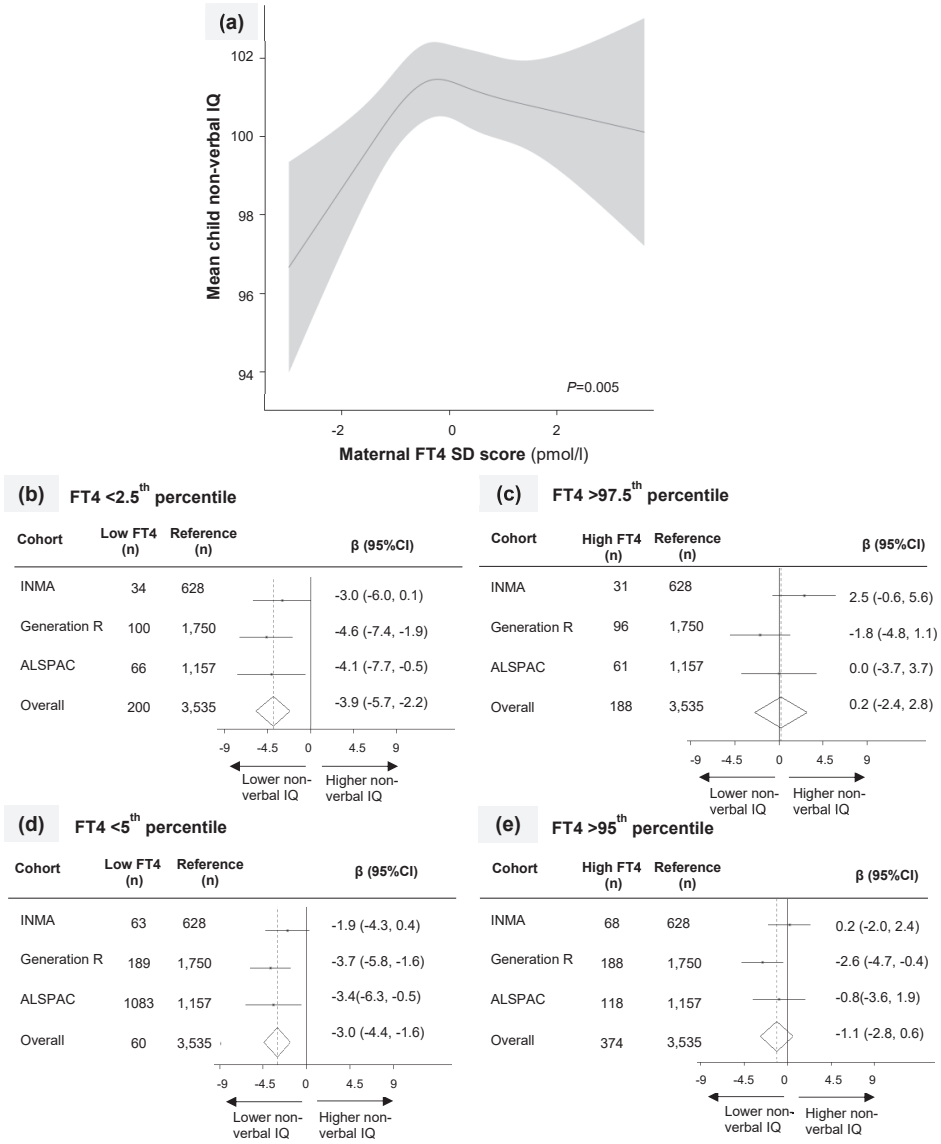

Figure 2 Association of maternal FT4 during early pregnancy with child non-verbal IQ.
Association shown as a) a continuous association depicted as the mean child non-verbal IQ (black line) with 95% CI (gray area) and by cohort-specific maternal FT4 concentrations in the (b) <2.5th percentile, (c) >97.5th percentile, (d), <5th percentile, and (e) >95th percentile compared with interquartile range (between 25th and 75th percentiles), depicted as effect estimate (dot) with the 95% CI per cohort and overall as estimated by random-effects meta-analysis (diamond). The I^2 for each model is as follows: for FT4<2.5th percentile, I^2=0.0%; for FT4>97.5th percentile, I^2=48.5%; for FT4<5th percentile, I^2=0.0%; for FT4>95th percentile, I^2=37.8%.

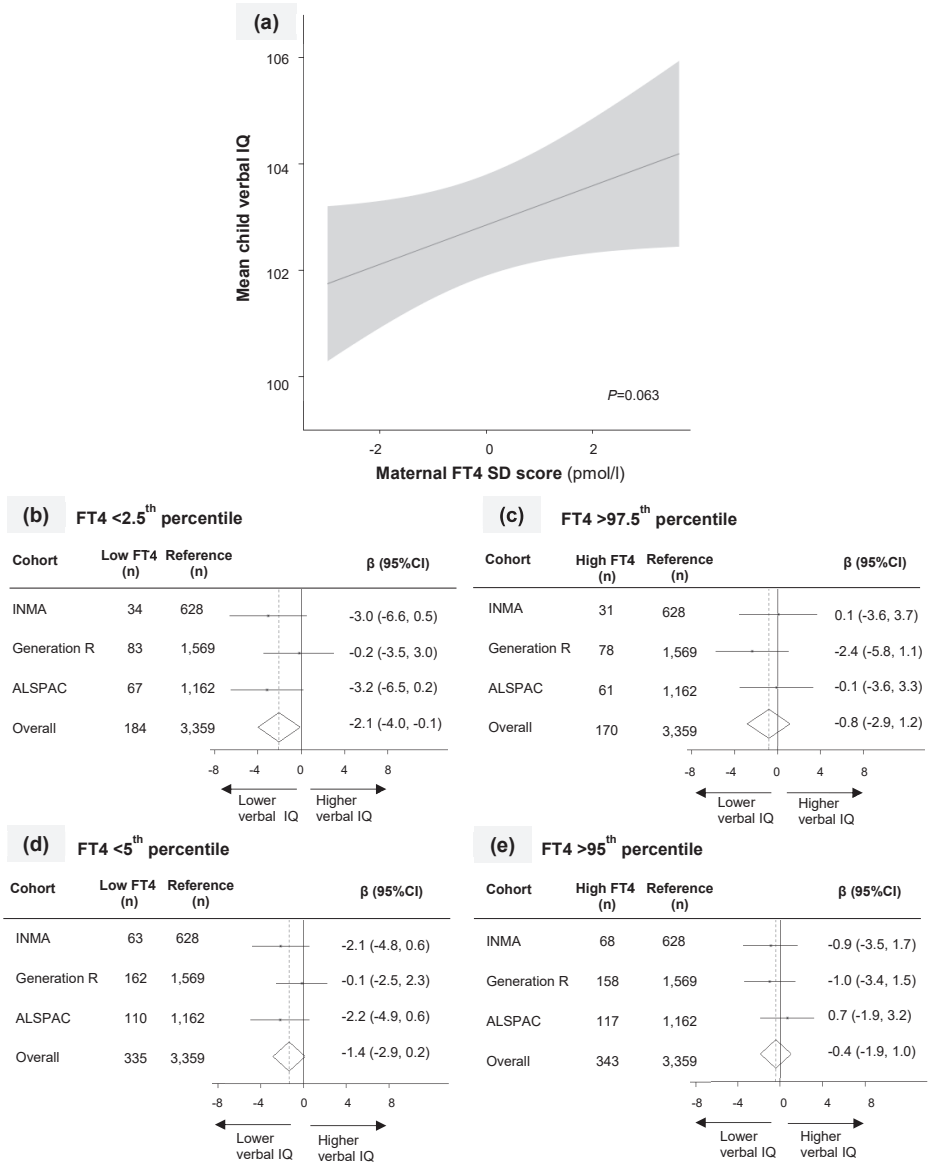

Figure 3 Association of maternal FT4 during early pregnancy with child verbal IQ.
Association shown as (a) a continuous association depicted as the mean child verbal IQ (black line) with 95% CI (gray area) and by cohort-specific maternal FT4 concentrations in the (b) <2.5th percentile, (c) >97.5th percentile, (d) <5th percentile, and (e) >95th percentile compared with interquartile range (between 25th and 75th percentiles), depicted as effect estimate (dot) with the 95% CI per cohort and overall as estimated by random-effects meta-analysis (diamond). The I^2 for each model is as follows: for FT4<2.5th percentile, I^2=0.0%; for FT4>97.5th percentile, I^2=0.0%; for FT4<5th percentile, I^2=0.0%; for FT4>95th percentile, I^2=0.0%.

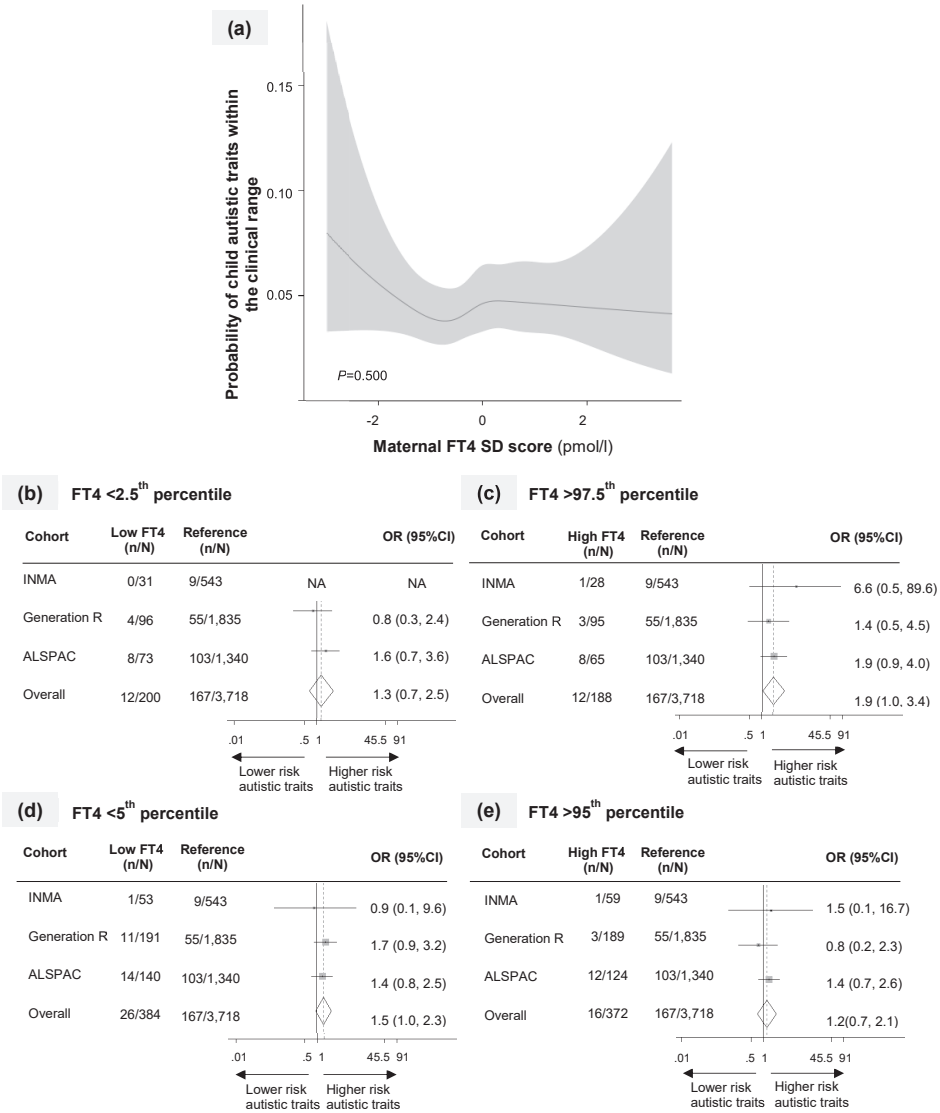

Figure 4 Association of maternal FT4 during early pregnancy with child autistic traits within the clinical range. Association shown as (a) a continuous association depicted as the mean risk of child autistic traits within the clinical range (black line) with 95% CI (gray area) and by cohort-specific maternal FT4 concentrations in the (b) <2.5th percentile (c) >97.5th percentile, (d) <5th percentile, and (e) >95th percentile compared with interquartile range (between 25th and 75th percentile), depicted as effect estimate (dot) with 95% CI per cohort and overall as estimated by random-effects meta-analysis (diamond). The I^2 for each model is as follows: for FT4<2.5th percentile, I^2=7.4%; for FT4>97.5th percentile, I^2=0.0%; for FT4<5th percentile, I^2=0.0%; for FT4>95th percentile, I^2=0.0%.

Discussion

To the best of our knowledge, the present study is the first individual participant data meta-analysis. We have demonstrated that low maternal FT4 in early pregnancy is associated with lower non-verbal and verbal child IQs. We also found a suggestive association between maternal hypothyroxinemia and high FT4 with a greater risk of autistic traits within the clinical range. In contrast to FT4, maternal TSH was not independently associated with non-verbal, verbal IQ, or autistic traits within the clinical range.

The association between low maternal FT4 and child IQ, specifically non-verbal IQ, was highly similar among the three cohorts, convincingly replicating the results of previous observational studies [4–9]. A recent randomized controlled trial studied the effects of levothyroxine treatment for women with subclinical hypothyroidism or hypothyroxinemia on child full IQ [21]. Although levothyroxine treatment of hypothyroxinemia or subclinical hypothyroidism started in mid-pregnancy (week 16 to 18), a statistically non-significant 3 points greater median child IQ was found after levothyroxine treatment compared with placebo. The associations of hypothyroxinemia with a 3.8- and 2.8-point lower non-verbal and verbal IQ, respectively, found in our study compared with euthyroid women might seem small on an individual level. However, on a population level, this might have effects on educational achievements and capita per income, among others [41].

The consistent association of low maternal FT4 with adverse child neurocognitive outcomes, specifically lower non-verbal IQ in three independent cohorts, is particularly relevant given that all three cohorts used a different immunoassay to measure FT4. The value of an FT4 measurement during pregnancy has been under debate, because the absolute values of FT4 might have been under- or overestimated when measured using immunoassays in pregnancy, especially in the third trimester [42–44]. However, these results suggest that FT4 is a reliable clinical marker of the fetal thyroid state in early pregnancy, a period when maternal FT4 is the sole source of thyroid hormones for the fetus and influences the developmental processes, including proliferation, migration, and differentiation of neuronal cells in various parts of the brain [45]. No conclusions about the use of FT4 assays during the later stages of pregnancy, when the fetal thyroid is fully functional, should be drawn from these data.

In our study, the effect estimates for non-verbal IQ were larger than for those for verbal IQ. Non-verbal IQ is a language- and culture-free measure of cognitive ability that is less dependent on the learning stimulus received by the child during the first years of life. Therefore, it might be a better neurodevelopmental outcome for detecting the effects of maternal exposures in early pregnancy, such as thyroid hormone levels.

Our results did not show an association between high maternal FT4 and non-verbal or verbal IQ across the three cohorts, although we confirmed the previously reported association with the Generation R data [4]. The discrepancies in the association of high FT4 and non-verbal IQ among the cohorts might have resulted from population differences such as maternal iodine

status, which differed considerably among the cohorts. Pregnant women in Generation R had an adequate iodine status according to the World Health Organization (median urinary iodine concentration, 229.6 μg/L [19]). In contrast, mild to moderate iodine insufficiency was present in the INMA and ALSPAC cohorts (median, 94 to 168 μg/L depending on the region and 91.1 μg/L, respectively [46,47]). Although mild-to-moderate iodine deficiency has been associated with adverse neurodevelopmental outcomes, such as lower verbal IQ, worse language skills, reduced educational outcomes, impaired executive function, more behavior problems, and worse fine motor skills, this was not found in iodine-deficient women in a iodine sufficient population [19,48–50]. It is unclear how much of the association of iodine deficiency with child neurocognitive outcomes can be attributed to impaired thyroid function in the mother or to impaired thyroid function in the fetus. Further studies should elucidate the mediating role of maternal and fetal thyroid function in the association between maternal iodine status and child neurodevelopment.

To date, only two studies have explored the association between maternal thyroid function and ASD diagnosis or autistic traits. The Danish study was based on registry linkage information and showed that maternal diagnosed or treated hypothyroidism was associated with a greater risk of a diagnosed ASD (hazard ratio, 1.30, 95% CI, 1.11 to 1.53) [51]. The Dutch study from the Generation R cohort found that severe hypothyroxinemia, defined as maternal FT4 $\leq 5^{th}$ percentile with normal TSH, was associated with a greater risk of autistic traits [9]. In the present meta-analysis, including data from Generation R, we also found an association between hypothyroxinemia using the FT4 fifth percentile or less cutoff and a greater risk of autistic traits. However, when using the FT4 $\leq 2.5^{th}$ percentile cutoff, no greater risk of autistic was found, suggesting the possibility of a chance finding. Likewise, high FT4 was associated with a greater risk of autistic traits, although only when the more stringent cutoff was used (*i.e.*, FT4 $\geq 97.5^{th}$ percentile). Considering the crucial role of thyroid hormones in key processes in the pathophysiology of ASD, including neuronal cell migration, synaptogenesis, synapse maintenance, neuronal activity, and fetal growth [52,53], it is biologically plausible that nonoptimal levels of maternal FT4 during early pregnancy are related to a greater risk of ASD. However, the inconsistent results across cohorts or cutoffs limited us from drawing firm conclusions regarding this potential association. Further studies focusing on autistic traits or ASD diagnosis are therefore needed to replicate and better understand the full extent of these results.

TSH is frequently used as a marker of thyroid status during pregnancy. Subclinical hypothyroidism has been associated with a greater risk of miscarriage and preterm delivery, and the beneficial effects of levothyroxine treatment for hypothyroid women have been shown in some trials, especially in TPOAb-positive women [54–57]. Therefore, the current international guidelines recommend screening for TSH first, either directly in combination with TPOAb status [27] or determining TPOAb status and FT4 only when TSH is elevated [58]. The results from the present study call into question the use of TSH as the only first-line parameter to screen

maternal thyroid status in early pregnancy. First, elevated human chorionic gonadotropin concentrations stimulate the thyroid directly to produce thyroid hormone, which induces a decrease in TSH in early pregnancy [59]. Therefore, TSH might not be the best marker for maternal thyroid status in this time window. Second, in our study, maternal TSH was not independently associated with non-verbal IQ, verbal IQ, or autistic traits, in contrast to FT4. However, owing to the absence of available randomized trials demonstrating the benefit of levothyroxine treatment for maternal hypothyroxinemia, screening for FT4 cannot be advocated.

One strength of the present study was that we investigated the association of maternal thyroid function with child neurodevelopmental outcomes in a prospective manner using a large dataset with detailed data on non-verbal IQ, verbal IQ, and autistic traits, assessed using validated tools. Furthermore, by combining data from three different countries, we were able to perform an external replication of previous studies and assess potential differences related to iodine status, after adjusting for many potential confounding variables. We also used advanced statistical methods, including multiple imputation combined with inverse probability weighting, to reduce possible selection bias.

One limitation of the present study was that the child neurodevelopmental outcomes were assessed with different tools at different ages. This might be, for example, reflected in the different prevalence of children with autistic traits within the clinical range across cohorts. The varying occurrence might have resulted from the different ages at the assessment and/or the different types of evaluator but most likely resulted from the different set of questions for assessing autistic traits. For instance, the CAST [33] contains 31 items and is therefore a more extensive questionnaire compared with the CBCL1½–5, with 13 items [34], and the SCDC, with 12 items [35]. The CAST and CBCL1½–5 cover questions on all three domains of ASD. In contrast to the CAST and CBCL1½–5, the SCDC was designed to assess deficits in social and communications skills but does not assess the ASD domain of restricted and repetitive behaviors and interests. To account for the differences as best as possible, we standardized all outcome scores and adjusted all analyses for child age at the IQ or autistic traits ascertainment. We observed little heterogeneity among the cohorts. Another limitation was the low prevalence children with autistic traits within the clinical range, which caused, especially in INMA, issues with statistical power. Furthermore, we only had a single thyroid function measurement available from early pregnancy. Hence, the results should not be generalized to thyroid function in late pregnancy, and the potential effects of individual variations in maternal thyroid hormone availability could not be studied.

In conclusion, the results from the present study have confirmed that a low FT4 is consistently associated with a lower child IQ. We also found a suggestive association of maternal hypothyroxinemia and high FT4 with a greater risk of autistic traits within the clinical range. FT4 seemed a reliably marker of the fetal thyroid state in early pregnancy, regardless of

the type of immunoassay. Further studies should replicate the findings of autistic traits and investigate the potential modifying role of maternal iodine status.

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Generation R, the Netherlands: The Generation R Study is conducted by the Erasmus Medical Center in close collaboration with the Faculty of Social Sciences of the Erasmus University Rotterdam, the Municipal Health Service Rotterdam area, Rotterdam, and the Stichting Trombosedienst & Artsenlaboratorium Rijnmond (STAR-MDC), Rotterdam. The Generation R Study is supported by the Erasmus Medical Center, Rotterdam, the Erasmus University Rotterdam, the Netherlands Organization for Health Research and Development (ZonMw), the Netherlands Organization for Scientific Research (NWO), the Ministry of Health, Welfare and Sport. A grant from the Sophia Children's Hospital Research Funds supports the neurodevelopmental work on thyroid; Robin P. Peeters is supported by a clinical fellowship from ZonMw, project number 90700412.

ALSPAC, United Kingdom: We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists, and nurses. The UK Medical Research Council and Wellcome (Grant ref: 102215/2/13/2) and the University of Bristol currently provide core support for ALSPAC. The publication is the work of the authors and Mònica Guxens will serve as guarantor for the contents of this paper. Data collection is funded from a wide range of sources, which are detailed on the ALSPAC website: http://www.bristol.ac.uk/alspac/about/.

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INMA, and Professor dr. Yolanda de Rijke, of the department of Clinical Chemistry, Erasmus MC, University Medical Center, the Netherlands for design and measurements of the thyroid measurements in Generation R.

Supplemental material

Supplemental Table 1 Type of immunoassay used by the cohorts.

Cohort	Assay	TPOAb positivity[a]
INMA	AutoDEL-FIA (PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland) and a lanthanide metal europium (Eu) label.	NA
Generation R	Vitros ECI immunodiagnostic (Ortho Clinical Diagnostics, Rochester, NY, USA)	≥60 IU/ml
ALSPAC	Abbott Architect i2000	≥6 IU/ml

[a] Assay cut-off. NA: not available

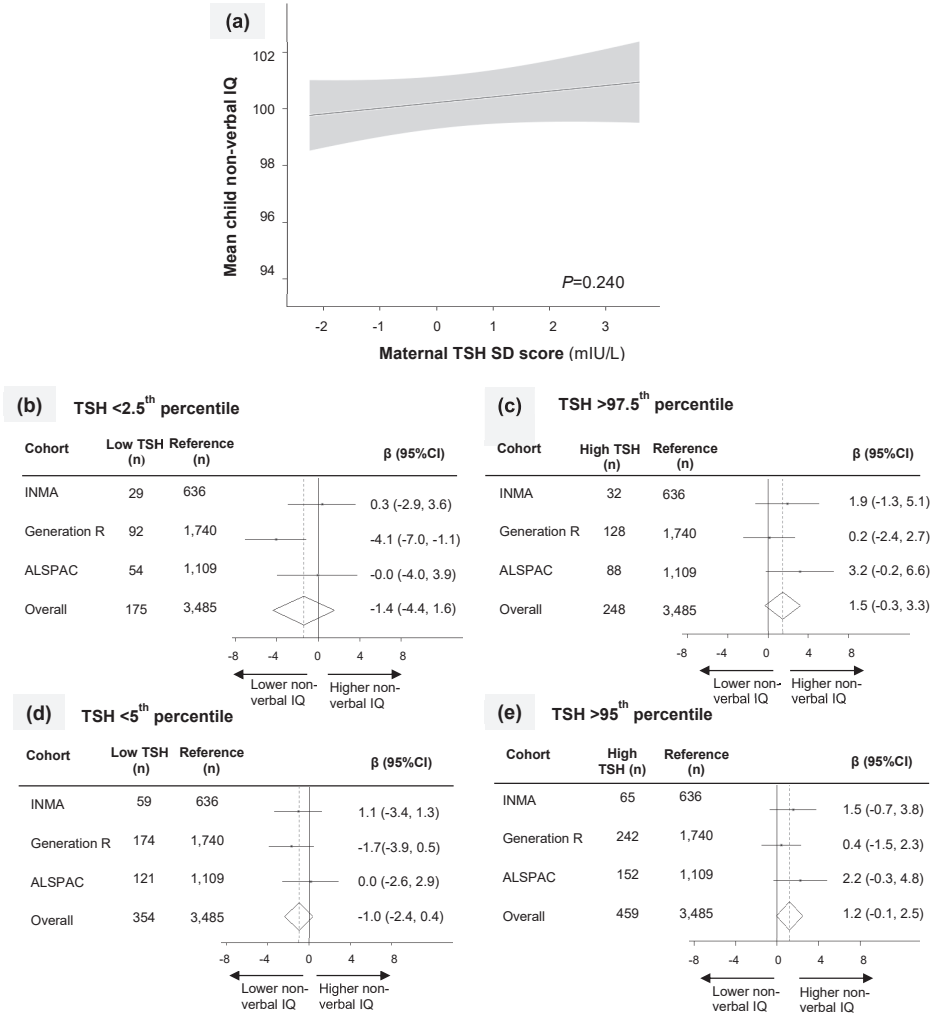
Supplemental Table 2 Distribution and comparison of maternal and child characteristics in the included and excluded population.

	INMA			Generation R			ALSPAC		
	Included (n=1,289)	**Excluded (n=679)**	***P*-value**	**Included (n=4,660)**	**Excluded (n=2,358)**	***P*-value**	**Included (n=3,087)**	**Excluded (n=1,790)**	***P*-value**
Maternal TSH, mIU/L	1.24 (0.84-1.81)	1.25 (0.77-1.91)	.660	1.36 (0.85-2.03)	1.28 (0.78-2.03)	.066	1.00 (0.64-1.46)	0.96 (0.62-1.43)	.088
Maternal FT4, pmol/L	10.6 (9.7-11.6)	10.4 (9.5-11.5)	.018	14.8 (13.2-16.7)	14.6 (12.9-16.6)	.071	16.2 (14.8-17.7)	16.3 (14.9-17.9)	.034
TPOAb positivity, n (%)	NA	NA		254 (5.8)	96 (6.7)	.253	392 (12.8)	212 (11.9)	.377
Gestational age at blood sampling, weeks	13.1 (1.3)	13.2 (1.3)	.074	13.4 (2.0)	13.8 (2.2)	<.001	11.0 (3.2)	11.0 (3.2)	.942
Maternal educational level, n (%)									
Low	281 (21.9)	216 (31.1)		353 (8.0)	295 (14.8)		736 (24.7)	562 (39.8)	
Medium	537 (41.8)	279 (40.1)	<.001	1,904 (42.9)	1,022 (51.3)	<.001	1,828 (61.3)	735 (52.0)	<.001
High	468 (36.4)	200 (28.8)		2,179 (49.1)	676 (33.9)		416 (14.0)	116 (8.2)	
Maternal ethnicity, n (%)									
Spanish	1,202 (93.4)	592 (85.2)		NA	NA		NA	NA	
Latin-american	60 (4.7)	68 (9.8)		NA	NA		NA	NA	
European/other	25 (1.9)	35 (5.0)		NA	NA		NA	NA	
Dutch	NA	NA		2,606 (56.7)	844 (41.0)		NA	NA	
Indonesian	NA	NA		150 (3.3)	50 (2.4)		NA	NA	
Cape verdian	NA	NA		170 (3.7)	108 (5.3)		NA	NA	
Moroccan	NA	NA		225 (4.9)	179 (8.7)		NA	NA	
Dutch Antilles	NA	NA	<.001	104 (2.3)	89 (4.3)	<.001	NA	NA	<.001
Surinamese	NA	NA		351 (7.6)	229 (11.1)		NA	NA	
Turkish	NA	NA		356 (7.8)	205 (10.0)		NA	NA	
Asian	NA	NA		51 (1.1)	40 (1.9)		NA	NA	
Other, non-western	NA	NA		162 (3.5)	135 (6.6)		NA	NA	
Other, western	NA	NA		418 (9.1)	178 (8.7)		NA	NA	
White	NA	NA		NA	NA		2,924 (98.6)	1,349 (96.5)	
Non-white	NA	NA		NA	NA		42 (1.4)	49 (3.5)	
Maternal age, years	31.5 (4.0)	30.8 (4.8)	<.001	30.3 (4.8)	28.6 (5.5)	<.001	28.0 (4.6)	26.3 (5.0)	<.001

Supplemental Table 2 Distribution and comparison of maternal and child characteristics in the included and excluded population (continued).

	INMA			Generation R			ALSPAC		
	Included (n=1,289)	**Excluded (n=679)**	***P*-value**	**Included (n=4,660)**	**Excluded (n=2,358)**	***P*-value**	**Included (n=3,087)**	**Excluded (n=1,790)**	***P*-value**
Parity, n (%)									
0	731 (56.8)	361 (51.9)		2,721 (58.4)	1,203 (52.7)		1,410 (47.2)	692 (43.9)	
1	472 (36.7)	275 (39.6)	.069	1,386 (29.7)	679 (29.7)	<.001	1,033 (34.6)	530 (33.6)	.002
≥2	84 (6.5)	59 (8.5)		553 (11.9)	402 (17.6)		543 (18.2)	354 (22.5)	
Maternal smoking, n (%)									
Never smoked	883 (69.4)	419 (66.4)		3,085 (73.5)	1,339 (68.6)		2,391 (79.2)	1,077 (67.0)	
Smoked at the beginning of pregnancy	174 (13.7)	90 (14.3)	.372	396 (9.4)	176 (9.0)	<.001	142 (4.7)	107 (6.7)	<.001
Continued smoking	216 (17.0)	122 (19.3)		719 (17.1)	437 (22.4)		486 (16.1)	424 (26.4)	
Pre-pregnancy BMI, kg/m^2	22.5 (20.8-25.1)	22.5 (20.7-25.2)	.845	22.6 (20.7-25.2)	22.7 (20.7-25.6)	.215	22.1 (20.5-24.2)	22.2 (20.5-24.5)	.380
Child female sex, n (%)	635 (49.3)	301 (47.6)	.490	2,313 (49.6)	1,155 (49.0)	.629	1,500 (48.6)	845 (47.2)	.360

Values represent median (interquartile range) or mean (SD), unless stated otherwise. *P*-value for differences calculated using Chi-square test for categorical variables, Student´s t-test for continuous normal-distributed variables, and Kruskal-Wallis test for continuous non-normal distributed variables. NA: not available. Numbers may not add up due to rounding.



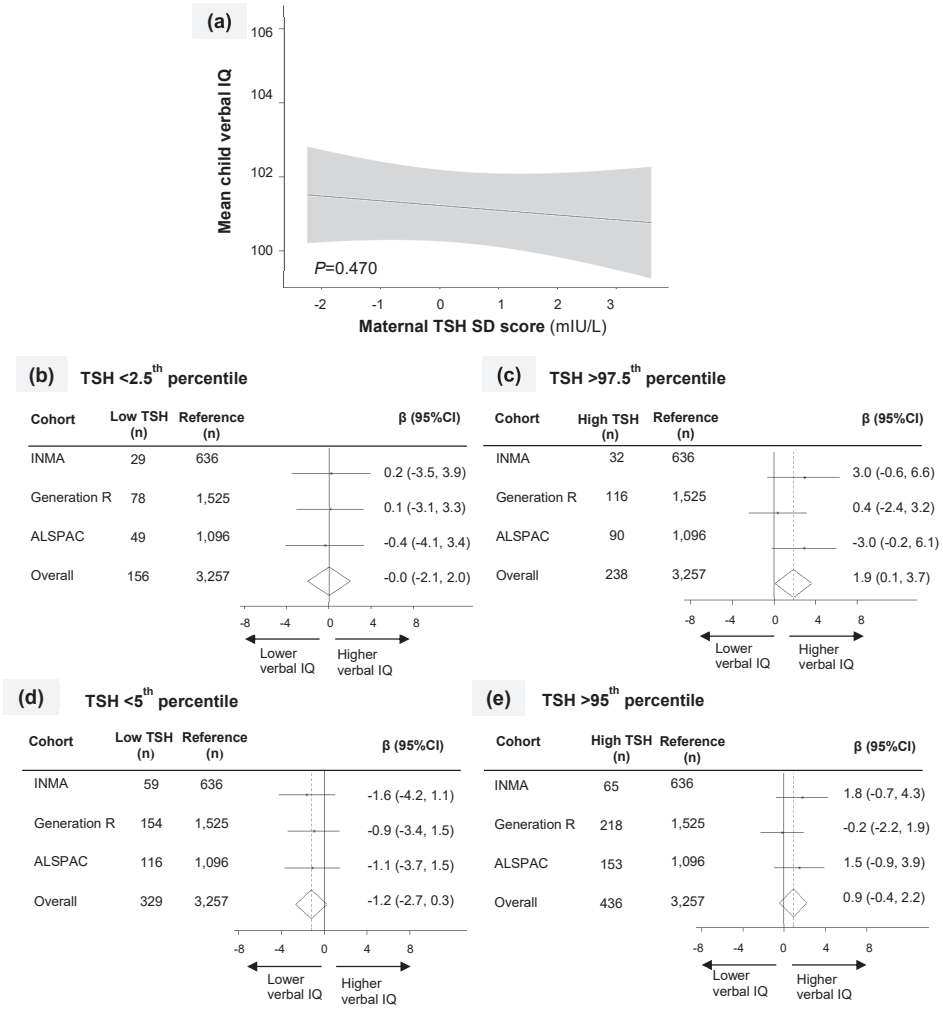
Supplemental Fig. 1 Association of maternal TSH during early pregnancy with child non-verbal IQ**.** Association shows as (a) a continuous association depicted as the child non-verbal IQ (black line) with 95% CI (gray area) and by cohort-specific maternal TSH concentrations in the (b) <2.5th percentile, (c) >97.5th percentile, (d) <5th percentile, and (e) >95th percentile compared with interquartile range (between 25th and 75th percentiles), depicted as effect estimate (dot) with the 95% CI per cohort and overall as estimated by random-effects meta-analysis (diamond). The I^2 for each model is as follows: for TSH<2.5th percentile, I^2=57.5%; for TSH>97.5th percentile, I^2=4.8%; for TSH<5th percentile, I^2=0.0%; for TSH>95th percentile, I^2=0.0%.

Supplemental Table 3 Association of maternal TSH with child non-verbal IQ stratified by FT4.

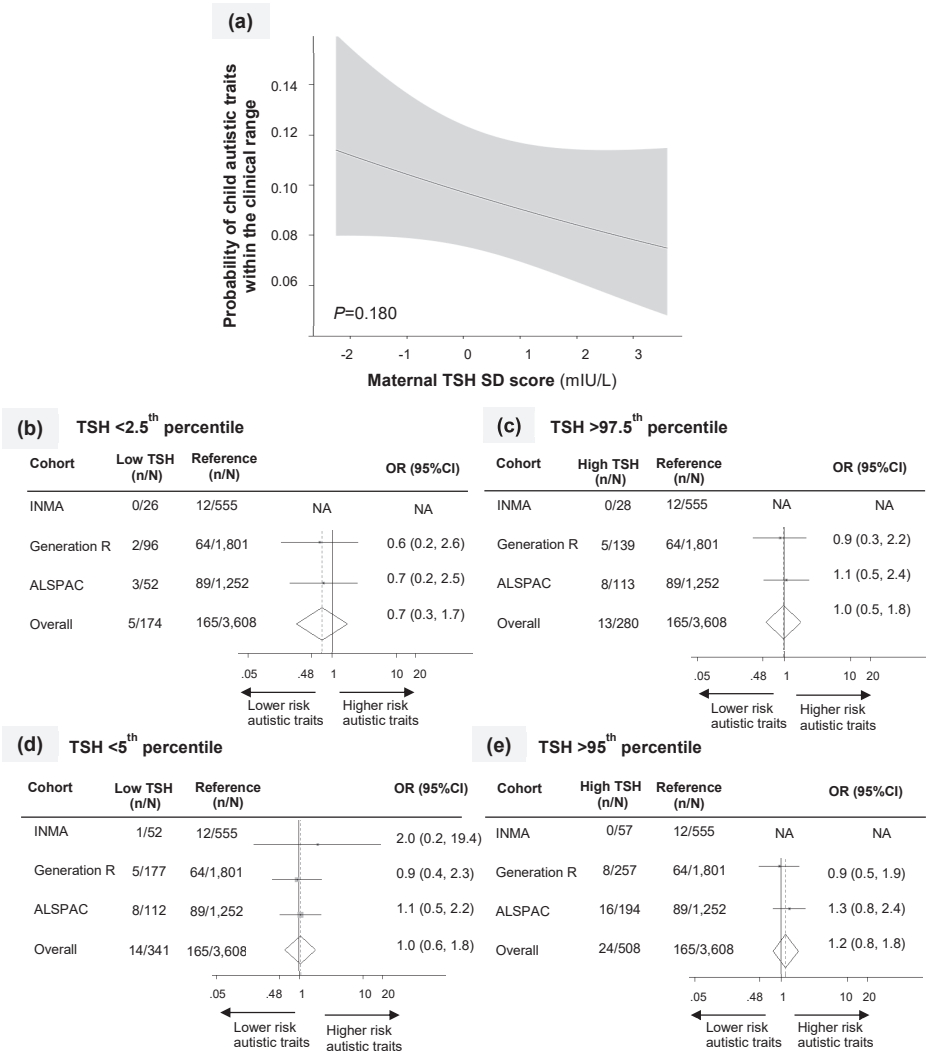
	β (95% CI)	*P*-value	*P* heterogeneity	I^2
High TSH (>P95) vs. normal TSH (P25-P75)	1.2 (-0.1 to 2.5)	0.063	0.503	0.0
in low FT4 group (<P25)	0.9 (-1.3 to 3.0)	0.421	0.522	0.0
in medium FT4 group (P25-P75)	2.1 (0.1 to 4.0)	0.038	0.343	6.5
in high FT4 group (>P75)	1.0 (-2.6 to 4.6)	0.578	0.926	0.0

Supplemental Table 4 Association of maternal TSH with child verbal IQ stratified by FT4.

	β (95% CI)	*P*-value	*P* heterogeneity	I^2
High TSH (>P97.5) vs. normal TSH (P25-P75)	1.9 (0.1 to 3.7)	0.039	0.388	0.0
in low FT4 group (<P25)	0.8 (-2.0 to 3.6)	0.565	0.780	0.0
in medium FT4 group (P25-P75)	1.7 (-1.0 to 4.5)	0.219	0.432	0.0
in high FT4 group (>P75)	3.3 (-5.0 to 11.6)	0.438	0.103	55.9

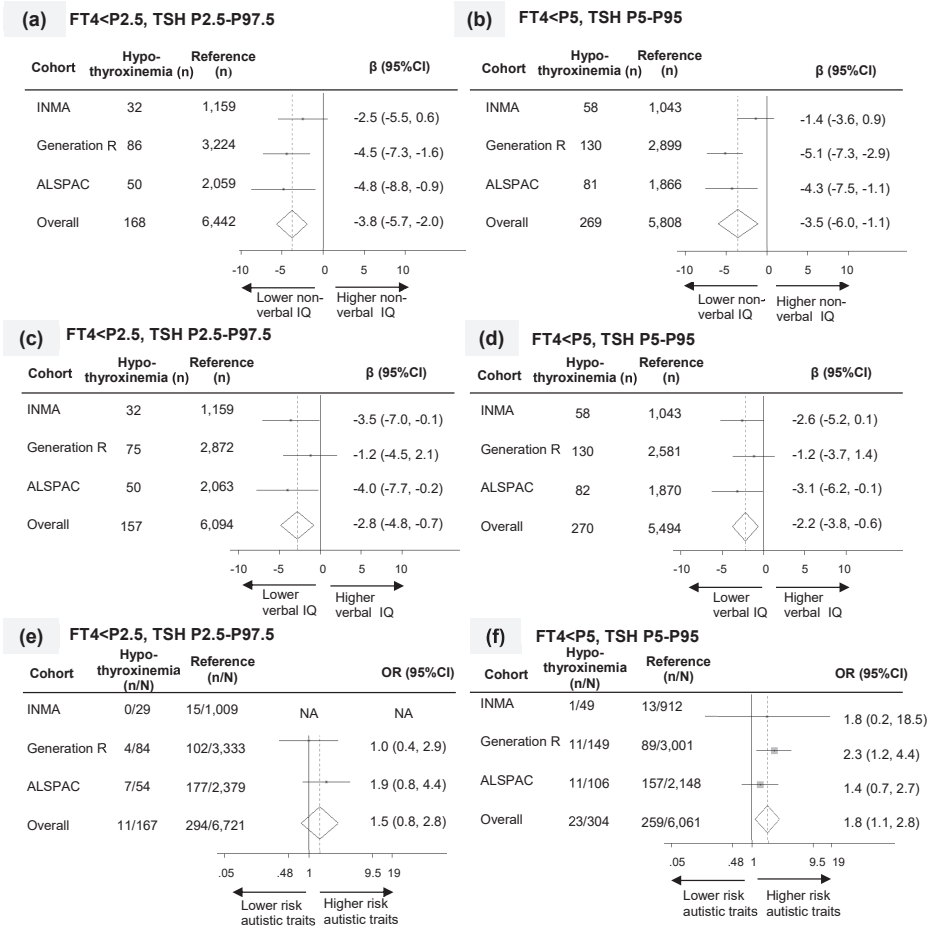


Supplemental Fig. 2 Association of maternal TSH during early pregnancy with child verbal IQ.
Association shown as (a) a continuous association depicted as the child verbal IQ (black line) with 95% CI (gray area); and by cohort-specific maternal TSH concentrations in the (b) <2.5th percentile, (c) >97.5th percentile, (d) <5th percentile, and (e) >95th percentile compared with interquartile range (between 25th and 75th percentiles), depicted as effect estimate (dot) with the 95% CI per cohort and overall as estimated by random-effects meta-analysis (diamond). The I^2 for each model is as follows: for TSH<2.5th percentile, I^2=0.0%; for TSH>97.5th percentile, I^2=0.0%; for TSH<5th percentile, I^2=0.0%; for TSH>95th percentile, I^2=0.0%.



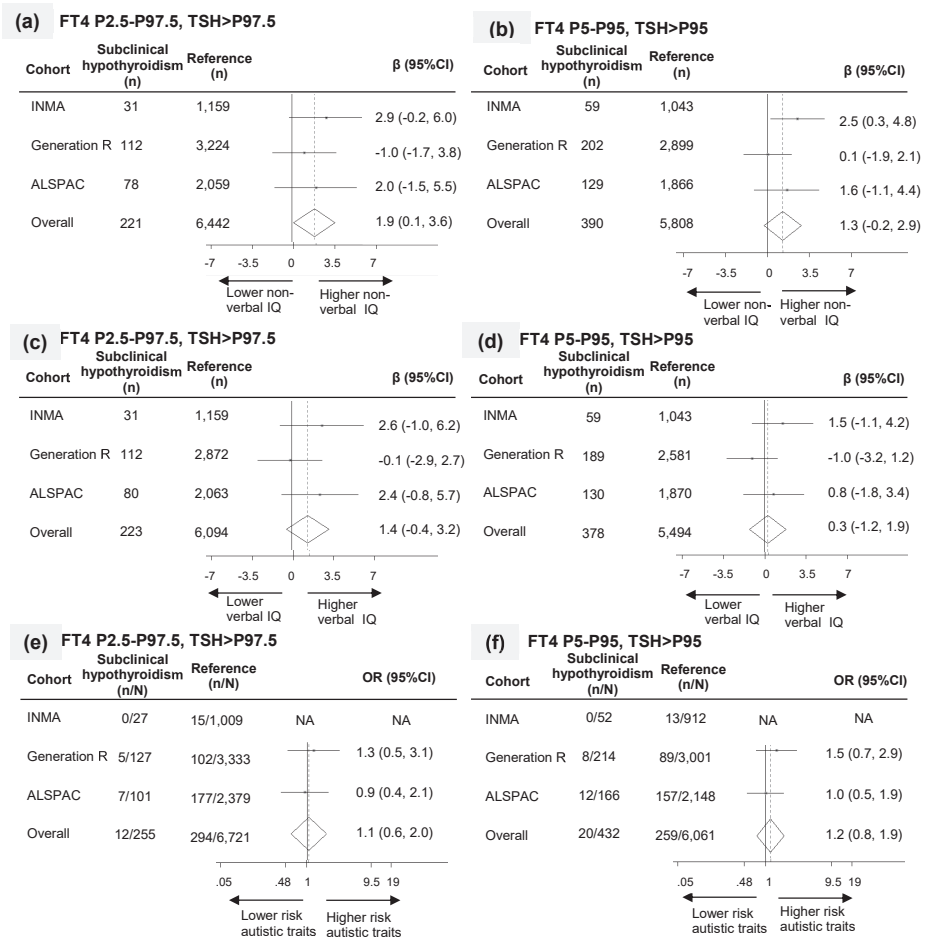
Supplemental Fig. 3 Association of maternal TSH during early pregnancy with child autistic traits within the clinical range.

Association shown a (a) a continuous association depicted as the child autistic traits within the clinical range (black line) with 95% CI (gray area) and by cohort-specific maternal TSH concentrations in the (b) <2.5th percentile, (c) >97.5th percentile, (d) <5th percentile, and (e) >95th percentile compared with interquartile range (between 25th and 75th percentiles), depicted as effect estimate (dot) with the 95% CI per cohort and overall as estimated by random-effects meta-analysis (diamond). The I^2 for each model is as follows: for TSH<2.5th percentile, I^2=0.0%; for TSH>97.5th percentile, I^2=0.0%; for TSH<5th percentile, I^2=0.0%; for TSH>95th percentile, I^2=0.0%.



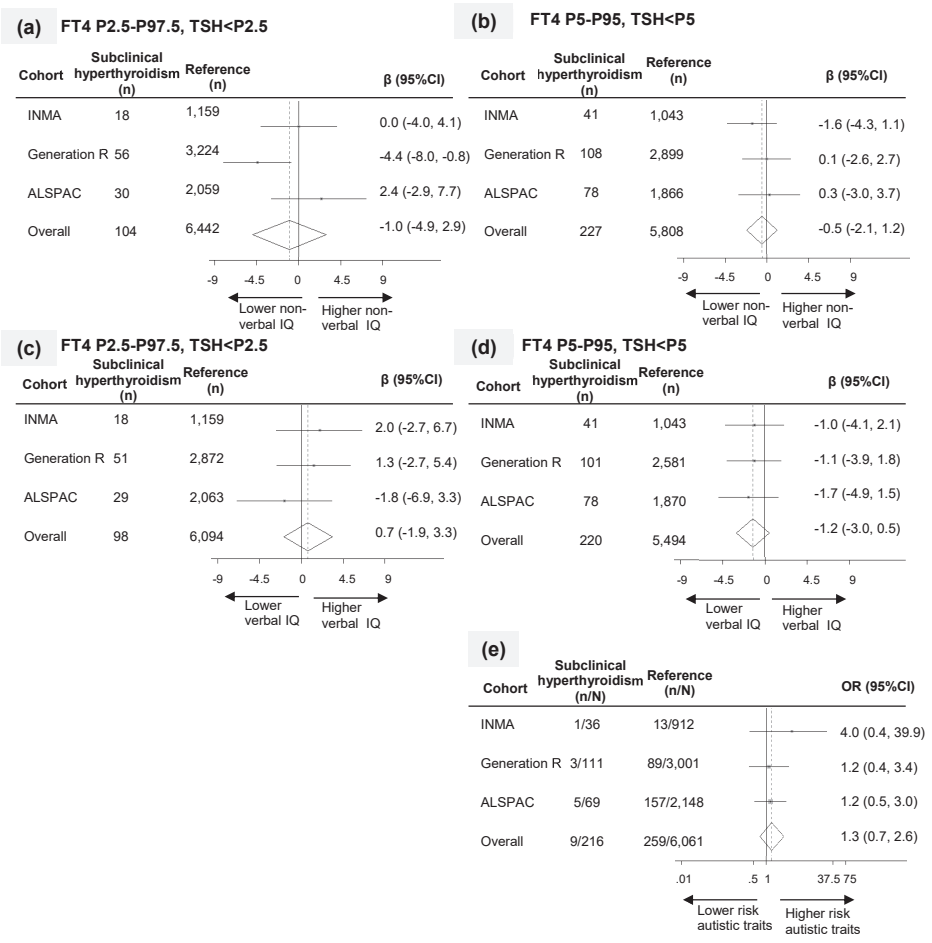
Supplemental Fig. 4 Association of maternal hypothyroxinemia during early pregnancy with child non-verbal IQ, verbal IQ, and autistic traits within the clinical range.

Figure shows the association of maternal hypothyroxinemia during early pregnancy with child non-verbal IQ (a,b), verbal IQ (c,d), and autistic traits within the clinical range (e,f), defined using the cohort-specific 2.5th and 97.5th population-based percentiles (a,c,e) or the 5th and 95th population-based percentiles (b,d,f), depicted as effect estimate (dot) with the 95% CI per cohort and overall as estimated by random-effects meta-analysis (diamond). The reference group consisted of euthyroid women. The I^2 for each model is as follows: for a, I^2=0.0%; for b, I^2=64.6%; for c, I^2=0.0%; for d, I^2=0.0%; for e, I^2=0.0%; for f, I^2=0.0%.



Supplemental Fig. 5 Association of maternal subclinical hypothyroidism during early pregnancy with child non-verbal IQ, verbal IQ, and autistic traits within the clinical range.

Figure shows the association of maternal subclinical hypothyroidism during early pregnancy with child non-verbal IQ (a,b), verbal IQ (c,d), and autistic traits within the clinical range (e,f), defined using the cohort-specific 2.5th and 97.5th population-based percentiles (a,c,e) or the 5th and 95th population-based percentiles (b,d,f), depicted as effect estimate (dot) with the 95% CI per cohort and overall as estimated by random-effects meta-analysis (diamond). The reference group consisted of euthyroid women. The I^2 for each model is as follows: for a, I^2=0.0%; for b, I^2=26.8%; for c, I^2=0.0%; for d, I^2=17.1%; for e, I^2=0.0%; for f, I^2=0.0%.



Supplemental Fig. 6 Association of maternal subclinical hyperthyroidism during early pregnancy with child non-verbal IQ, verbal IQ, and autistic traits within the clinical range.

Figure shows the association of maternal subclinical hyperthyroidism during early pregnancy with child non-verbal IQ (a,b), verbal IQ (c,d), and autistic traits within the clinical range (e), defined using the cohort-specific 2.5th and 97.5th population-based percentiles (a,c) or the 5th and 95th population-based percentiles (b,d,e), depicted as effect estimate (dot) with the 95% CI per cohort and overall as estimated by random-effects meta-analysis (diamond). The reference group consisted of euthyroid women. The I^2 for each model is as follows: for a I^2=60.5%; for b, I^2=0.0%; for c, I^2=0.0%; for d, I^2=0.0%; for e, I^2=0.0%.

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Chapter 5.2

Maternal Thyroid Function in Early Pregnancy and Child Attention-Deficit Hyperactivity Disorder: An Individual-Participant Meta-Analysis.

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Abstract

Background: Thyroid hormone is essential for optimal fetal brain development. Evidence suggests that both low and high maternal thyroid hormone availability may have adverse effects on child neurodevelopmental outcomes, but the effect on behavioral problems remains unclear. We studied the association of maternal thyrotropin (TSH) and free thyroxine (FT4) concentrations during the first 18 weeks of pregnancy with child attention-deficit hyperactivity disorder (ADHD).

Methods: A total of 7669 mother-child pairs with data on maternal thyroid function and child ADHD were selected from three prospective population-based birth cohorts: INfancia y Medio Ambiente (INMA; N=1073, Spain), Generation R (N=3812, the Netherlands), and Avon Longitudinal Study of Parents and Children (ALSPAC; N=2784, United Kingdom). Exclusion criteria were multiple pregnancy, fertility treatment, usage of medication affecting the thyroid, and pre-existing thyroid disease. We used logistic regression models to study the association of maternal thyroid function with the primary outcome, ADHD, assessed via the *Diagnostic and Statistical Manual of Mental Disorders, fourth edition* (DSM-IV) criteria by parents and/or teachers at a median child age of 4.5 to 7.6 years, and with the secondary outcome, an ADHD symptom score above the 90th percentile. Effect modification by gestational age and sex was tested with interaction terms and stratified analyses.

Results: Overall, 233 (3%) children met the criteria for ADHD. When analyzed continuously, neither FT4 nor TSH was associated with a higher risk of ADHD [odds ratio (OR) 1.1, 95% confidence Interval (95% CI 1.0-1.3), p=0.060 and OR 0.9, (95% CI 0.9-1.1), p=0.385, respectively] or with high symptom scores. When investigating effect modification by gestational age, a higher FT4 was associated with symptoms above the 90th percentile but only in the first trimester [for FT4 per 1 SD: OR 1.2 (95% CI 1.0-1.4), p=0.027]. However, these differential effects by gestational age were not consistent. No significant effect modification by sex was observed.

Conclusions: We found no clear evidence of an association between maternal thyroid function and child ADHD.

Introduction

Thyroid hormone regulates important brain developmental processes including early neuronal proliferation and migration [1]. Until mid-gestation, fetal thyroid hormone availability largely depends on the supply of maternal thyroid hormone *via* the placenta [2]. Relatively mild deficits in thyroid hormone availability have been associated with adverse child neurodevelopmental outcomes, such as lower IQ, lower psychomotor development scores, and lower gray matter and cortex volume [3–5]. Evidence from mainly animal studies also suggests that high-normal thyroid hormone availability may have similar adverse effects on neurodevelopmental outcomes [5–11]. However, whether mild changes in thyroid hormone concentrations also play a role in the etiology of child behavioral problems is less well established.

Attention-deficit hyperactivity disorder (ADHD) is a "persistent pattern of inattention and/or hyperactivity-impulsivity that is more frequently displayed than is typically observed in individuals at a comparable level of development" [12] and occurs in ~ 5.9-7.1 percent of children and adolescents [13]. The exact cause of ADHD is unknown, but susceptibility is thought to depend on genetic predisposition, environmental factors and their interactions [14,15]. There is some indication that high thyroid hormone availability may increase the risk of ADHD. Patients with resistance to thyroid hormone beta (RTHβ), in which a defective thyroid hormone receptor beta (TRβ) results in lifelong exposure to a high thyroid hormone concentration in tissues that predominantly express thyroid hormone receptor alfa (TRα) such as the brain, have a higher risk of ADHD [16]. Furthermore, individuals with ADHD have a delay in cortical maturation, reduced neuronal activity, and brain volume [17–19] compared with healthy controls; some of the same abnormalities are also seen in the offspring of mothers with gestational thyroid dysfunction [5,20–22]. However, further studies are required to clarify whether too low and/or too high thyroid hormone availability in pregnancy is consistently associated with the development of ADHD.

So far, evidence from epidemiological studies is inconsistent with regard to an association of maternal thyroid function with child ADHD [23–31]. Some studies indicated that the effects of maternal thyroid function on ADHD may be more prominent in girls [23,31], but further studies are needed to replicate and further clarify sex-specific effects. In addition, it would be relevant to investigate whether there are differential effects by gestational age, as the relatively late start of levothyroxine therapy in women with subclinical hypothyroidism or hypothyroxinemia in two randomized control trials has been suggested to be a reason for the negative findings for those trials [32,33]. We therefore combined data from three prospective birth cohorts to study the association between free thyroxine (FT4) and thyrotropin (TSH) in early pregnancy and the risk of ADHD, as well as the effect modification by child sex and gestational age.

Material and Methods

Study design and populations

We used data from three population-based birth cohort studies: INfancia y Medio Ambiente (INMA; Spain, sub-cohorts of Valencia, Sabadell, and Gipuzkoa), Generation R (the Netherlands), and the Avon Longitudinal Study of Parents and Children (ALSPAC, United Kingdom). Information on the designs of the three cohort studies can be found elsewhere [34–37]; the ALSPAC study website contains details of all the data that is available through a searchable data dictionary and variable search tool [38]. Briefly, all three cohorts were designed to understand the role of environmental exposures and/or genetic characteristics for child growth, development, and health from fetal life until (young) adulthood. The INMA Project is a network of seven birth cohorts in Spain with different recruitment periods. Pregnant women included in the Valencia (N=855), Sabadell (N=657), and Gipuzkoa (N=638) cohorts enrolled from November 2003 until June 2005, July 2004 until July 2006, and April 2006 until January 2008, respectively. In Generation R, 9778 mothers with a delivery date from April 2002 until January 2006 were enrolled in the study. In ALSPAC, pregnant women resident in Avon, UK with expected dates of delivery between April 1991 and December 1992 were invited to take part in the study. The initial number of pregnancies enrolled was 14541, of which 13998 children were alive at the first year of age. For the current study, mother-child pairs were eligible if they had a thyroid measurement in the first half of pregnancy (≤18 weeks) and had data on an assessment of ADHD symptoms during childhood. Exclusion criteria were multiple pregnancy, fertility treatment, usage of medication affecting thyroid function during pregnancy, and pre-existing thyroid disease. Ethical approval was obtained before recruitment and during the follow-up waves of data collection from a number of bodies: the Ethical Committee of the Municipal Institute of Medical Investigation, the Ethical Committees of the hospitals involved in the study (INMA; reference numbers G03/176, 2005/2106/I, and 2009/3432/I), the Medical Ethical Committee of the Erasmus Medical Center (Generation R; reference numbers: MEC 198.782.2001.31, MEC-2007-413), the ALSPAC Ethics and Law Committee, and the Local Research Ethics Committees (ALSPAC; reference numbers available elsewhere [39]); approval by parents or guardians of the children was given by a signed informed-consent form.

Maternal thyroid function

The procedures and methodologies by which maternal thyroid function was measured differed among cohorts. In INMA, serum samples were collected at a mean [± standard deviation (SD)] gestational age of 13.1 ± 1.3 weeks and stored at -80°C after collection. FT4 and TSH were measured using a solid-phase, time-resolved sandwich fluoro-immunoassay (AutoDEL-FIA, PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland) and a lanthanide metal europium (Eu) label. Thyroid peroxidase antibodies (TPOAbs) were not

measured. In Generation R, serum samples were collected at a mean ± SD age of 13.4 ± 1.9 weeks, centrifuged, and stored at -80°C after collection. FT4 and TSH were measured using the Vitros ECI immunodiagnostic (Ortho clinical Diagnostics, Rochester, NY). Maternal TPOAbs were measured using the Phadia 250 immunoassay (Phadia AB, Uppsala, Sweden), and a TPO titre ≥ 60 IU/mL was considered as positive. In ALSPAC, serum samples were collected at a mean ± SD age of 11.0 ± 3.2 weeks and stored at -20°C. FT4, TSH, and TPOAb measurements were performed using Abbott Architect i2000. A TPO titre ≥ 6 IU/mL was considered as positive. Additional information about the serum measurements is provided in the Supplementary Material.

ADHD symptoms

In INMA, ADHD symptoms were assessed by teachers at a median age of 4.5 years [interquartile range (IQR) 4.4-5.7 years] using the ADHD Criteria of *Diagnostic and Statistical Manual of Mental Disorders, fourth edition* (ADHD-DSM-IV) list [12]. The DSM criteria are a valid tool for diagnosing ADHD, already at a pre-school age [40]. The DSM-IV consists of questions on nine inattention symptoms and nine hyperactivity-impulsivity symptoms on a 4-point Likert scale (never or rarely, sometimes, often, or very often), designed to score ADHD symptoms present in the last six months before the assessment. A symptom was defined as present if the question was answered with "often" or "very often". The total symptom score consisted of the sum of all the inattention and hyperactivity-impulsivity symptoms. Based on the symptom criteria of the DSM-IV, an ADHD diagnosis was given when the child had at least six inattention and/or six hyperactivity-impulsivity symptoms.

In Generation R, parents assessed their child's behavior in the last two months at a median age of 6.0 years (IQR 5.9-6.2 years) using the Child Behavior Checklist 1½–5 (CBCL/1½–5) [41]. From this 99-item questionnaire, the sum score of six questions on a 3-point Likert scale (not true, somewhat or sometimes true, very true or often true) made up the total ADHD symptom score. In addition, the computer-assisted Diagnostic Interview Schedule for Children - Young Child version [42], which is a DSM-IV based interview, was administered by research assistants at a median age of 6.6 years (IQR 6.3-7.1 years) to the parents or caregivers of a selected group of children. This subgroup consisted of a random selection of negative controls and children with a high probability of a psychiatric disorder, e.g., children who scored in the top 15th percentile of the CBCL1½–5 total problem score and/or in the top 2% of the syndrome scale scores. Algorithms provided by the developers of this tool were used to derive a DSM-IV diagnosis based on the symptom criteria of the DSM-IV. More information on the procedures and assessment is described elsewhere [43]. In our study, the reference group consisted of children that were not identified as having ADHD and children who did not have data on the Diagnostic Interview Schedule-Young Child version, but did have data on ADHD symptoms as assessed with the CBCL/1½–5 (e.g., mostly children with a low probability of a psychiatric disorder).

In ALSPAC, the Development and Well-Being Assessment (DAWBA) was used to evaluate child psychological disorders at a median age of 7.6 years (IQR 7.6-7.7 years) [44]. This validated tool consists of questions based on the diagnostic criteria described in the *International Classification of Diseases, Tenth Edition* (ICD-10) or the DSM-IV. A semi-structured interview was administered to parents relating to inattention and hyperactivity symptoms present in the six months before the assessment. Teachers in the geographically defined study area were also requested to fill out the DAWBA questionnaire on all children with a birth date between April 1991 and December 1992. The completion rate by teachers was 37% for ALSPAC children [45]. Clinical raters reviewed all available ratings on ADHD symptoms and assigned an ADHD diagnosis using the DSM-IV criteria as if in clinical settings.

Covariates

During pregnancy, questionnaires were used to collect information on maternal age, parity (zero, one, and two or more), Snijders Oomen Nonverbal Intelligence Test body mass index, smoking during pregnancy (never, smoked in the beginning or until pregnancy confirmed, continued smoking), ethnicity/country of birth (cohort-specific categories), and maternal educational level (low, middle, and high). Gestational age at blood sampling was defined using ultrasound and/or last menstrual period. Information on sex of the child was obtained from community midwives, obstetricians, hospital registries, clinical records, or questionnaires. Age at ADHD assessment was obtained during the study visit.

Statistical analyses

Owing to the differences in assays, the absolute values of TSH and FT4 across the cohorts cannot be compared. We therefore calculated cohort-specific SD scores with a mean of 0 and a SD of 1 using logarithmically transformed values of FT4 and TSH based on TPOAb-negative women when possible. Values outside the mean ± 4 SD range were considered as outliers and excluded from further analyses. We assessed the association of maternal TSH and FT4 SD scores within the mean ± 4 SD range with child ADHD by performing multivariable logistic regression models using pooled data. We used the same model to study the association with our secondary outcome, an ADHD symptom score above the 90th percentile, which was generated in each cohort separately. This extra harmonized cut-off was chosen *a priori* to increase statistical power owing to the low prevalence of children meeting the criteria of ADHD and has been used previously to define children at risk [46,47]. Negative binomial regression models were used to assess the association of FT4 and TSH with total ADHD symptom scores on a continuous scale per cohort. We could not analyze these associations using pooled data since the symptom scores did not share a common metric between cohorts and therefore were not comparable.

We tested for non-linearity by adding a quadratic term to our models and we assessed multicollinearity between covariates by the variance inflation factor. All cohort-specific models were adjusted for all the mentioned covariates. However, in the pooled analyses, we could not adjust for child age at ADHD ascertainment, maternal ethnicity/country of birth, and cohort simultaneously. The categories or the range of these variables did not overlap i.e., they were cohort-specific, and adjusting for several of these variables at the same time causes multicollinearity. Therefore, in the pooled analyses, we chose to adjust for maternal ethnicity/country of birth because it is known that there are ethnic differences in maternal thyroid parameters during pregnancy [48–50], and race/ethnicity is intertwined with social economic status, which has been associated with behavioral problems [51] and ADHD [13,52]. We investigated whether adjusting for cohort instead of ethnicity/country of birth yielded different results. Because the results were similar, we chose to show the results based on the ethnicity/country of birth adjustment. We decided to present standard logistic regression models after comparison with multilevel models using the Akaike information criterion.

We performed several sensitivity analyses. First, we tested for heterogeneity between cohorts using the Cochran Q test and the I^2 statistic (Supplementary Fig. 1; [53]). Second, we also compared the effect estimates before and after excluding TPOAb-positive women in mother-child pairs, of which TPOAb status was known, e.g., Generation R and ALSPAC, since TPOAb positivity was associated with a higher risk of ADHD irrespective of maternal TSH [54]. Third, we tested for possible effect modification by sex and/or gestational age at blood sampling by adding product terms into the models. To identify potential relevant effect modifiers, we screened for interaction terms with a *p*-value of <0.15 and subsequently performed stratified analyses to verify and quantify any relevant differences. For gestational age, analyses were stratified by tertiles based on pooled data (< 11.8 weeks, ≥ 11.8 to ≤ 13.5 weeks, and > 13.5 weeks).

The percentage of missing covariate data ranged from 0% to 15.6% (Table 1). Before pooling the cohort data, missing values in these covariates were imputed using chained equations, generating 25 data sets. Additionally, to alleviate the potential bias that arises when only the population with available data on maternal thyroid function and child ADHD symptoms is included, we applied inverse probability weighting [55]. Briefly, we used data on characteristics available for all participants at recruitment to predict the probability of participation in the study, and an inverse of those probabilities as weights in the analyses so that results would be representative for the initial populations of the cohorts. Statistical analyses were performed in STATA (version 15.0; Stata Corporation, College Station, TX).

Table 1 Distribution of maternal and child characteristics.

	INMA (N=1073)	Generation R (N=3812)	ALSPAC (N=2784)	*p*-value
ADHD, %	5.3	3.2	2.0	<0.001
ADHD symptoms > P90, %	10.4	10.3	10.3	0.990
Maternal TSH, median (IQR), mIU/L	1.25 (0.82-1.82)	1.38 (0.87-2.07)	1.02 (0.64-1.49)	<0.001
Maternal FT4, median (IQR), pmol/L	10.6 (9.7-11.5)	14.8 (13.2-16.7)	16.1 (14.8-17.6)	<0.001
TPOAb positivity, %	NA	5.8	12.8	<0.001
Gestational age at blood sampling, mean (SD), weeks [a]	13.1 (1.3)	13.4 (1.9)	11.0 (3.2)	<0.001
Maternal educational level, %[a]				<0.001
Low	20.8	6.2	24.0	
Medium	42.5	40.8	61.6	
High	36.6	53.0	14.4	
Maternal ethnicity/country of birth, % [a]				<0.001
Spanish	93.8	NA	NA	
Other, non-Spanish	6.2	NA	NA	
Dutch	NA	60.6	NA	
Indonesian	NA	3.3	NA	
Cape Verdean	NA	3.0	NA	
Turkish	NA	6.9	NA	
Other, non-Western	NA	17.0	NA	
Other, Western	NA	9.2	NA	
White	NA	NA	98.6	
Non-white	NA	NA	1.4	
Maternal age, mean (SD), years	31.6 (3.9)	30.7 (4.6)	28.1 (4.6)	<0.001
Parity, % [a]				<0.001
0	57.0	59.6	47.9	
1	36.7	29.9	34.7	
≥2	6.3	10.5	17.4	
Smoking during pregnancy, % [a]				<0.001
Never	70.2	74.9	79.6	
At the beginning of pregnancy	12.9	9.6	4.5	
Continued smoking	16.9	15.5	15.9	
Pre-pregnancy BMI, median (IQR), kg/m^2 [a]	22.5 (20.8-25.0)	22.5 (20.8-25.1)	22.1 (20.5-24.2)	<0.001
Child female sex, % [a]	48.9	50.1	48.5	0.410

p-value for differences was calculated using the Chi-square test for categorical variables, one-way ANOVA for continuous normal-distributed variables, and Kruskal-Wallis test for continuous non-normal distributed variables.

[a] Values do not take into account missing data (0%, 0.2%, and 0% for gestational age; 0.3%, 3.2%, and 2.5% for maternal education; 0.1%, 0%, and 2.9% for maternal ethnicity/country of birth; 0.2%, 0%, and 2.9% for parity; 1.4%, 9.0%, and 1.7% for smoking; 0%, 15.6%, and 8.8% for pre-pregnancy BMI; 0.1%, 0%, and 0% for child sex in INMA, Generation R, and ALSPAC, respectively). ADHD, attention-deficit hyperactivity disorder; ALSPAC, Avon Longitudinal Study of Parents and Children; ANOVA, analysis of variance; BMI, body mass index; FT4, free thyroxine; INMA, INfancia y Medio Ambiente; IQR, interquartile range; NA, not available; SD, standard deviation; TPOAb, thyroid peroxidase antibody; TSH, thyrotropin.

Results

After exclusions, data for a total of 7669 mother-child pairs were available for analyses (Fig. 1). An overview of the characteristics of the study population is given in Table 1. The prevalence of ADHD was 5.3% (n=57) in INMA, 3.2% (n=121) in Generation R, and 2.0% (n=55) in ALSPAC. Compared with the study population, women who were not included in the analysis had a lower education level, were less often native or Caucasian, and were younger in all three cohorts (Supplementary Table 1). There were similar differences in characteristics when comparing mothers whose children had an ADHD symptom score and those whose children did not undergo an ADHD assessment (Supplementary Table 2). No clinically relevant differences were found in TSH and FT4 concentrations between these two groups.

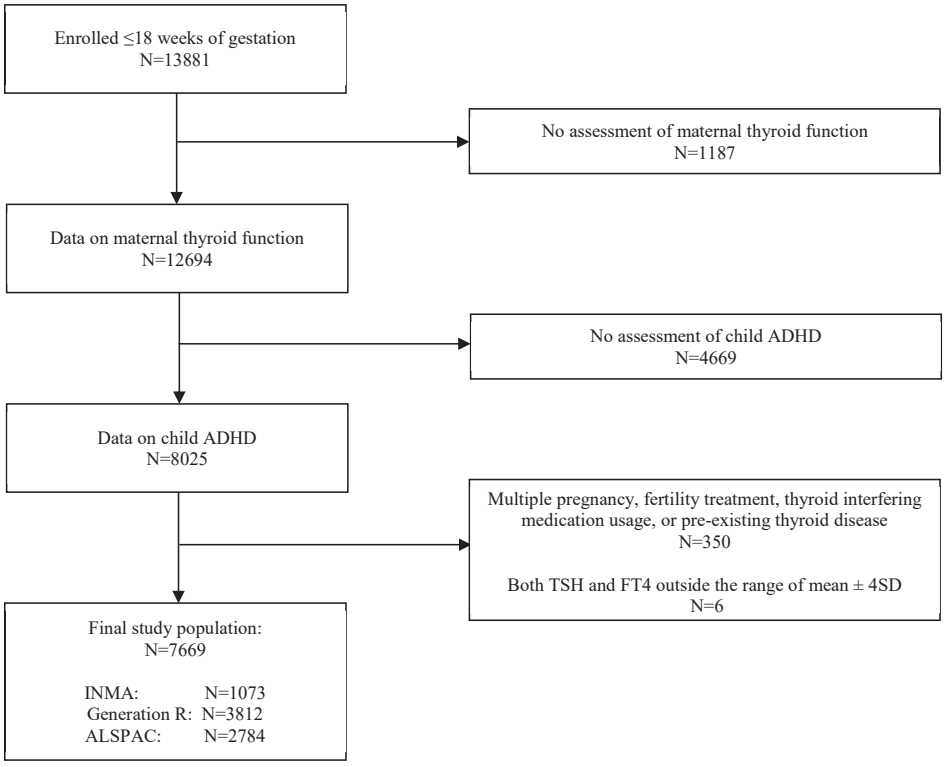

Figure 1 Flowchart of the selection of the study population.
ADHD, attention-deficit hyperactivity disorder; ALSPAC, Avon Longitudinal Study of Parents and Children; FT4, free thyroxine; INMA, Infancia y Medio Ambiente; SD, standard deviation; TSH, thyrotropin.

Attention-deficit hyperactivity disorder

FT4 was not associated with ADHD [odds ratio (OR) 1.1, 95% confidence interval (95% CI 1.0 to 1.3), p=0.060; Table 2 and Supplementary Fig.1]. The effect estimate remained similar

after excluding TPOAb-positive women. There was no effect modification by gestational age (*p* for interaction term=0.581). While there was no indication of significant effect modification by child's sex (*p* for interaction term=0.144), we did observe a significant association in girls only [OR 1.3 (95% CI 1.0 to 1.7), *p*=0.042; for boys: OR 1.1 (95% CI 0.9 to 1.3), *p*=0.352]. The association in girls became stronger after excluding TPOAb-positive women from mother-child pairs, of which TPOAb status was known [from OR 1.2 (95 % CI 0.9 to 1.7), *p*=0.187 to OR 1.5 (95% CI 1.1 to 2.1), *p*=0.020], while the effect estimate remained similar in boys.

TSH was not associated with ADHD [OR 0.9 (95% CI 0.8 to 1.1), *p*=0.385; Table 2 and Supplementary Fig. 1) and the effect estimate remained similar after excluding TPOAb-positive women. There was no effect modification by gestational age (*p* for interaction term= 0.757; Table 3) or child sex (*p* for interaction term= 0.474).

Table 2 Association of maternal FT4 and TSH concentrations during pregnancy with child ADHD and with an ADHD symptom score ≥90th percentile

	ADHD			ADHD symptoms score ≥90th percentile		
	n/N[a]	OR (95% CI)	*p*-value	n/N[b]	OR (95% CI)	*p*-value
FT4						
Pooled	232/7355	1.1 (1.0-1.3)	0.060	779/6768	1.0 (0.9-1.1)	0.554
INMA	57/1016	1.3 (0.9-1.7)	0.126	111/952	1.3 (1.0-1.6)	0.040
Generation R	121/3658	1.0 (0.8-1.3)	0.747	390/3384	1.0 (0.8-1.1)	0.420
ALSPAC	54/2681	1.2 (0.9-1.7)	0.225	278/2432	1.1 (0.9-1.3)	0.294
TSH						
Pooled	230/7299	0.9 (0.8-1.1)	0.385	777/6712	0.9 (0.9-1.0)	0.073
INMA	56/1009	0.9 (0.7-1.3)	0.562	110/945	0.8 (0.7-1.0)	0.114
Generation R	119/3633	0.9 (0.8-1.1)	0.323	388/3359	1.0 (0.9-1.1)	0.943
ALSPAC	55/2657	0.9 (0.7-1.2)	0.641	279/2408	0.9 (0.8-1.0)	0.046
		1.2 (1.0-1.4)[c]	0.027			

Reported OR's and 95% CIs are increase in odds per SD of log-transformed FT4 or TSH. Analyses were performed using logistic regression and adjusted for gestational age at blood sampling, maternal education, maternal ethnicity/country of birth, age, parity, smoking during pregnancy, pre-pregnancy body mass index, and child sex. Cohort-specific estimates were also adjusted for child age at ADHD ascertainment and subcohort in INMA.

[a] n=children with ADHD, N=children without ADHD

[b] n=children with a symptom score ≥90th percentile, N=children with a symptom score < 90th percentile.

[c] A quadratic term (TSH SD score^2) was added to the model, indicating a non-linear association.

CI, confidence interval; OR, odds ratio.

ADHD symptom score above the 90th percentile

There was no association of FT4 with an ADHD symptom score above the 90th percentile [OR 1.0 (95% CI 0.9 to 1.1), *p*=0.554, respectively; Table 2 and Supplementary Fig. 1] and no indication of effect modification by child's sex. We did identify a possible effect modification

by gestational age for the association of FT4 with symptoms above the 90th percentile (p for interaction term: 0.013); a higher FT4 was associated with a significant 1.2-fold higher risk of ADHD symptoms above the 90th percentile in the early-pregnancy FT4 measurements (95% CI 1.0 to 1.4, p=0.027; < 11.8 weeks), while a higher FT4 was associated with a lower risk in mother-child pairs with relatively late-pregnancy FT4 measurements [OR 0.9 (95% CI 0.7 to 1.0), p=0.045; Table 3]. The effect estimates remained similar after excluding TPOAb-positive women.

There was no association of TSH with an ADHD symptom score above the 90th percentile [OR 0.9 (95% CI 0.9 to 1.0), p=0.073, respectively; Table 2 and Supplementary Fig. 1] and no indication of effect modification by child's sex. We identified a possible effect modification by gestational age for the association of TSH with symptom scores above the 90th percentile (p for interaction term: 0.082). Stratified analyses by tertile of gestational age showed that a higher TSH was associated with a significant 0.8-fold lower risk of ADHD symptoms above the 90th percentile in the relatively early-pregnancy TSH measurements (95% CI 0.7 to 0.9, p=0.006; Table 3; < 11.8 weeks). The effect estimates remained similar after excluding TPOAb-positive women.

ADHD symptoms on a continuous scale

No associations were identified between FT4 or TSH across the full range and the total ADHD symptom score as investigated in each cohort separately (Supplementary Table 3 and 4).

Table 3 Pooled association of maternal FT4 and TSH concentrations during pregnancy with child ADHD and with an ADHD symptom score above the 90th percentile, stratified by tertile of gestational age.

		ADHD				ADHD symptoms above the 90th percentile			
	gestational age median (range)	N	% with ADHD	OR (95% CI)	*p*-value	N	% score >P90	OR (95% CI)	*p*-value
FT4									
Tertile 1	10.0 (4.0-11.8)	2523	2.4	1.1 (0.9-1.5)	0.356	2503	9.8	1.2 (1.0-1.4)	0.027
Tertile 2	12.6 (11.8-13.5)	2580	3.5	1.2 (1.0 -1.6)	0.064	2580	11.1	1.0 (0.9-1.2)	0.884
								1.1 (1.0-1.2)[a]	0.027
Tertile 3	15.1 (13.5-18.0)	2480	3.2	1.0 (0.8-1.3)	0.799	2470	10.1	0.9 (0.7-1.0)	0.045
TSH									
Tertile 1	10.0 (4.0-11.8)	2500	2.4	0.9 (0.7-1.2)	0.510	2480	9.9	0.8 (0.7-0.9)	0.006
Tertile 2	12.6 (11.8-13.5)	2562	3.5	1.0 (0.8-1.2)	0.836	2562	11.1	0.9 (0.8-1.1)	0.284
Tertile 3	15.1 (13.5-18.0)	2461	3.2	0.9 (0.7-1.2)	0.436	2451	10.1	1.0 (0.9-1.2)	0.595

Reported ORs and 95% CIs are increase in odds per SD of log-transformed FT4 or TSH. Analyses were performed using logistic regression and adjusted for gestational age at blood sampling, maternal education, maternal ethnicity/country of birth, age, parity, smoking during pregnancy, pre-pregnancy body mass index, and child sex.

[a] A quadratic term (FT4 SD score^2) was added to the model, indicating a non-linear association.

Discussion

We did not identify an association between FT4 and TSH and ADHD in the overall study population. There was inconsistent evidence that the associations investigated were different depending on gestational age.

While both low and high-normal maternal FT4 concentrations have been associated with adverse outcomes related to fetal brain development [3–11], the role of thyroid hormone in the development of ADHD is unknown. Exposure to high thyroid hormone concentrations might contribute to the etiology of this disorder, since individuals with generalized resistance to thyroid hormone were shown to be more prone to meet the criteria for ADHD than subjects without this mutation [16]. Fetuses without the mutation but born to a mother with RTHβ have reduced sensitivity to thyroid hormone compared with fetuses born to mothers without RTHβ [56]. The reduced sensitivity to thyroid hormone was explained by increased deiodinase 3 (D3) expression in the anterior pituitary. Other studies have also shown that untreated hyperthyroidism during pregnancy may lead to persistent changes in the thyroid function of the child [57,58]. In addition, children born to mothers with RTHβ were found to have lower birth weight [59]. Lower birth weight and small size for gestational age, which has been already shown to be associated with a higher FT4 concentration in pregnancy in Generation R and INMA [60,61], could be on the causal pathway to ADHD symptomatology [62].

Thus far, the results of studies on the association between maternal thyroid function and child ADHD are inconsistent. While one study reported a positive association between FT4 and ADHD symptoms (26), other studies either report no association [23,24,28,30] or a negative association [27,29,31]. Likewise for TSH, studies show either a positive association between TSH and attention problems or ADHD symptoms [23–25], no association [26–29,31], or a possible protective effect of a higher TSH concentration [30]. There may be different explanations as to why some studies found an association between FT4 or TSH and ADHD, while others did not. First of all, although FT4 and TSH both reflect thyroid status, TSH is a more sensitive reflection of thyroid autoimmunity, which is associated with ADHD [54]. Second, the inconsistent study results may also be explained by several methodological points. ADHD symptoms were assessed differently between studies, with some earlier studies using tools that did not include an extensive set of items as the DSM-IV uses to classify ADHD. In addition, ADHD was defined differently across studies – by medical diagnosis [26,31], symptoms on a continuous scale [24,27,30], or by ADHD or inattention symptom cut-offs [23,25,28,29] – hence the comparability between studies is limited. The latter studies did not use a symptom score above the 90th percentile, as used in our study. Third, iodine status may also explain part of the heterogeneity between studies [63]. Results in a small study population showed that ADHD prevalence was higher in an iodine-deficient area than in an iodine-sufficient area [64].

The importance of maternal thyroid hormone for the fetal brain changes throughout pregnancy. Before the fetal thyroid is functionally mature at 18-20 weeks gestation, the fetus

depends on thyroid hormone from the mother [1]. During the second half of pregnancy, the maternal thyroid remains an important source, but to a lesser extent as considerable amounts of thyroid hormone are produced by the fetus itself [2]. We investigated whether the effect of maternal thyroid hormone on ADHD (symptoms) differed by gestational age. The proportion of mother-child pairs were not equally distributed across the different groups of gestational age; in roughly the first 12 weeks of gestation, two thirds of the mother-child pairs were from the ALSPAC cohort. Stratified analyses showed that the association of higher FT4 with a higher risk of symptom score above the 90th percentile was prominent in roughly the first 12 weeks of gestation, but not thereafter. Given these findings and the physiologically interrelation between FT4 and TSH, we could also observe that a higher TSH was associated with a lower risk of symptom scores above the 90th percentile only in the first trimester. However, there was no significant interaction with gestational age in the association of FT4 or TSH with ADHD. From our analyses, we therefore have no strong evidence for differential effects by gestational age.

Interestingly, in the current study, a higher maternal FT4 concentration during pregnancy was associated with a higher risk of ADHD in girls only. Differential effects of thyroid hormone on brain-development-related outcomes by sex have been reported previously [23,31], and it is well known that ADHD is more often diagnosed in males. While it remains unclear why this association may be sex-specific, it is known that child sex modifies the thyroidal response to human chorionic gonadotropin (hCG); women pregnant with a female fetus have a higher thyroidal response to high hCG concentrations, resulting in lower TSH and higher FT4, than those with a male fetus [65]. However, our results have to be interpreted with caution as no significant interaction with sex was found. Further studies are needed to replicate if there are sex-specific effects of thyroid hormone on brain development.

Observational studies showed that both low and high maternal thyroid function are associated with lower child IQ and lower gray matter volume [3,5,25]. Randomized trials thus far failed to show a benefit of levothyroxine treatment in pregnant women with subclinical hypothyroidism or hypothyroxinemia on child IQ [32,33], but these trials have been limited by a suboptimal timing and dose of treatment [66]. Behavioral problems such as ADHD could also be considered an outcome that may reflect suboptimal fetal brain development during pregnancy. Our study shows no association between maternal thyroid function and child ADHD. However, owing to the observational design of this study, we cannot make statements on what the potential effects of this treatment on behavior problems in children would be.

We were able to combine data from three large cohorts, enabling us to investigate associations between thyroid function and ADHD in a large number of mother-child pairs. All three cohorts used the DSM, internationally applied, diagnostic criteria, which are the most used clinically for diagnosing psychiatric disorders. Using individual-participant data instead of aggregate data facilitated the use of consistent inclusion and exclusion criteria and standardized statistical analyses across cohort studies. Combining individual participant

data into a meta-analysis offers further advantages over individual studies. Irrespective of a replication of findings as found in individual studies, findings from meta-analyses enriches our knowledge on the theory tested and increases confidence in generalization.

In our study, ADHD occurred in only 3% of children, which may have been a low proportion in which to detect an association. This prevalence was lower than that reported in children and adolescents in a systematic review [13]. The prevalence of ADHD in Generation R was lower than previously reported due to a different sample size, and we did not report a weighted prevalence to represent the full sample with CBCL1½–5 data [43]. Prevalence estimates are known to vary widely by individual studies. Part of this variability may be explained by the diagnostic criteria used to define ADHD, the method used to assess ADHD symptoms, the incorporation of functional impairments as part of the definition of ADHD, and possibly also by social economic status of the population [13]. In our study, mother-child pairs with a lower social economic status (e.g., lower maternal educational level and less often native or Caucasian) were more likely to be lost to follow-up. We accounted for potential differential loss to follow-up by applying inverse probability weighting. To increase statistical power, we used the highest 10% of ADHD symptoms as a secondary outcome. It should be noted that this extra cut-off was arbitrarily chosen and has not been validated.

A limitation of this study is that ADHD symptoms were assessed at different ages using different methods, including inconsistency in the type of assessor used by the different cohorts, which may have contributed to heterogeneity in results. The heterogeneity by the latter was shown by a recent study showing that maternal thyroid function was associated with teacher-rated ADHD symptoms, but not with parental ratings [29]. Due to low-to-moderate agreement between teacher and parental observations, multi-informant assessment has been recommended in order to understand the behavior of a child in different settings [67,68]; this was most closely adhered to in ALSPAC. We did not have the opportunity to re-score the data that was collected of a sample of children using the three methods. In addition, we had no data on clinical diagnosis of ADHD by a physician, which may have caused outcome misclassification.

In conclusion, the results of the current study do not show an association between maternal thyroid function and ADHD in the overall study population. As the etiology of ADHD as well as the potential role of thyroid hormone availability remains poorly understood, further studies are warranted.

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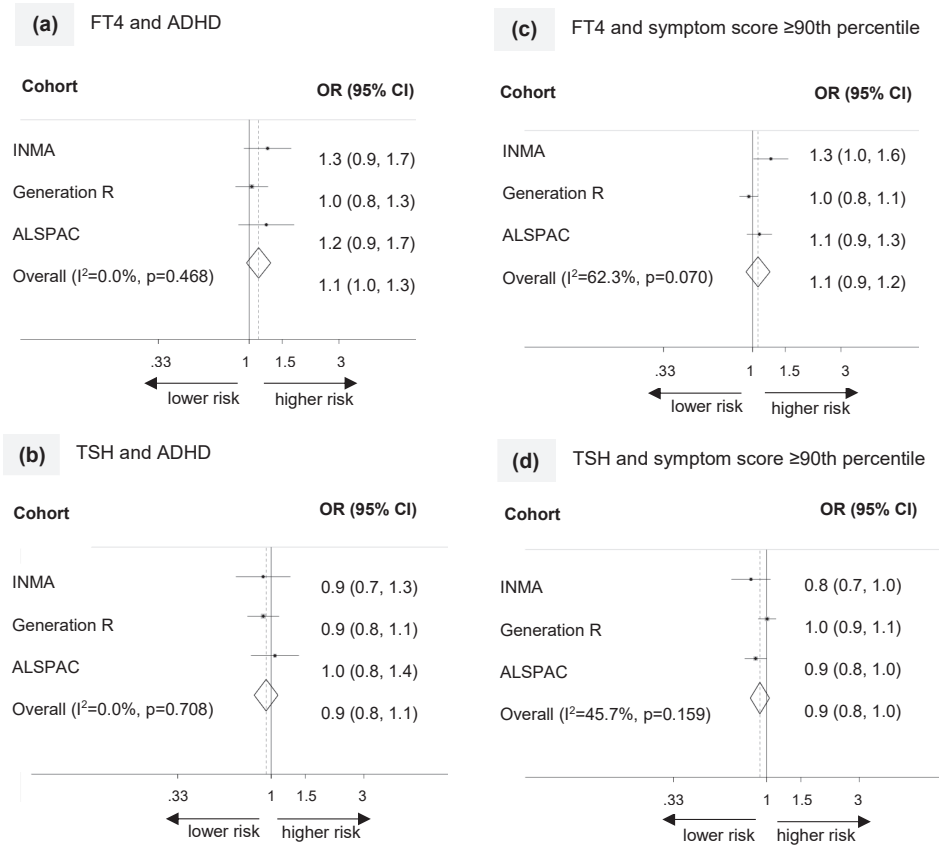
Supplemental material

Serum measurements

In INfancia y Medio Ambiente (INMA), the between-assay coefficients of variation for low, medium, and high hormone concentrations were 6.1%, 4.1%, and 4.0% for free thyroxine (FT4) respectively, and 3.0%, 3.1%, and 2.6% for thyrotropin (TSH). The intra-assay coefficients of variation were 3.7%. 3.0%, and 3.3% for FT4, and 7.7%, 2.1%, and 1.7% for TSH, respectively (for AutoDEL-FIA, PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland). In Generation R, the intra- and interassay coefficients of variation were <5.4% for FT4 with a range of 14.3-25.0 pmol/L and <4.1% for TSH with a range of 3.97-22.7 mIU/L (for Vitros ECI immunodiagnostic; Ortho Clinical Diagnostics, Rochester, NY). In ALSPAC, FT4, TSH, and thyroid peroxidase antibody measurements were performed using Abbott Architect i2000 with functional sensitivity of 0.05 mIU/L or less. Inter- and intra-assay coefficients of variation were <5% for all analytes.



Supplementary Fig. 1 Association of maternal FT4 and TSH concentrations during pregnancy with child ADHD and with an ADHD symptom score ≥90th percentile.

Associations shown as effect estimate (dot) with the 95% CI per cohort and overall as estimated by a random-effects meta-analysis (diamond). Statistical heterogeneity was explored and quantified using the Cochran Q test and the I^2 statistic, which is the proportion of total variation in the estimates that is due to heterogeneity between studies. The models were adjusted for maternal age, parity, pre-pregnancy body mass index, smoking during pregnancy, ethnicity/country of birth, maternal educational level, gestational age at blood sampling, child sex, and subcohort in INMA. (a) FT4 and ADHD, (b) TSH and ADHD, (c) FT4 and symptom score ≥90th percentile, (d) TSH and symptom score ≥90th percentile. ADHD, attention-deficit hyperactivity disorder; ALSPAC, Avon Longitudinal Study of Parents and Children; CI, confidence interval; FT4, free thyroxine; INMA, Infancia y Medio Ambiente; OR, odds ratio; TSH, thyrotropin.

Supplementary Table 1 Distribution and comparison of maternal and child characteristics in the included and excluded population.

	INMA			Generation R			ALSPAC		
	Included (n=1073)	**Excluded (n=913)**	***p*-value**	**Included (n=3812)**	**Excluded (n=3206)**	***p*-value**	**Included (n=2784)**	**Excluded (n=2093)**	***p*-value**
Maternal TSH, median (IQR), mIU/L	1.25 (0.82-1.82)	1.23 (0.79-1.87)	0.886	1.38 (0.87-2.07)	1.27 (0.78-1.97)	<0.001	1.02 (0.64-1.49)	0.97 (0.62-1.43)	0.013
Maternal FT4, median (IQR), pmol/L	10.6 (9.7-11.5)	10.5 (9.5-11.6)	0.170	14.8 (13.2-16.7)	14.6 (12.9-16.7)	0.072	16.1 (14.8-17.6)	16.4 (14.9-17.9)	0.003
TPOAb positivity, %	NA	NA		5.8	6.4	0.360	12.8	12.1	0.415
Gestational age at blood sampling, mean (SD), weeks	13.1 (1.3)	13.2 (1.3)	0.165	13.4 (1.9)	13.7 (2.1)	<0.001	11.0 (3.2)	11.0 (3.2)	0.997
Maternal educational level, %									
Low	20.8	30.1		6.2	15.3		24.0	38.5	
Medium	42.5	39.6	<0.001	40.8	51.8	<0.001	61.6	53.1	<0.001
High	36.6	30.3		53.0	32.9		14.4	8.4	
Maternal ethnicity/country of birth, %									
Spanish	93.8	86.7		NA	NA		NA	NA	
Other, non-Spanish	6.2	13.3		NA	NA		NA	NA	
Dutch	NA	NA		60.6	40.1		NA	NA	
Indonesian	NA	NA		3.3	2.6		NA	NA	
Cape Verdean	NA	NA	<0.001	3.0	5.8	<0.001	NA	NA	<0.001
Turkish	NA	NA		6.9	10.5		NA	NA	
Other, non-Western	NA	NA		17.0	32.3		NA	NA	
Other, Western	NA	NA		9.2	8.7		NA	NA	
White	NA	NA		NA	NA		98.6	96.9	
Non-white	NA	NA		NA	NA		1.4	3.1	
Maternal age, mean (SD), years	31.6 (3.9)	30.9 (4.6)	<0.001	30.7 (4.6)	28.6 (5.4)	<0.001	28.1 (4.6)	26.4 (5.0)	<0.001

Parity, %									
0	57.0	52.9		59.6	52.8		47.9	43.5	
1	36.7	38.9	0.106	29.9	29.5	<0.001	34.7	33.6	<0.001
≥2	6.3	8.2		10.5	17.7		17.4	22.9	
Maternal smoking, %									
Never smoked	70.2	66.1		74.9	68.0		79.6	68.3	
Smoked at the beginning of pregnancy	12.9	15.1	0.144	9.6	9.0	<0.001	4.5	6.6	<0.001
Continued smoking	16.9	18.8		15.5	23.0		15.9	25.1	
Pre-pregnancy BMI, median (IQR), kg/m^2	22.5 (20.8-25.0)	22.5 (20.7-25.2)	0.738	22.5 (20.8-25.1)	22.7 (20.7-25.7)	0.119	22.1 (20.5-24.2)	22.2 (20.5-24.6)	0.145
Child female sex, %	48.9	48.6	0.898	50.1	48.6	0.226	48.5	47.6	0.520

p- value for differences calculated using Chi-square test for categorical variables, Student´s t-test for continuous normal-distributed variables, and Wilcoxon rank-sum test for continuous non-normal distributed variables. Numbers are based on unimputed data; percentages add up to 100% without taking into account missing values. ALSPAC, Avon Longitudinal Study of Parents and Children; BMI, body mass index; FT4, free thyroxine; INMA, INfancia y Medio Ambiente; IQR, interquartile range; NA: not available; SD, standard deviation; TPOAb, thyroid peroxidase antibody; TSH, thyrotropin.

Supplementary Table 2 Distribution and comparison of maternal and child characteristics among the mother-child pairs with available data on thyroid function during pregnancy and child ADHD symptoms and those mothers whose children did not underwent an ADHD assessment.

	INMA			Generation R			ALSPAC		
	ADHD data (n=1130)	no ADHD data (n=817)	*p*-value	ADHD data (n=3935)	no ADHD data (n=1954)	*p*-value	ADHD data (n=2960)	no ADHD data (n=1898)	*p*-value
Maternal TSH, median (IQR), mIU/L	1.26 (0.83-1.87)	1.21 (0.79-1.83)	0.354	1.38 (0.86-2.08)	1.27 (0.80-1.95)	<0.001	1.01 (0.63-1.49)	0.97 (0.63-1.42)	0.054
Maternal FT4, median (IQR), pmol/L	10.6 (9.7-11.6)	10.5 (9.5-11.5)	0.025	14.9 (13.2-16.7)	14.6 (12.9-16.6)	0.007	16.2 (14.8-17.7)	16.3 (14.9-17.9)	0.053
TPOAb positivity, %	NA	NA	NA	6.3	5.7	0.401	13.1	11.6	0.115
Gestational age at blood sampling, mean (SD), weeks	13.1 (1.3)	13.2 (1.3)	0.202	13.7 (2.1)	13.4 (1.9)	<0.001	11.0 (3.2)	10.9 (3.2)	0.502
Maternal educational level, %									
Low	20.9	31.3		6.3	18.0		23.8	40.6	
Medium	42.2	39.4	<0.001	40.7	55.3	<0.001	61.6	52.1	<0.001
High	36.9	29.3		53.0	26.7		14.7	7.2	
Maternal ethnicity/country of birth, %									
Spanish	94.1	85.8		NA	NA		NA	NA	
Other, non-Spanish	5.9	14.3		NA	NA		NA	NA	
Dutch	NA	NA		61.0	33.3		NA	NA	
Indonesian	NA	NA		3.3	2.4		NA	NA	
Cape Verdean	NA	NA	<0.001	3.0	6.5	<0.001	NA	NA	<0.001
Turkish	NA	NA		6.9	11.2		NA	NA	
Other, non-Western	NA	NA		16.9	37.8		NA	NA	
Other, Western	NA	NA		9.1	8.8		NA	NA	
White	NA	NA		NA	NA		98.5	96.7	
Non-white	NA	NA		NA	NA		1.5	3.3	
Maternal age, mean (SD), years	31.6 (4.0)	30.8 (4.6)	<0.001	30.7 (4.6)	27.8 (5.3)	<0.001	28.2 (4.5)	26.0 (4.9)	<0.001

Parity, %									
0	56.7	52.8		59.4	51.2		48.4	42.2	
1	37.0	38.7	0.090	30.1	29.0	<0.001	34.5	33.8	<0.001
≥2	6.4	8.6		10.5	19.5		17.1	24.0	
Maternal smoking, %									
Never smoked	70.6	64.9		74.9	65.6	<0.001	79.7	66.8	
Smoked at the beginning of pregnancy	12.6	15.8	0.029	9.6	8.9		4.5	6.8	<0.001
Continued smoking	16.9	19.3		15.5	25.5		15.8	26.4	
Pre-pregnancy BMI, median (IQR), kg/m2	22.6 (20.8-25.1)	22.4 (20.7-25.2)	0.401	22.5 (20.8-25.1)	22.7 (20.6-25.9)	0.195	22.1 (20.5-24.2)	22.2 (20.5-24.6)	0.155
Child female sex, %	48.7	48.4	0.895	50.0	47.9	0.136	48.2	47.9	0.797

The distribution of characteristics is compared between the group "enrolled ≤18 week and data on maternal thyroid function N=12694" and the group "maternal thyroid function and child ADHD data collected N=8025", as shown in Figure 1. *p*-value for differences calculated using Chi-square test for categorical variables, Student´s t-test for continuous normal-distributed variables, and Wilcoxon rank-sum test for continuous non-normal distributed variables. Numbers are based on unimputed data; percentages add up to 100% without taking into account missing values. ALSPAC, Avon Longitudinal Study of Parents and Children; ADHD, attention-deficit hyperactivity disorder; BMI, body mass index; FT4, free thyroxine; INMA, INfancia y Medio Ambiente; IQR, interquartile range; NA: not available; SD, standard deviation; TPOAb, thyroid peroxidase antibody; TSH, thyrotropin.

Supplementary Table 3 Association of maternal FT4 during early pregnancy with child ADHD symptom scores on a continuous scale.

	n	IRR (95% CI)	*p*-value
INMA	1063	1.05 (0.98 to 1.14)	0.187
Generation R	3657	0.98 (0.95 to 1.01)	0.204
ALSPAC	2710	0.99 (0.92 to 1.06)	0.692

Reported IRR and 95% CIs represent the change in ADHD symptoms per SD of log-transformed FT4 in terms of a percentage increase or decrease, with the precise percentage determined by the amount the IRR is either above or below 1. Analyses were performed using negative binomial regression models and adjusted for gestational age at blood sampling, maternal education, maternal ethnicity/country of birth, age, parity, smoking during pregnancy, pre-pregnancy body mass index, child sex, child age, and sub-cohort in INMA. CI, confidence interval; IRR, incidence rate ratio.

Supplementary Table 4 Continuous association of maternal TSH during early pregnancy with child ADHD symptom scores on a continuous scale.

	n	IRR (95% CI)	*p*-value
INMA	1055	0.94 (0.87 to 1.02)	0.144
Generation R	3630	1.00 (0.97 to 1.03)	0.928
ALSPAC	2687	0.97 (0.91 to 1.03)	0.282

Reported IRR and 95% CIs represent the change in ADHD symptoms per SD of log-transformed TSH in terms of a percentage increase or decrease, with the precise percentage determined by the amount the IRR is either above or below 1. Analyses were performed using negative binomial regression models and adjusted for gestational age at blood sampling, maternal education, maternal ethnicity/country of birth, age, parity, smoking during pregnancy, pre-pregnancy body mass index, child sex, child age, and sub-cohort in INMA. CI, confidence interval; IRR, incidence rate ratio.

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Chapter 6

General discussion

Severe maternal iodine deficiency during pregnancy can lead to dwarfism and intellectual disability (i.e., cretinism). Despite increasing efforts to eliminate iodine deficiency and prevent thyroid-related diseases world-wide, mild-to-moderate iodine deficiency is still common, especially in pregnant women. The potential effects of mild-to-moderate iodine deficiency during pregnancy for thyroid function and child neurodevelopment are less well known. For this reason, the aims of this thesis were to explore the determinants of iodine status, to examine the association of maternal iodine status with thyroid function during pregnancy, and to investigate if maternal iodine status and thyroid function during pregnancy are associated with child neurodevelopment outcomes. In this chapter the main results will be discussed in light of methodological considerations, together with the clinical implications and suggestions for further research.

Main findings and interpretation

Determinants of iodine status

In chapter 2, we demonstrated that younger women, with a higher pre-pregnancy BMI, and with low intake of milk and dairy products are more likely to have a low urinary iodine-to-creatinine ratio (UI/Creat) during pregnancy. We also identified cohort-specific determinants of maternal UI/Creat, such as intake of fish and shellfish in INMA and ALSPAC, and intake of eggs and cereal products in Generation R. The cohort-specific associations may be a reflection of differences between populations in the consumption of dietary iodine sources (i.e., the average fish consumption is higher in INMA and ALSPAC than in Generation R), the availability of iodized salt (i.e., the estimated penetration rate of iodized salt in household is 60-70% in the Netherlands, 16% in Spain, and 2% in the UK [1]; eggs are assumed to be eaten with salt), and the implemented iodine fortification program in the specific countries (i.e., bread is fortified with iodized salt in the Netherlands, and therefore a great source of iodine in this population [2]). These cohort-specific associations of maternal characteristics and dietary habits with iodine status suggest that public health interventions targeted to achieve iodine sufficiency in pregnancy probably need to be country-specific.

Iodine status and maternal thyroid function

In Chapter 3, we showed that in a population of 2009 pregnant women with a median UI/Creat of 85 μg/g (indicating mild-to-moderate iodine deficiency during pregnancy), the clinical reference ranges of thyroid function tests were not meaningfully different among subgroups of women differing in iodine status. We therefore question the current recommendation to calculate reference ranges for thyroid function during pregnancy solely in iodine sufficient populations. Furthermore, we examined the cross-sectional associations of maternal UI/Creat with TSH, FT4, FT3, TT4, TT3, TPOAb and TgAb positivity. Lower

iodine availability was associated with a slightly higher TT4 and a lower TSH, while women with adequate iodine intake (i.e., UI/Creat of 150-249 μg/g) had the lowest risk of TPOAb positivity. The latter results are in line with the current recommendations that define an iodine concentration between 150-249 μg/L as optimal during pregnancy [3]. The lack of an association of UI/Creat with the FT4/FT3 and TT4/TT3 ratios indicated that there was no increase in T3 over T4 secretion or higher peripheral deiodination of T4, both well-known adaptive mechanisms to chronic low iodine intake. The severity of iodine deficiency in this population may have been too mild to deplete the intra-thyroidal iodine storage and thus caused no shift in these ratios. Unfortunately, we could not study if the mild changes in thyroid function that were found in our study (i.e., higher TT4 and TPOAb positivity), were also associated with neurodevelopmental outcomes in the offspring from the SELMA cohort. Hence, we can only speculate about the clinical relevance of the observed associations. The association of TT4 during pregnancy with child neurodevelopmental outcomes has not yet been studied in great detail, though research in the iodine sufficient Generation R cohort showed that TT4 was not associated with child IQ after correction for FT4 [4]. TPOAb positivity during pregnancy has been associated with pregnancy outcomes, such as preterm birth [5], and with a higher risk of ADHD [6], and a lower IQ score in the offspring [7]. The latter was however only observed in the iodine sufficient population of Generation R but not in the mild-to-moderate iodine deficient population of ALSPAC.

Maternal iodine status and child neurodevelopment

In chapter 4.1, a pooled analysis of individual-participant data in 6180 mother-child pairs showed a positive curvilinear association of UI/Creat with mean verbal IQ. The results further suggested that particularly the first 14 weeks of gestation constitute a vulnerable period for fetal exposure to maternal iodine deficiency. This supports the notion that it is important for pregnant women to have adequate iodine stores in early or even before pregnancy. We were unable to show that the association of maternal iodine status with child verbal IQ was mediated through maternal thyroid function; UI/Creat was not associated with TSH or FT4. The absence of such an association might be explained through possible flaws of UI/Creat as a proxy for individual iodine status or thyroidal iodine availability, or suggest that the association is mediated by fetal thyroid function. Iodine “deficiency” (i.e., UI/Creat below 150 μg/g) and iodine “excess” (i.e., UI/Creat above 150 μg/g) were not associated with lower verbal or non-verbal IQ. In chapter 4.2, we describe that there is no evidence for an association between iodine deficiency during pregnancy and the child’s ADHD or autistic traits. The associations of maternal FT4 or TSH with ADHD or autistic traits did not depend on maternal iodine status.

Maternal thyroid function and child neurodevelopment

In chapter 5.1, our meta-analysis of individual-participant data in 9036 mother-child pairs showed that low maternal FT4 in early pregnancy was associated with lower non-verbal and verbal child IQ scores. The association of low FT4 with the non-verbal IQ score was robust in all three cohorts, showing a 3.9-point lower non-verbal IQ score in children born to women with FT4 <2.5th percentile as compared with those born to women with FT4 between the 25th to 75th percentile range. We could not replicate the association of high FT4 with lower non-verbal IQ that had previously been demonstrated in the Generation R cohort [8]. Firm conclusions about the association of maternal thyroid function with autistic traits could not be made due to the small number of children scoring within the clinical range and the inconsistency of the results when using different cut-off values in the extreme ends of the FT4 distribution. In chapter 5.2, we show that in 7669 mother-child pairs maternal TSH or FT4 was not associated with child ADHD or with high ADHD symptom scores. Further investigation of effect modification by gestational age showed no consistent differential effect across the two outcomes.

No association was observed between TSH and IQ, ADHD, or autistic traits in any of the studies published in chapter 5. We therefore question whether maternal FT4 is a better marker for fetal thyroid state than maternal TSH. However, recent analysis in the Generation R cohort showed that maternal TSH was associated with brain imaging by MRI, which is an objective reproducible measure of brain development [9].

While non-verbal IQ is a language- and culture-free measure of cognitive ability which is less dependent on the learning stimulus received by the child during the first years of life, it is somewhat surprising that maternal iodine status was not associated with child non-verbal IQ, considering that low FT4 was associated with lower child non-verbal IQ. Our results show an association of lower maternal UI/Creat with lower child verbal IQ instead, suggesting that maternal iodine status potentially affects child neurodevelopment in a specific manner. Why this would be the case, requires further investigation regarding the underlying mechanisms. We speculate that the association between maternal iodine status and child verbal IQ may be explained through effects of mild iodine deficiency, via thyroid hormone, on the auditory system [10,11], but this requires further study.

Methodological considerations

Exposure assessment

As a proxy measurement for thyroidal iodine availability, we have used one to four measurements of urinary iodine concentration in pregnant women, which we divided by the creatinine concentration to take urine dilution into account. The urinary iodine concentration in a single spot urine sample is known to fluctuate based on diet, and reaches a peak concentration ap-

proximately 4-5 hours after meals [12]. In large populations, this day-to-day variation in iodine intake, and thus the random measurement error, is balanced out in a median value of spot urinary iodine concentration [13]. The precision by which the iodine status of a population can be determined, increases with the number of individuals included. For instance, a study showed that the mean iodine excretion in a population can be estimated with a 5% or 2% precision range if from 489 or 3054 individuals a spot urine sample is collected, respectively [14]. Because the number of individuals included in the birth cohort studies in this thesis is large, it seems safe to say that we have estimated the iodine status of these populations with high precision. Another important factor in determining individual iodine status, is timing of urine collection. For example, early morning urine void tends to underestimate daily iodine excretion [15]. In SELMA, early morning urine void was collected in all women, and in INMA (i.e., INMA Valencia) fasting urine samples were collected [16], but it is unsure whether these were overnight fasting samples. In Generation R and ALSPAC, urine was obtained at random moments during the day. It remains unsure whether potential timing of sampling significantly affected the population status, and whether this error was random or more common in subgroups of women that gave birth to children with lower IQ scores or neurodevelopmental problems.

In general, random measurement error in the exposure flattens the slope of the regression line, resulting in a regression coefficient biased towards the null [17]. To minimize random measurement error in the exposure variable, one could obtain repeated measurements or use a biomarker, if available, that better reflects the exposure. For individual iodine status, more research should be performed into thyroglobulin, which is a protein from which thyroid hormones are formed [18], as a biomarker for individual iodine status. In children, reference ranges of blood spot thyroglobulin have been made available in iodine-sufficient children, and a median blood spot thyroglobulin concentration above 40 Ig/L has been suggested to reflect adequate iodine status [19]. In pregnant women, thyroglobulin may be a sensitive biomarker of individual iodine status [20], but references ranges have yet to be defined. Alternatively, individual iodine status can be determined by repeated measurements. Research suggests that if 10 or 55 urine spot samples were obtained from each individual over a period of time, we could calculate the mean iodine excretion for this individual with a 25% or 10% precision range (e.g., if the mean iodine excretion was 200 µg/L, then the true iodine excretion with a 25% precision range would be between 150-250 µg/L) [14]. Determining individual iodine status by a limited number of repeated measurements is unlikely to be very precise (i.e., 1 to 4 samples were obtained from mothers from the cohorts included in this thesis; the precision range would be approximately 40 to 50%).

Next, as a proxy of the fetal exposure to maternal thyroid hormone we used maternal serum TSH or FT4 concentrations measured before mid-gestation. FT4 was measured using immunoassays and may potentially have under- or overestimated the true concentration due to immunoassay interference [21–23]. Owing to the use of different assays and reference ranges

between the cohorts, the absolute value of the thyroid function tests could not be compared across cohorts and standardization of both the TSH and FT4 concentration was necessary.

Outcome assessment

The assessment of the outcome is likely subject to measurement error. We have used IQ scores and child behavioral problems as outcomes related to fetal brain development. These outcomes were assessed with validated tests or questionnaires. While each child was administered an IQ test, their behavior, i.e., ADHD symptoms and autistic traits, was assessed through questionnaires filled in by parents and/or teachers. The assessment of child behavior may be a more subjective dimension of health than IQ, because every informant may have different perspectives on behavior. This may be influenced by the behavior required in a particular setting. With regards to ADHD, the international DSM-IV criteria require that attention and/or hyperactivity symptoms cause some impairment in at least two settings, e.g., at home, work, or school [24]. Due to low-to-moderate agreement between teacher and parental observations, multi-informant assessment has been recommended in order to understand the behavior of a child more completely [25,26]. However, ADHD symptoms were assessed by a single informant in the cohorts included in this thesis, with exception of a proportion of children in ALSPAC. Potentially, random error in the assessment of ADHD may therefore have been higher in INMA and Generation R than in ALSPAC. In general, random error in the outcome measurements may lead to wider confidence ranges as a result of higher standard error, and as such, there is a higher chance to obtain statistically non-significant associations. By increasing the sample size, precision of the estimates can be retained. Again, bias can only be introduced if the degree of random measurement error is uneven between the cases [i.e., those with ADHD or autistic traits above the (validated) cut-off] and the controls [i.e., those without ADHD or autistic traits below the (validated) cut-off]. However, due to the natural course of cohort studies, it is unlikely that the misclassification of children depended on the exposure status during pregnancy, since the exposure status was unknown to the assessors.

The accuracy of a meta-analysis

With the growing number of individual studies, meta-analyses are useful to summarize the existing evidence from often small studies into an overall pooled estimate. This may enhance evidence-based policy decisions, a re-evaluation of clinical practice guidelines, or be an incentive for further research. The individual studies that are used for a meta-analysis will, to some extent, be heterogeneous. The pregnant populations of INMA, Generation R, and ALSPAC differed in maternal and child characteristics, maternal iodine status, the assays that were used to measure iodine status and thyroid function, and in the outcome assessment. Heterogeneity across studies can be minimized by using individual participant data, so that similar in- and exclusion criteria can be applied and the data and analyses can be harmonized. But even then, there will be some degree of heterogeneity. In this thesis, we

have performed random-effects meta-analyses instead of fixed-effects meta-analyses, because a random-effects meta-analysis does not assume that differences in effect estimates across cohorts is due to chance only. There are three types of heterogeneity: clinical (e.g., differences related to the population characteristics, interventions, and outcomes), methodological (e.g., differences in how the study was conducted and the risk of bias), and statistical. There are currently no criteria to check the degree of clinical and methodological heterogeneity [27]. In contrast, statistical heterogeneity can be assessed by the I^2 statistic, which indicates the percentage of variability across studies due to heterogeneity rather than chance[28]. If the degree of heterogeneity is unknown, a high percentage of I^2 (i.e., ≥75%) indicates that studies are highly heterogeneous and in the absence of strict criteria, it is up to the meta-analysist to decide whether the meta-analysis is meaningful or if it is better to present the cohort-specific effect estimates only[27]. While several meta-analyses included in this thesis showed moderate statistical heterogeneity (i.e., $I^2 \sim 50\%$), the only meta-analysis which showed a high I^2 of 77.7% was of the association of maternal UI/Creat with child autistic traits in those with at least one measure of UI/Creat in the first 14 weeks of pregnancy (chapter 4.2). This finding should therefore be interpreted with caution.

Clinical implications

Should we be concerned about mild-to-moderate iodine deficiency during pregnancy?

There has been increased international support for the elimination of iodine deficiency disorders since 1990, which led to the endorsement of universal salt iodization as the most cost-effective preventive measure in 1994. Although great improvement has been made in the last 2-3 decades, iodine deficiency is still common among pregnant women, even in countries with a mandatory or voluntary iodine fortification program [29–31]. According to the global iodine status in 2017, 39 out of 72 countries (54.2%) reported insufficient iodine intake in pregnant women (i.e., median UIC < 150 μg/L) [31,32]. Because this public health problem remains to exist, the EUthyroid consortium, and other signatories of the Krakow Declaration on Iodine, have called on governments, public health authorities, the food industry, scientists, and health professionals for what they think is needed towards the elimination of iodine deficiency disorders: obligatory universal salt iodization, harmonized monitoring and evaluation of fortification programs across countries, and public information campaigns [33].

The results of this thesis indicate that mild-to-moderate iodine deficiency during pregnancy is associated with a higher risk of TPOAb positivity, but not with meaningful alterations in other thyroid function tests. Lower iodine status in the first 14 weeks of gestation was associated with lower mean child verbal IQ scores. Our research therefore suggests that adequate iodine intake in early pregnancy is important for fetal brain development. One of the means

of achieving adequate iodine intake in pregnancy, is iodine supplementation. In the United Kingdom, a country without an iodine fortification program or supplementation recommendation, universal iodine supplementation before, during, and right after pregnancy has been estimated to hypothetically increase child IQ with 1.2 IQ points [34]. However, randomized controlled trials have thus far failed to show any benefit of daily iodine supplementation during pregnancy for child IQ. The most recent randomized, placebo-controlled trial conducted in Thailand and India, showed no statistical difference in child non-verbal or verbal IQ scores at a mean age of 5.4 years of daily supplementation with 200 µg iodine as potassium iodide of mildly iodine-deficient women [35]. It should be noted however that pregnant women from India included in this trial were not iodine deficient at baseline (i.e., median UIC of 188 µg/L) and that treatment only started at a mean gestational age of 10.7 weeks [36]. Two other randomized trials also showed no benefit of treatment, but these were underpowered [37,38]. Several non-randomized trials in mild-to-moderate iodine deficient regions showed mixed results for benefits of iodine supplementation for neurodevelopment [39–41]. Two of the studies suggest benefits of a maternal intake of 200 or 300 µg of iodine supplements for child neurodevelopment up to 2 years of age [39,40]. Another study, without placebo group, found no difference in child neurodevelopmental outcomes if the mother used iodized salt or took iodine containing supplements of either 150 µg or 230 µg per day [41].

If a future randomized placebo controlled trial were to be designed, then our results indicate that supplementation should not start later than the first trimester. Iodine supplementation may even need to be started before conception, as there is evidence that a late start of iodine supplementation (i.e., between week 13-20 of gestation) is associated with a lower FT4 concentration than when supplementation is initiated preconceptionally [42], and pre-pregnancy iodine status has been associated with child IQ [43]. A future randomized placebo controlled trial should be performed in a country which has not yet introduced iodine supplementation recommendations.

Should mild maternal thyroid dysfunction be treated?

Our results clearly indicate that mild maternal thyroid dysfunction, particularly low FT4, is associated with lower IQ scores in the offspring. From an individual point of view, lower IQ may lead to poorer academic achievements. From a public health perspective, IQ loss in a population may have economic implications. A loss of 5 IQ points has been estimated to cost $30 billion per year in Canada and $275 billion to $326 billion per year in the United States [44]. In clinical practice, TSH, but not FT4, is used as the first line parameter to screen maternal thyroid status at the first prenatal consultation. Even though our results suggest that screening for FT4 may need to be considered, evidence is first needed from randomized controlled trials on the benefits of screening and subsequent levothyroxine treatment of women with mild thyroid dysfunction during pregnancy for child neurodevelopment.

Randomized controlled trials have yet to show benefit of treatment. The CATS trial was the first to investigate the treatment effect of levothyroxine (LT4) administration in pregnant women with subclinical hypothyroidism or hypothyroxinemia on child neurodevelopment [45]. Women with these mild types of thyroid dysfunction were randomized to either standard care (control group) or treatment with 150 µg of levothyroxine per day, which was initiated at a median gestational age of 13 weeks and 3 days. The offspring of these women underwent neuropsychological testing when they were 3 and 9.5 years of age [45,46]. This trial found no statistical significant difference in IQ score between the control and treatment group at both time points in childhood. One of the explanations for the absence of effect may be that the dose of levothyroxine was not optimal. During the course of pregnancy, the dose had to be lowered or increased in 10% and 5% of women due to signs of side-effects, respectively. In two other randomized controlled trials, women with subclinical hypothyroidism were randomized to placebo or instructed to take 100 µg of levothyroxine daily, while women with hypothyroxinemia were randomized to placebo or treatment with a dose of 50 µg of levothyroxine per day [47]. The primary outcome was the child IQ score at the age of 3 or 5 years. Though a 3-point difference in IQ was observed in both trials between the treatment and the placebo group, this difference did not reach statistical significance. The absence of a statistical difference may have to do with the fact that these trials were only powered to detect a difference of 5 IQ points; an expected effect size which was based on the 7 IQ point difference between children born to treated or untreated women with overt hypothyroidism [48]. Another concern was that treatment was started relatively late - week 16 and 4 days of gestation for subclinical hypothyroidism and week 18 for hypothyroxinemia. This may have been too late to result in a beneficial effect because adequate maternal thyroid hormone transfer to the fetus is especially important for fetal brain development during the period when the fetal thyroid is not functionally mature yet (i.e., before mid-gestation).

How could observational studies help to design future randomized clinical trials?

Observational studies can inform on what is a realistic IQ difference between the treated and untreated group. A realistic expected effect size is essential for adequate power calculations. The study in chapter 5.1 showed that hypothyroxinemia during pregnancy is associated with a 2 to 4 point IQ difference, depending on the cut-off values used and the type of IQ score. As a consequence, a marked difference of 5 IQ points or higher may not be a realistic aim for studies that investigate the effect of treatment of mild gestational thyroid dysfunction. In addition, observational studies can help to identify vulnerable periods for exposure to inadequate thyroid hormone concentrations. This can provide more insight into the optimal timing of treatment. The most vulnerable periods could be investigated by testing for differential effects by gestational age. If thyroid function tests are performed repeatedly during pregnancy, observational studies can also provide a better understanding of how the duration of thyroid

dysfunction is associated with offspring neurodevelopmental outcomes. Unfortunately, most cohorts only have a single measurement available. To our knowledge, a study with repeated thyroid function measurements in relation to child neurocognitive outcomes has only been performed once and showed that low a FT4 concentration at week 12, but which increased during pregnancy, was associated with better mental and motor function scores at the age of 2 years than when FT4 remained low at week 24 and week 32 of gestation [49].

Future research

The urinary iodine concentration is commonly used as a marker for iodine status of populations. However, due to high day-to-day variability of urinary iodine concentrations [14,50,51], a reliable marker of individual iodine status is currently missing. Future studies should investigate whether thyroglobulin (and possible other new potential biomarkers) could be used during pregnancy as a marker for individual iodine status. Studies need to examine the dose-dependent effects of iodine or levothyroxine supplementation on thyroid function to investigate the optimal dose for treatment and examine if treatment during pregnancy, well before the fetal thyroid is fully functional (i.e., at mid-gestation), would be beneficial for child neurodevelopment.

Conclusion

The results of this thesis suggest:

- that public-health interventions focusing on improving the dietary iodine intake of pregnant women need to follow a country-specific approach, taking into account differences in dietary habits.
- that mild-to-moderate deficiency are not associated with meaningful variation in clinical reference ranges of thyroid function tests.
- that mild-to-moderate iodine deficiency may not be severe enough to evoke adaptive mechanisms to chronic low iodine intake (e.g., preferential production of T3).
- that maternal iodine status in particularly the first 14 weeks of gestation constitute a vulnerable period for fetal exposure to maternal iodine deficiency.
- that there is consistent evidence for a role of maternal thyroid hormone in normal brain development.

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Chapter 7

Summary/samenvatting

Summary

Chapter 1 provides a brief background about the importance of thyroid hormone for fetal brain development and the role of severe iodine deficiency therein. The regulation of thyroid hormone production through the hypothalamic-pituitary-thyroid axis is explained as well as the scientific evidence underlying the guidelines for the management of thyroid dysfunction during pregnancy. Relevant knowledge gaps concerning the potential effects of mild-to-moderate iodine deficiency for maternal thyroid function during pregnancy and fetal brain development are mentioned. Lastly, the aims of the thesis, the setting, and the outline of the thesis are described.

In **chapter 2**, we explore what are the determinants of maternal iodine status during pregnancy and whether these are similar across mild-to-moderate iodine deficient and iodine sufficient pregnant populations. We show that younger women, with a higher pre-pregnancy BMI, and with low intake of milk and dairy products were more likely to have low urinary iodine excretion during pregnancy. Cohort-specific determinants were also identified, such as the intake of fish and shellfish in INMA and ALSPAC (mild-to-moderate iodine deficient populations), and the intake of eggs and cereal products in Generation R (iodine sufficient population). These cohort-specific associations of maternal characteristics and dietary habits with urinary iodine excretion suggest that public health interventions targeted at achieving iodine sufficiency in pregnancy may need to be country-specific.

In **chapter 3**, we investigate the association between maternal iodine status and maternal thyroid function in a mild-to-moderate iodine deficient population and determine whether there is variation in thyroid function reference ranges according to iodine status. We show that lower iodine availability is associated with a slightly higher total thyroxine (TT4) and a lower thyroid stimulating hormone (TSH) concentration, while women with adequate iodine intake have the lowest risk of thyroid peroxidase antibody positivity. The lack of an association of iodine status with the free T4/free triiodothyronine (FT4/FT3) and TT4/TT3 ratios indicate no evidence for an increase in T3 secretion or higher peripheral de-iodination of T4, which are adaptive mechanisms to chronic low iodine intake. The clinical reference ranges of thyroid function tests are found not to be meaningfully different in subgroups of women differing in iodine status.

In **chapter 4**, we study the association between maternal iodine status and child neurodevelopmental outcomes. Chapter 4.1 shows that in a pooled analysis of individual-participant data, lower iodine availability is associated with a lower mean child verbal IQ score. Also differential effects by gestational age are detected, suggesting that the first 14 weeks of gestation potentially constitute a vulnerable period for fetal exposure to maternal iodine deficiency. In chapter 4.2, we describe that we find no evidence for an association between iodine deficiency during pregnancy and a greater risk of offspring ADHD or autistic traits. The associations of

the maternal FT4 or TSH concentrations with ADHD or autistic traits did not depend on maternal iodine status.

In **chapter 5**, we examine the association of maternal thyroid function with child neurodevelopmental outcomes. In chapter 5.1, our meta-analysis of individual-participant data shows that a low maternal FT4 concentration in early pregnancy is associated with lower mean child verbal and non-verbal IQ scores; the latter association being robust in mild-to-moderate iodine deficient and iodine sufficient populations. Firm conclusions about the association of maternal thyroid function with autistic traits could not made. In chapter 5.2, we show that the maternal TSH or FT4 concentrations during pregnancy are not associated with child ADHD.

Chapter 6 provides a general discussion of the main results and their interpretation, methodological considerations, clinical implications, and suggestions for further research.

Samenvatting

Hoofdstuk 1 geeft een korte achtergrond over het belang van schildklierhormoon voor de ontwikkeling van de foetale hersenen en de rol die ernstige jodium deficiëntie daarin speelt. De regulatie van de schildklierhormoon balans door de hypothalamus-hypofyse-schildklier-as wordt uitgelegd, evenals het wetenschappelijk bewijs dat ten grondslag ligt aan de richtlijnen voor de behandeling van schildklierafwijkingen tijdens de zwangerschap. Relevante ontbrekende kennis wordt genoemd met betrekking tot de potentiële effecten van milde tot matige jodium deficiëntie voor de schildklierfunctie van de moeder tijdens de zwangerschap en de ontwikkeling van de foetale hersenen. Ten slotte worden de doelen en de opzet van het proefschrift besproken.

In **hoofdstuk 2** onderzoeken we wat de determinanten zijn van de jodiumstatus van zwangere populaties en of deze vergelijkbaar zijn in populaties die gemiddeld mild tot gematigd jodium deficiënt of jodium sufficiënt zijn. We laten zien dat jongere vrouwen, met een hogere BMI vóór de zwangerschap en met een lage inname van melk en zuivelproducten, een grotere kans hadden om tijdens de zwangerschap een lage urine-excretie van jodium te hebben. Cohort-specifieke determinanten werden ook geïdentificeerd, zoals de inname van vis en schaaldieren in INMA en ALSPAC (mild tot matig jodium deficiënte populaties), en de inname van eieren en graanproducten in Generation R (jodium sufficiënte populatie). Deze cohort-specifieke associaties van maternale kenmerken en voedingsgewoonten met urine-excretie van jodium suggereren dat interventies gericht op het bereiken van jodium sufficiënte tijdens de zwangerschap mogelijk per land moeten verschillen.

In **hoofdstuk 3** onderzoeken we het verband tussen maternale jodiumstatus en maternale schildklierfunctie in een milde tot matige jodium deficiënte zwangere populatie en bepalen we of er verschillen zijn in de schildklier referentiewaarden op basis van de jodiumstatus. We laten zien dat een lagere urine-excretie van jodium geassocieerd is met een iets hogere totale thyroxine concentratie (TT4) en een lagere concentratie van schildklier stimulerend hormoon (TSH), terwijl vrouwen met een adequate jodiuminname het laagste risico op positieve schildklierautoantistoffen hebben. Het ontbreken van een verband tussen de jodiumstatus en de vrije T4 tot vrije trijodothyronine (FT4 / FT3) verhouding of de TT4 tot TT3 verhouding duiden niet op aanwijzingen voor een toename in de T3-productie of een hogere perifere dejodinatie van T4, wat adaptieve mechanismen zijn bij een chronische lage jodiuminname. De klinische referentiewaarden van schildklierfunctietests blijken niet erg anders te zijn in subgroepen van vrouwen met een verschillende jodiumstatus.

In **hoofdstuk 4** bestuderen we de associatie tussen maternale jodiumstatus en neurologische uitkomsten van het kind. Hoofdstuk 4.1 laat zien dat in een gepoolde analyse van gegevens van individuele deelnemers, lagere urine-excretie van jodium geassocieerd is met een lager gemiddeld verbale IQ score van kinderen. Deze associatie lijkt afhankelijk te zijn van de week van de zwangerschap, want de resultaten suggereren dat de eerste 14 weken

van de zwangerschap mogelijk een kwetsbare periode vormen voor foetale blootstelling aan maternale jodiumdeficiëntie. In hoofdstuk 4.2 beschrijven we dat we geen bewijs vinden voor een verband tussen jodiumtekort tijdens de zwangerschap en een groter risico op ADHD of autistische trekken bij het kind. De associaties van de maternale FT4 of TSH concentraties met ADHD of autistische trekken waren niet afhankelijk van de jodiumstatus van de moeder tijdens de zwangerschap.

In **hoofdstuk 5** onderzoeken we de associatie van maternale schildklierfunctie met neurologische uitkomsten van het kind. In hoofdstuk 5.1 laat onze meta-analyse van gegevens van individuele deelnemers zien dat een lage maternale FT4 concentratie in de vroege zwangerschap geassocieerd is met een lager gemiddeld verbale en non-verbale IQ score van het kind, waarbij de laatste associatie robuust is in milde tot matige jodium-deficiënte en jodium sufficiënte populaties. Harde conclusies over de associatie van maternale schildklierfunctie met autistische trekken konden niet worden getrokken. In hoofdstuk 5.2 laten we zien dat de maternale TSH of FT4 concentraties tijdens de zwangerschap niet geassocieerd zijn met ADHD van het kind.

In **hoofdstuk 6**, de discussie van het proefschrift, worden de belangrijkste resultaten en hun interpretatie besproken, als ook enkele methodologische overwegingen, de klinische implicaties en suggesties voor toekomstig onderzoek.

Chapter 8

Appendices

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List of publications

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List of publications

This thesis

Dineva M, Rayman MP, **Levie D**, Guxens M, Peeters RP, Vioque J, González L, Espada M, Ibarluzea JM, Sunyer J, Korevaar TIM, Bath SC. Similarities and differences of dietary and other determinants of iodine status in pregnant women from three European birth cohorts. *Eur J Nutr. 2019.* doi: 10.1007/s00394-019-01913-w.

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Levie D, Korevaar TIM, Bath SC, Dalmau-Bueno A, Murcia M, Espada M, Dineva M, Ibarluzea JM, Sunyer J, Tiemeier H, Rebagliato M, Rayman MP, Peeters RP, Guxens M. Thyroid Function in Early Pregnancy, Child IQ, and Autistic Traits: A Meta-Analysis of Individual Participant Data. *J Clin Endocrinol Metab. 2018;103(8):2967-2979.*

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Hafkamp-de Groen E, Lingsma HF, Caudri D, **Levie D**, Wijga A, Koppelman GH, Duijts L, Jaddoe VW, Smit HA, Kerkhof M, Moll HA, Hofman A, Steyerberg EW, de Jongste JC, Raat H. Predicting asthma in preschool children with asthma-like symptoms: validating and updating the PIAMA risk score. *J Allergy Clin Immunol. 2013;132(6):1303-10.*

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PhD portfolio

PhD student:	Deborah Levie
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Promotors:	Prof. dr. Robin P. Peeters, Prof. dr. Henning Tiemeier
Co-promotors:	dr. Mònica Guxens, dr. Tim I.M. Korevaar

	Year	ECTS
Courses		
Stata advanced course	2016	0.2
Becoming a scientific writer: Putting the “why ”before the “how”	2016	0.3
Write it clearly: fundamentals of good scientific writing	2017	0.4
Causal Inference with Directed Graphs	2017	0.3
Causal Mediation and Interaction Analysis	2017	0.4
Methods to deal with attrition and missing data	2017	0.1
Research Integrity	2018	0.3
Conferences – oral presentations		
13ª Jornadas Científicas INMA, Sabadell: *Assocation between maternal thyroid function in early pregnancy and offspring cognitive and psychomotor function.*	2016	1.0
EUthyroid project meeting, Copenhagen: *WP4: Maternal iodine status during pregnancy and neuropsychological development of the offspring.*	2016	1.0
European Thyroid Association, Copenhagen: *Association between maternal thyroid function in early pregnancy and offspring cognitive and psychomotor function.*	2016	1.0
EUthyroid project meeting, Belgrade: *WP4: Maternal iodine status during pregnancy and neuropsychological development of the offspring.*	2017	1.0
European Thyroid Association, Belgrade: *Association between maternal thyroid function in early pregnancy and offspring autistic traits.*	2017	1.0
DOHaD, Rotterdam: *Association between maternal thyroid function in early pregnancy and offspring autistic traits.*	2017	1.0
World Iodine Association, Pisa: *Maternal iodine status during pregnancy and child IQ : an individual level meta-analysis.*	2017	1.0
EUthyroid project meeting, Krakow: *WP4: Maternal iodine status during pregnancy and neuropsychological development of the offspring.*	2018	1.0
European Thyroid Association, Newcastle: *Maternal iodine status during pregnancy and child IQ: A meta-analysis of individual participant data.*	2018	1.0
Dutch Thyroid Club, Nijmegen: *Maternal iodine status during pregnancy and child IQ: A meta-analysis of individual-participant data.*	2019	1.0
European Society of Endocrinology, Lyon (invited speaker): *Mild thyroid dysfunction and offspring development.*	2019	1.0
Seminars, symposia or meetings		
EUthyroid kick-off meeting, Vienna	2015	0.6
2nd ISGlobal-CREAL PhD symposium, Barcelona	2015	0.3
3rd ISGlobal PhD symposium, Barcelona	2016	0.3

A Clinical Update on Thyroid and Pregnancy, Rotterdam	2017	0.1
Childhood and Environment meetings, Barcelona	2015-2017	1.0
ISGlobal Seminars-Campus Mar, Barcelona	2015-2017	1.0
Dutch Thyroid Club, Rotterdam	2018	0.1
The Krakow Declaration on Iodine, Krakow	2018	0.1
Dutch thyroid Research Foundation annual symposium, Amsterdam	2018	0.3
Health sciences research day, Rotterdam	2019	0.3
Thyroid lab meetings, Rotterdam	2017-2019	1.0
Generation R meetings, Rotterdam	2017-2019	1.0
Teaching		
VO Onderwijs Diagnose en behandeling van schildklieraandoeningen	2018	0.4
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INMA general tasks	2015-2017	13
Generation R general tasks	2017-2018	20
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European Thyroid Association Young Investigator Travel Grant	2017	
European Thyroid Association Young Investigator Travel Grant	2018	

About the author

Deborah Levie was born on February 16th, 1991 in Rotterdam, the Netherlands. She received her Bachelor and Master degree in Health Sciences with a specialization in Prevention and Public Health at the Vrije Universiteit in Amsterdam. After receiving her Master of Science in 2014, she worked at the Netherlands Organisation for applied scientific research (TNO). She started as a PhD student in 2015 at the Barcelona Institute for Global Health under the supervision of dr. Mònica Guxens, Prof. dr. Robin Peeters, Prof. dr. Henning Tiemeier, and dr. Tim Korevaar. In 2017, she moved back to the Netherlands to continue her PhD at the Department of Internal Medicine, Academic Center for Thyroid Diseases at the Erasmus University Medical Center in Rotterdam. During her PhD, most of her studies were embedded within EUthyroid, which was a EU-funded research project (2015-2018) with the goal of harmonizing and sustainably improving the iodine intake in Europe (grant agreement No 6344543).