Iodine nutrition in pregnancy, maternal thyroid function and child neurodevelopment

- Results from the Norwegian Mother and Child Cohort Study

Marianne Hope Abel

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"Iodine deficiency is the world's most prevalent, yet easily preventable, cause of brain damage. Today we are on the verge of eliminating it – an achievement that will be hailed as a major public health triumph that ranks with getting rid of smallpox and poliomyelitis."

World Health Organization webpage (www.who.int, 2018) (1)

«*Scientists fear* ***up to 50% of all new-borns in Europe do not reach their full cognitive potential due to iodine deficiency****. Iodine is a micronutrient critical for children's brain development. Today with the Krakow Declaration on Iodine presented at the Jagiellonian University, scientists from the EU-funded project EUthyroid, supported by several stakeholder organisations, call on European policy-makers to support measures to eliminate iodine deficiency. (…) The adverse effects of iodine deficiency are diverse and impose a significant burden on public healthcare systems. Although this fact is well established,* ***in Europe prevention programmes for iodine deficiency disorders receive surprisingly little attention*** *from policy makers, opinion leaders and citizens. »*

From press release: Krakow Declaration on Iodine 18.04.2018 by EUthyroid – a project within Horizon2020 involving 27 European countries

http://www.iodinedeclaration.eu/wp-ontent/uploads/2018/04/EUthyroid_Declaration_PR_INTERNATIONAL.pdf

«Jodinntaket er urovekkende lavt i deler av befolkningen, og nasjonale myndigheter bør igangsette tiltak som sikrer adekvat jodstatus i hele befolkningen og spesielt i sårbare grupper som kvinner i fertil alder, gravide, ammende og små barn.»

Nasjonalt råd for ernæring, rapport om jodstatus i Norge 2016 (2)

Acknowledgements

In 2006, I contacted Helle Margrete Meltzer at the Norwegian Institute of Public Health (NIPH) and asked if there was a possibility for doing a PhD-project in The Norwegian Mother and Child Cohort Study (MoBa). Since 2009, we applied for money each year and continuously worked on improving the proposal. Thank you Helle Margrete, Anne Lise Brantsæter, Margareta Haugen, Jan Alexander, Heidi Aase, and NIPH for giving me this opportunity and for never giving up hope and effort to get funding.

In 2014, we decided to try a new strategy and apply for an Industrial PhD. I want to thank my employer, TINE SA, for supporting this project. A very special thanks to Anne Sofie Biong, Johanne Brendehaug, Kirsti Wettre Brønner, Eirik Selmer Olsen, and others in TINE who supported the idea and helped make it happen. I am also grateful to NIPH, OsloMet – Oslo Metropolitan University, and to the Research Council of Norway for joining this collaboration. And, to all my dear colleagues in the Department of Nutrition in TINE, thank you for covering for me while I have been a PhD-student and for cheering me on.

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In total, there have been 16 co-authors on papers 1-4 in this project. I want to express my gratitude for your valuable input to the publications and great collaboration. Thank you Heidi Aase and Ragnhild Eek Brandlistuen for contributing with your expertise on child mental health and for being open to, and curious about, nutritional epidemiology. Thank you, Jan Alexander, for many fruitful discussions on biological mechanisms. Thank you, Margaretha Haugen, for answering all possible questions on the MoBa nutrition data. Thank you Ted Reichborn-Kjennerud, Eivind Ystrøm, and Ragna Bugge Askeland for giving me the opportunity to study ADHD diagnosis in the Norwegian Patient Registry, an outcome that was not initially included in the project protocol. Thank you Gro Dehli Villanger, Tim Korevaar, Iris Erlund, and Petra Arohonka for contributing with your knowledge on maternal thyroid function, and a special thanks to Tim Korevaar for spending a whole day with me at the Erasmus Medical Center in Rotterdam planning paper 3.

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Dear family and friends. Thank you for your support and for showing interest in my project. I am especially grateful to my husband, Kim. You always believed that I could do it, and you never complained when I talked endlessly about my project, but managed to show enthusiasm and be supportive. Thank you, Sandra and Simen, for reminding me what is most important in my life.

Last but not least, I would like to thank all the participating mothers, fathers and children in MoBa who continuously contribute to this unique dataset, -the largest study of its kind in the world! It has really been an honour to work on these high-quality data.

Oslo, January 2019
Marianne Hope Abel

Short summary in Norwegian

Bakgrunn: Mild- til moderat jodmangel er utbredt blant unge kvinner, gravide og ammende i Norge og i mange andre land. Jod inngår i stoffskiftehormonene som dannes i skjoldbruskkjertelen (thyreoidea), og disse er særlig viktige i hjernens utvikling hos foster og barn. Alvorlig jodmangel i fosterlivet påvirker hjernens utvikling negativt, mens det er mindre kunnskap om konsekvenser av mild- til moderat jodmangel. Enkelte observasjonsstudier og dyrestudier indikerer at selv mildere former for mangel kan gi varig redusert nevrokognitiv utvikling, f.eks. lavere IQ. Det er også manglende kunnskap om effekt av å ta kosttilskudd med jod i svangerskapet i befolkninger med mild- til moderat jodmangel.

Mål: Å bruke data fra Den norske mor og barn-undersøkelsen (MoBa) til å undersøke sammenhengen mellom mors jodinntak i svangerskapet og hjernens utvikling hos barn.

Materiale og metode: Studien inkluderer deltakere i MoBa, en prospektiv kohort-undersøkelse der deltakere ble rekruttert fra hele landet i første del av svangerskapet i årene 1999-2008. Inklusjonskriterier var enkeltfødsel og ingen rapportert bruk av stoffskiftemedisiner i svangerskapet. Eksponeringer var mors jodinntak i svangerskapet beregnet ut fra et validert matvarefrekvensskjema (i bruk fra mars 2002) og mors bruk av kosttilskudd i svangerskapsuke 0-22 (ja/nei og tid for oppstart av tilskudd). Utfall var mors thyreoideafunksjon i svangerskapet (n=2910), mål for barnets utvikling ved tre år (n=48,297) og åtte års (n=39,471) og barnets risiko for ADHD-diagnose (n=77,164). Regresjonsanalyse ble brukt for å undersøke assosiasjoner kontrollert for kovariater.

Resultater: Et lavt jodinntak fra kost i svangerskapet (tilsvarende lavere inntak enn anbefalt for ikke-gravide: 150 µg/dag) var assosiert med endringer i mors nivåer av thyreoideahormoner og at barnet fikk redusert språkutvikling, dårligere finmotorikk, mer atferdsproblemer og ADHD-symptomer, dårligere skoleprestasjoner og hadde økt sannsynlighet for å motta spesialundervisning. Mors jodinntak var ikke signifikant assosiert med barnets grovmotorikk eller risiko for ADHD-diagnose. Det ble ikke funnet evidens for gunstige effekter av å ta jodtilskudd i svangerskapet. Å starte å ta jodtilskudd i første trimester var assosiert med økt risiko for atferdsproblemer, ADHD-symptomer og ADHD-diagnose, men ikke med de andre utfallene.

Konklusjon: Resultatene understøtter at mild- til moderat jodmangel i svangerskapet er assosiert med redusert nevrokognitiv utvikling hos barn. Effektene var generelt små, men omfattet en betydelig andel av populasjonen og er derfor likevel relevante. Tilskudd med jod i svangerskapet ser ikke ut til å kunne kompensere for et lavt langtidsinntak av jod fra kost.

Abstract

Background: Iodine is an essential micronutrient through being an integral part of the thyroid hormones synthesized in the thyroid gland. Thyroid hormones are important in regulating nerve cell- and brain development. Suboptimal iodine nutrition can affect thyroid function and consequently also foetal brain development, and the first trimester is identified as a particularly vulnerable time window. Although great effort has been made during the last decades to eradicate iodine deficiency (ID), mild-to-moderate ID remains one of the most common nutritional deficiencies worldwide. The World Health Organization (WHO) estimates that ID is the most common cause of preventable impaired cognitive development, and up to 50% of babies born in Europe today are estimated to be at risk. Severe ID has detrimental effects on brain development, but less is known about the potential impact of mild-to-moderate ID and about what is the optimal range of iodine intake in pregnancy. Studies have indicated that the optimal range is narrow. A high iodine intake can also affect thyroid function negatively. In areas of ID, WHO recommends iodine supplements for women of childbearing age, pregnant and lactating women until salt iodization is implemented. In severe ID, iodine supplements are effective in preventing thyroid dysfunction and child impairments, but in mild-to-moderate ID studies show conflicting results.

Aim: The aim of this project was to take full advantage of the potential within the Norwegian Mother and Child Cohort Study (MoBa) (within the frames of a Ph.D.-project) to explore the association between maternal iodine intake and child neurodevelopment in a population characterized with mild-to-moderate ID in pregnant women. Specifically, we aimed to explore if maternal iodine intake from food was associated with maternal thyroid function in pregnancy (plasma thyroid hormones and antibodies) and with child neurodevelopment up to age 8 years (language, motor, behaviour problems, school performance, special educational services, symptoms of attention deficit/hyperactivity disorder (ADHD), and ADHD diagnosis). A second aim was to explore the potential impact of maternal use of iodine containing supplements on the same outcome measures.

Material and methods: The study sample included participants in the Norwegian Mother and Child Cohort Study (MoBa) recruited in pregnancy, all over Norway in 1999-2008. Inclusion criteria were singleton pregnancy, no reported use of thyroid medication in pregnancy, available data on exposure(s) and outcome(s). Maternal habitual iodine intake was calculated based on an extensive and validated food frequency questionnaire (FFQ, in use from 2002) covering the first half of pregnancy. Outcomes included maternal thyroid function in

pregnancy (*n*=2910), measures of child neurodevelopment at 3 years (*n*=48,297) and 8 years (*n*=39,471), and child risk of ADHD diagnosis (*n*=77,164). Associations were explored by multivariable regression analysis controlling for confounding factors.

Results The median calculated habitual iodine intake from food was 121 µg/day (IQR: 89, 161 µg/day)[1], and the majority of the participants did not reach the recommended intake of iodine in pregnancy[2]. UIC was measured in a subsample of women (*n*=2910, mean gestational week 18.5, SD: 1.3), and median UIC was 59 µg/L in non-users of iodine supplements and 98 µg/L in current iodine supplement users. Both groups were well below what is considered adequate by WHO (i.e. median ≥150 µg/L). UIC in the subsample of 8 year old children (*n*=279) indicated adequate iodine status in the children (i.e. median UIC ≥100 µg/L; median UIC was 110 µg/L, IQR: 79, 155 µg/L).

UIC, but not iodine from food by the FFQ, was inversely associated with maternal thyroid hormones (plasma free thyroxine (FT4) and free triiodothyronine (FT3)) in gestational week 18. A recent introduction of an iodine supplement (within the last 5 weeks) was associated with lower FT4.

A low maternal iodine intake from food (below ~150 µg/day) was associated with poorer language skills at 3 and 8 years, poorer fine-motor skills at 3 years, with more behaviour problems at 3 years and ADHD-symptoms at 8 years, with poorer reading and writing skills at 8 years, and with increased likelihood of child receiving special educational services at 8 years. It was not associated with gross motor skills at 3 years or child ADHD diagnosis registered in the Norwegian Patient Registry (NPR) by Dec. 2015.

There was no evidence of beneficial effects of maternal use of an iodine-containing supplement in the first half of pregnancy. Introducing an iodine-containing supplement in the first trimester was associated with more behaviour problems at 3 years, ADHD symptoms at 8 years, and ADHD diagnosis in the NPR, but not with the other outcomes on neurodevelopment.

Conclusion: Overall, the results from MoBa indicate that mild-to-moderately insufficient iodine intake in pregnancy (less than ~150 µg/day) was associated with changes in maternal thyroid function, poorer child cognitive development, and more child behaviour problems.

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[1] All singleton pregnancies in MoBa with data from the food frequency questionnaire (*n*=84,327).
[2] Recommended iodine intake for pregnant women varies and ranges from 175 µg/day in the Nordic recommendations to 250 µg/day in the WHO recommendations.

There was no evidence of beneficial effects of maternal use of iodine-containing supplements. Initiating iodine supplement use might lead to a temporary inhibition of thyroid hormone production/release in mild-to-moderate ID which potentially can affect neurodevelopment negatively. This was indicated for child behaviour problems, including ADHD, when supplement use was initiated in the first trimester.

Impact: Preventing even mild-to-moderate ID in women of childbearing age seems critical to secure optimal foetal brain development. Prevention by taking iodine supplementation initiated in pregnancy might be too late, and can potentially cause a transient inhibition of the thyroid function. Effective prevention strategies should therefore aim to secure an optimal iodine status in *all* women of reproductive age. ID is easily preventable, yet it remains an important risk factor for impaired neurodevelopment. Given the relatively high prevalence of mild-to-moderate ID in Norwegian pregnant women (69% in MoBa had iodine intake from food <150 µg/day), results from this study suggests that ID is an important risk factor for impaired neurodevelopment in children born in Norway and that actions to prevent ID are urgently needed.

For the purpose of prevention, it is important to gain knowledge about what intake is needed to secure an optimal foetal brain development while at the same time not aiming too high putting pregnant women and also other groups of the population at risk of iodine excess. The results of this study does *not* support either an increased recommended intake of iodine in pregnancy or recommending pregnant women with mild-to-moderate ID to take iodine-containing supplements. However, more research is needed to elucidate this.

Scientific environment

The PhD-candidate in this project is employed by the Norwegian dairy company TINE SA, Department of Nutrition. This project is funded partially by TINE SA and partially by the Research Council of Norway through a four year grant (grant no. 241430) under their Industrial PhD-program (3).

The overall objectives of the Industrial PhD-program are "to increase the recruitment of researchers to Norwegian industry, to boost long-term competence-building and increase research efforts in business and industry and to enhance interaction between academia and industry" (3).

The PhD-project is a collaboration between the Norwegian Institute of Public Health (NIPH), Oslo Metropolitan University (OsloMet), and TINE SA, but only the PhD-student and collaborators at NIPH had access to the study data. The project was conducted at NIPH, Department of Exposure and Environmental Epidemiology, Division of Infection Control and Environmental Health where also the main supervisor, Anne Lise Brantsæter (PhD), is affiliated. The PhD-candidate, Marianne Hope Abel, is a student in the PhD program in Health Sciences at OsloMet, where co-supervisor Professor Liv Elin Torheim is affiliated to the Department of Nursing and Health Promotion, Faculty of Health Sciences, OsloMet.

This project has involved collaboration between researchers from multiple countries and fields of research including nutritional epidemiology and public health (NIPH, OsloMet), mental health (Division of Mental and Physical Health, NIPH), statistics (NIPH), and endocrinology (Genomics and Biomarkers Unit, National Institute for Health and Welfare, Helsinki, Finland, and Academic Center for Thyroid Diseases, Erasmus Medical Center, Rotterdam, the Netherlands).

Abbreviations

ADHD	Attention-deficit/hyperactivity disorder
BMI	Body mass index
CI	Confidence interval
DAG	Directed acyclic graph
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FFQ	Food frequency questionnaire
FT3	Free triiodothyronine (T3)
FT4	Free thyroxine (T4)
GW	Gestational week
HELIX	Human Early-Life Exposome Project
ICD-10	International Classification of Diseases-10
ID	Iodine deficiency
IQ	Intelligence quotient
IQR	Interquartile range (or 25^{th} and 75^{th} percentile)
MoBa	Norwegian Mother and Child Cohort Study
NIPH	Norwegian Institute of Public Health
NPR	Norwegian Patient Registry
RCT	Randomised controlled trial
SD	Standard deviation
T3	Tri-iodothyronine
T4	Tetra-iodothyronine (or thyroxine)
TSH	Thyroid stimulating hormone (or thyrotropin)
TgAb	Thyroglobulin antibodies
TPOAb	Thyroid peroxidase antibodies
UIC	Urinary iodine concentration
WHO	World Health Organization
UNICEF	United Nations Children's Fund

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Dissemination: List of papers and communication

List of papers

1. **Abel MH**, Caspersen IH, Meltzer HM, Haugen M, Brandlistuen RE, Aase H, Alexander J, Torheim LE, Brantsaeter AL. Suboptimal maternal iodine intake is associated with impaired child neurodevelopment at 3 years of age in the Norwegian Mother and Child Cohort Study. J Nutr 2017;147(7):1314-24. doi: 10.3945/jn.117.250456.
2. **Abel MH**, Ystrom E, Caspersen IH, Meltzer HM, Aase H, Torheim LE, Askeland RB, Reichborn-Kjennerud T, Brantsaeter AL. Maternal iodine intake and offspring attention-deficit/hyperactivity disorder: Results from a large prospective cohort study. Nutrients 2017;9(11). doi: 10.3390/nu9111239.
3. **Abel MH**, Korevaar TIM, Erlund I, Villanger GD, Caspersen IH, Arohonka P, Alexander J, Meltzer HM, Brantsæter AL. Iodine intake is associated with thyroid function in mild to moderately iodine deficient pregnant women. Thyroid 2018;28(10):1359-71. doi: 10.1089/thy.2018.0305.
4. **Abel MH**, Brandlistuen RE, Caspersen IH, Aase H, Torheim LE, Meltzer HM, Brantsæter AL. Language delay and poorer school performance in children of mothers with inadequate iodine intake in pregnancy: results from follow-up at 8 years in the Norwegian Mother and Child Cohort Study. Eur J Nutr 2018. doi: 10.1007/s00394-018-1850-7.

Abstracts/posters/presentations at international meetings/conferences

- The 1st international conference by the World Iodine Association November 2017 in Pisa, Iodine in Food Systems and Health:
    - **Abel MH**, Torheim LE, Meltzer HM, Brantsæter AL. Maternal iodine nutrition in pregnancy is associated with likelihood of special education for the child at age 8 years in the Norwegian Birth Cohort Study. Abstract and oral presentation. Awarded best student oral presentation.
    - Dahl L, Torheim LE, Brantsæter AL, **Abel MH**, Madar A, Meltzer HM. Risk of iodine deficiency in Norway. Identification of an urgent need for action. Abstract.
- The 10th World Congress on Developmental Origins of Health and Disease (DOHaD) October 2017 in Rotterdam, abstract, poster and oral poster-presentation:
  Abel MH, Ystrom E, Caspersen IH, Aase H, Torheim LE, Meltzer HM, Askeland RB, Reichborn-Kjennerud T, Brantsæter AL. Maternal use of iodine containing supplements in pregnancy is associated with increased risk of child attention-deficit/hyperactivity disorder

- Bilateral meeting between iodine researchers in Norway and Portugal in Lisbon, September 2017. Presentation of the iodine project within MoBa.
- Nutrition during the reproductive cycle (NRCycle) meeting in Oslo May 2017, presentation: Iodine and neurodevelopment
- Nordic iodine meeting Reykjavik May 2017, presentation:
  Iodine intake in pregnancy and neurodevelopment in MoBa (results so far)
- The Nordic Nutrition Conference, Gothenburg June 2016, abstract and oral presentation:
  Abel MH, Torheim LE, Haugen M, Brandlistuen RE, Aase H, Caspersen IH, Alexander J, Meltzer HM, Brantsæter AL. Iodine intake in pregnancy is associated with neurodevelopment at age 3 years: Results from The Norwegian Birth Cohort Study
- Nordic iodine meeting Bergen September 2015, presentation:
  Iodine intake in pregnancy and neurodevelopment in MoBa (project protocol)

Presentations at Norwegian seminars

- Seminar on iodine for public health nurses, Oslo and Akershus University College of Applied Sciences, February 2017, presentation:
  [Why is iodine important?] (Hvorfor er jod så viktig?)
- Norwegian Association for Nutritionists (Norsk forening for ernæringsfysiologer – NFE) Autumn seminar, November 2016, presentation:
  [Iodine and the brain] (Jod og hjernen)
- MoBa Seminar, Norwegian Institute of Public Health, Oslo, October 2015, presentation:
  Iodine intake in pregnancy and neurodevelopment in MoBa (project protocol)

Press releases

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  (Archived by WebCite® at http://www.webcitation.org/7014SzRa3)
- The American Society for Nutrition. Low maternal iodine consumption associated with impaired neurodevelopment of children. The Journal of Nutrition Media Alerts. 2017-07. URL:http://nutrition.org/wp-content/uploads/2017/02/JN-Media-Alert-July-2017.pdf. Accessed: 2018-06-28.
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Media coverage (examples)

- Nutraingredients.com: Cutcliffe, T. Food-based iodine during pregnancy important for child brain development. 2018-06-08. URL:https://www.nutraingredients.com/Article/2017/07/25/Food-based-iodine-during-pregnancy-important-for-child-brain-development. Accessed: 2018-06-08. (Archived by WebCite® at http://www.webcitation.org/7015heK9K)
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- NRK P1: Norgesglasset (radio program), 24 May 2017, interview with Brantsæter, AL. https://radio.nrk.no/serie/norgesglasset/dmta01010317/24-05-2017#t=35m34s

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- Forskning.no: Torheim, LE; Meltzer, HM; Dahl, L; Brantsæter, AL; Madar, A; **Abel, MH**. Gode grunner til å ha melk og sjømat i kostholdet. 2018-06-08. URL:http://forskning.no/meninger/debattinnlegg/2017/07/jod-gode-grunner-til-ha-melk-og-sjomat-i-kostholdet. Accessed: 2018-06-08. (Archived by WebCite® at http://www.webcitation.org/7013Pe3S8)
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Other relevant publications (not included in the thesis)

- **Abel MH**, Caspersen IH, Sengpiel V, Jacobsson B, Meltzer HM, Magnus P, Alexander J, Brantsæter AL. Inadequate maternal iodine intake is associated with sub-fecundity and adverse pregnancy outcomes in the Norwegian Mother and Child Cohort Study. Manuscript in preparation. To be submitted in 2019.
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- **Abel MH**, Brantsæter AL. [Iodine deficiency is prevalent in Norwegian pregnant women and may be a risk factor for child ADHD] (Original title: Jodmangel er utbredt blant gravide i Norge og kan ha betydning for barnets risiko for ADHD). BestPractice Psykiatri/Nevrologi. 2018;9(34):25-7.
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Introduction

Iodine – an essential micronutrient

Like fluorine, chlorine and bromine, iodine is a halogen in the periodic table of elements. The name halogen means "salt producing", and the halogens react easily to form different salt compounds. Examples are sodium chloride (NaCl) in table salt, and potassium iodide (KI) which is often added in small amounts to salt to increase the iodine intake in populations (4).

In all vertebrates, including humans, iodine is an essential micronutrient through being an intrinsic component of the thyroid hormones, thyroxine (T4) and tri-iodothyronine (T3) (5, p. 26). In fact, this is the only known function of iodine in the human body. Thyroid hormones are important in regulating cell metabolism and in controlling tissue growth and maturation, particularly of the nervous system. The hormones are synthesized in the thyroid gland situated at the front base of the neck.

If iodine intake is adequate, the body of an adult contains about 15-20 mg of iodine, 70-80% of this within the thyroid gland (6). In a situation of long-term insufficient iodine intake, the iodine store in the thyroid may fall to less than 20 µg (6). The recommended daily intake of iodine for adolescents and adults is 150 µg/day (7), and to cover the recommended intake of iodine for a whole lifetime (0-85 years), a total of less than 5 gram (or one teaspoon) of iodine is needed.

Prevalence of iodine deficiency

Iodine is very abundant in the sea, but it is unevenly distributed in the soil. In many regions of the world the natural content of iodine in the soil, farming produce and drinking water is low. The World Health Organization (WHO) estimates that almost 2 billion people worldwide are dependent on iodine fortification or supplementation to prevent iodine deficiency (ID) (8). To monitor iodine status in a population, WHO recommends measuring UIC in a random sample of school aged children (9). About 90% of ingested iodine is excreted in the urine within 24-48 hours, and if population median urinary iodine concentration (UIC) falls below 100 µg/L, the population is considered iodine deficient by WHO criteria (**Table 1**) (10). Tremendous effort has been made during the last few decades to prevent ID, and severe ID has been almost completely eradicated (11). Still, mild-to-moderate ID remains one of the most prevalent nutrient deficiencies in the world. Surprisingly, Europe is the continent with the highest

estimated prevalence of ID and about 1/3 of school aged children have an inadequate iodine intake (12).

Table 1 Epidemiological criteria for assessing iodine nutrition in population groups based on median urinary iodine concentration by the World Health Organization (9)

Population group	Median urinary iodine (µg/L)	Iodine intake/status	Iodine status
School-age children (≥ 6 years) [a]	< 20	Insufficient	Severe iodine deficiency
	20 - 49	Insufficient	Moderate iodine deficiency
	50 – 99	Insufficient	Mild iodine deficiency
	100 – 199	Adequate	Adequate iodine nutrition
	200 – 299	Above requirements	Likely adequate for pregnant and lactating, but a slight risk of more than adequate intake in the overall population
	≥ 300	Excessive [b]	Risk of adverse consequences
Pregnant women	< 150	Insufficient	
	150 – 249	Adequate	
	250 – 499	Above requirements	
	≥ 500	Excessive [b]	
Lactating women	< 100	Insufficient	
Children < 2 years	< 100	Insufficient	

[a] Applies also to adults, but not pregnant and lactating women.
[b] Excessive, meaning in excess of the amount required to prevent and control iodine deficiency.

In pregnancy, the iodine turnover increases due to an upregulated production of thyroid hormones, transfer of iodine and thyroid hormones to the developing foetus, and an increase in renal clearance (6). Therefore, iodine recommendations for pregnant women are higher, and a median UIC ≥150 µg/L is the recommended cut-off for defining iodine sufficiency in pregnant women according to WHO (13). There is, however, a lack of knowledge about what represents the optimal level of iodine intake in pregnancy, and the recommendations vary. In the UK the recommendation is the same as for non-pregnant women (140 µg/day) (14), in the Nordic countries it is 175 µg/day (7), the European Food Safety Authority recommends 200 µg/day (15), in the U.S. 220 µg/day is recommended (16), and WHO recommend 250 µg/day (13). The U.S. Institute of Medicine has also set an estimated average requirement of iodine for pregnant women of 160 µg/day (16). In Europe, more than half of pregnant women are probably ID by WHO criteria (see **Figure 1**). Mild-to-moderate ID in pregnancy can be defined as median UIC of 50-150 µg/L (17).

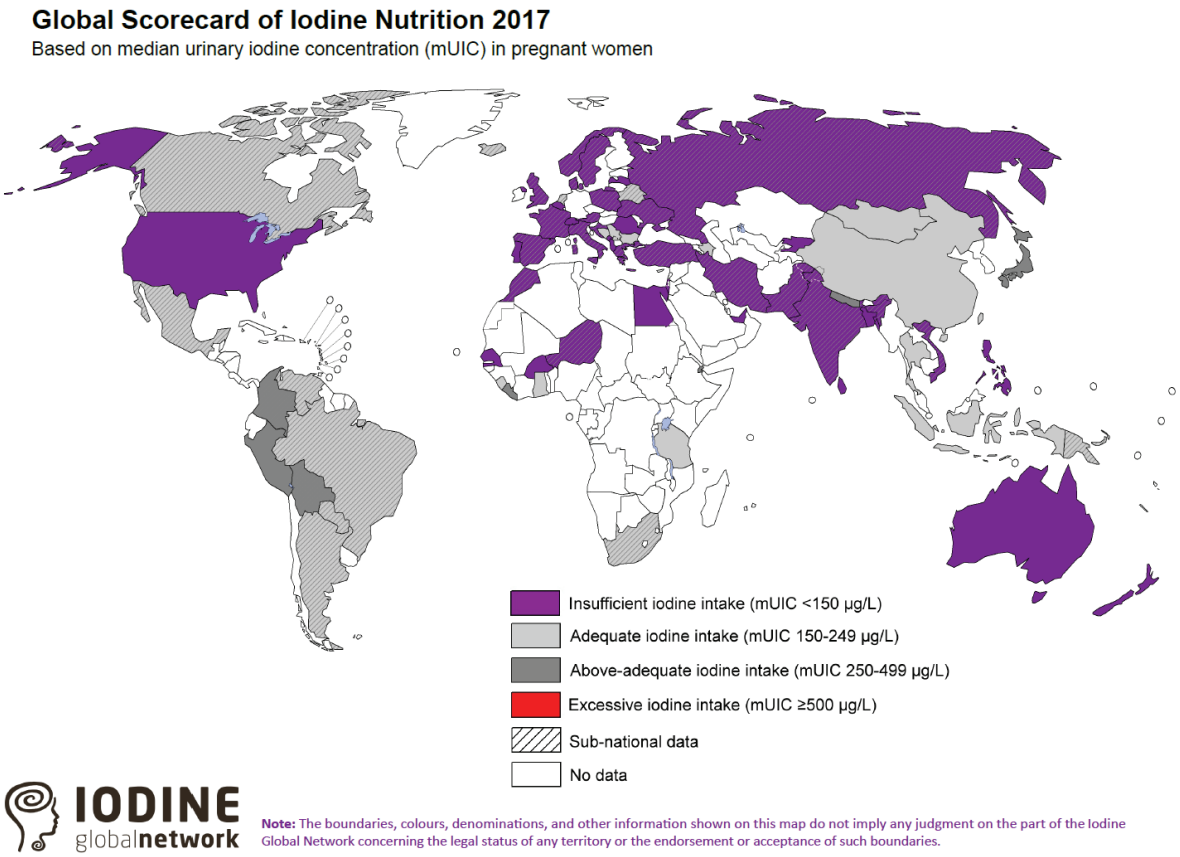

Figure 1 Global scorecard on iodine nutrition in pregnant women 2017 illustrating median urinary iodine concentration (mUIC) by country. Published by the Iodine Global Network (18). Retrieved from http://www.ign.org/cm_data/IGN_Global_Map_PW_30May2017_1.pdf Accessed: 2018-05-07. (Archived by WebCite® at http://www.webcitation.org/6zEimmPjZ)

Thyroid hormones and brain development

A human brain contains of over 80 billion nerve cells (neurons) and more than a hundred trillion contact points between neurons (synapses) (19, p. 185). In addition, a comparable number of non-neuronal brain cells provide support to the brain (19, p. 24). The "wiring" connecting the nerve cells is estimated to add up to a distance equalling four trips around the world (19, p. 2), and a typical number of contact points for a single nerve cell is about 5000-10,000 (19, p. 31). At birth, the human brain already contains most of the neurons it will ever have (19, p. 190).

Thyroid hormones are essential in guiding the extremely complex process of brain development (20). They are involved in regulating many key steps such as neurogenesis, cell migration, cell differentiation and synaptogenesis (20). Disturbances in the levels of thyroid hormones may result in irreversible impairments since the brain-cells develop in a strictly timed sequence of events (20, 21). Recent evidence suggests that even moderate changes in

maternal thyroid function, especially during early gestation, might have negative and irreversible effects on child cognitive development and increase the risk of neurodevelopmental disorders (22).

Before the foetal thyroid begins to function from gestational week (GW) 18-20, the foetus is entirely dependent on supply of maternal thyroid hormone, especially of T4 (20). Also after GW 20, the foetal thyroid hormone production is not adequate, and the transfer of maternal T4 to the foetus continues until birth (20). The placental transfer of maternal T3, the active form of the thyroid hormones, is extremely low, protecting the foetus from fluctuations in T3 (23). Instead, T3 is generated locally in the foetal brain from maternal T4 through a process that is tightly regulated (23). This makes the foetus vulnerable to a low maternal T4 (20). Animal studies have shown that a low maternal T4 is associated with lower foetal brain T3 (23), which in turn is associated with irreversible changes in foetal brain cytoarchitecture characterized by blurred neocortical layering (i.e. nerve cells not reaching their destination causing distortion or blurring in the layers of cells that are usually more distinctly organized) (24). In 2018, Levie *et al.* published a meta-analysis of individual participant data from three European prospective birth cohorts (INMA, ALSPAC, and Generation R, n=9036 mother-child pairs) demonstrating that a low maternal free T4 (FT4) (below the 5th percentile) in the first half of pregnancy was associated with lower verbal and non-verbal child intelligence quotient (IQ) (21). They also found a non-significant, but suggestive association with increased risk of autistic traits (21). Their results confirmed findings of previous human observational studies (25-30). However, no effect on child IQ was seen in two randomised controlled trials (RCTs) investigating T4-treatment (levothyroxine medication) of pregnant women with low T4 (31, 32). Explanations for the null findings may be that the treatment was initiated too late in pregnancy (GW 13-18), the dosage might have been too high, and that the studies were underpowered to detect small changes in IQ (21).

Figure 2 shows changes in thyroid physiology in pregnancy. The maternal blood concentration of thyroid hormones are highly gestational age specific, and changes in maternal hormone levels are especially large during the first half of pregnancy.

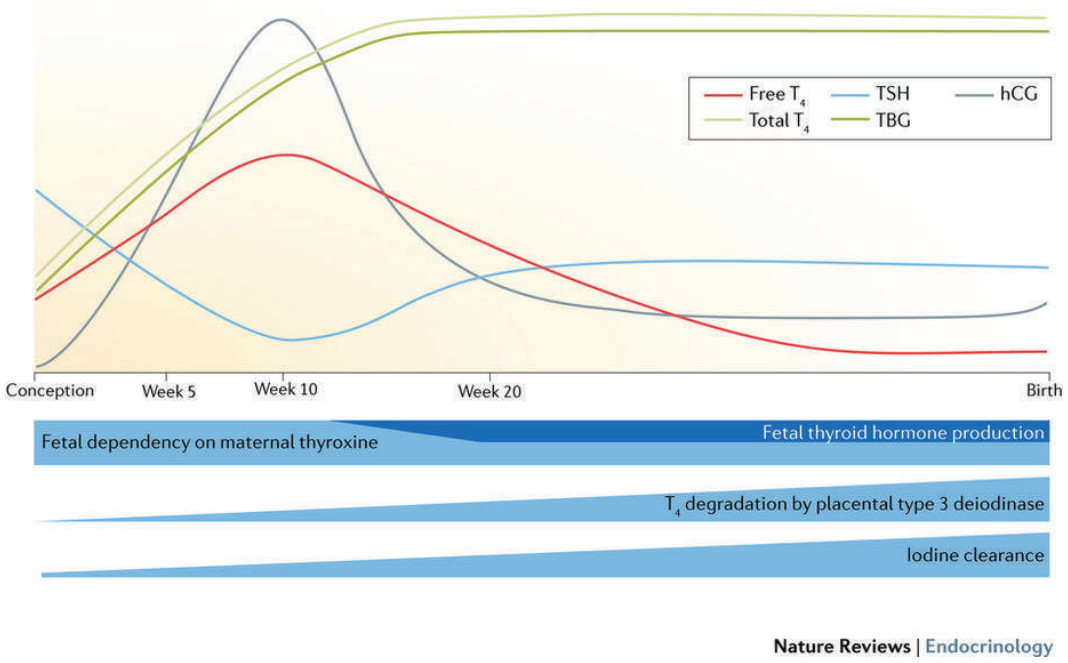

Figure 2 Changes in thyroid physiology during the course of pregnancy. The coloured lines show normal levels of maternal thyroid function parameters. To maintain adequate thyroid hormone availability, human chorionic gonadotropin (hCG) stimulates an increase in maternal thyroid hormone production to cover the needs of the mother and foetus, and to compensate for an increased breakdown of thyroid hormones in the placenta. The foetus is entirely dependent on placental transfer of maternal thyroid hormones, particularly in the first half of pregnancy before the foetal thyroid gland is functional.
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Consequences of ID in pregnancy

In ID, the pregnancy-related increased demand for thyroid hormones may not be met adequately. Indeed, this is well documented in severe ID which is associated with goitre (adaptive swelling of the thyroid gland), maternal and foetal hypothyroidism (i.e. low levels of thyroid hormones and high levels of thyroid stimulating hormone (TSH)), growth retardation, and serious neurologic and cognitive deficits in children, in addition to increased risk of pregnancy loss and infant mortality (33).

Less is known about the potential consequences of mild-to-moderate ID (33) which can be defined as a median UIC in pregnant women of 50-150 µg/L (17). Although the association between iodine intake and thyroid disorders is documented to be U-shaped (34-36), and both low and high intakes are associated with increased risks, *mild* ID is *not* associated with an increased prevalence of thyroid disorder. However, when iodine intake is marginally low,

auto-regulatory processes are effectively initiated in the body in order to save iodine. This includes a shift towards a higher production of T3 at the cost of T4, saving one iodine atom and securing an adequate level of active thyroid hormones in the mother. However, it may at the same time result in a reduced thyroid hormone-supply to the foetus (23).

Results from two observational studies published in 2013 indicated that even mild-to-moderate ID in pregnancy might be harmful and affect child IQ and school performance (37, 38). However, studies on iodine supplementation in pregnancy in mild-to-moderate ID show conflicting results (39). This might be explained by several factors. The studies on supplementation varied greatly in design and in iodine status of the study populations. As for the T4 treatment studies mentioned above, most studies on iodine supplementation was initiated after the first trimester, and the first trimester is identified as the time period were the foetus seems to be most vulnerable to maternal thyroid dysfunction. Also, in some studies, rather high doses of iodine supplements were given which may increase the risk of thyroid dysfunction caused by iodine excess. A few studies have indicated that in mild-to-moderate ID, the thyroid seems to be vulnerable to an abrupt increase in iodine supply causing a temporary "stunning effect" with lower maternal thyroid hormone production (34, 40). Finally, in studies where the median UIC is only slightly below the recommended cut-off for defining adequacy of 150 µg/L, probably very few of the women were truly iodine deficient. This might for example explain the null-finding in the most recent RCT published, an impressive study from India and Thailand following 315 children of supplemented and non-supplemented mothers up to age 5-6 years (41).

Velasco *et al.* published a review in March 2018 summarizing the available evidence from animal and human studies for the effect of ID on brain development (24). Animal studies support the human observational studies and show that even mild-to-moderate ID can lead to irreversible changes in brain development (24). However, effects might be small and difficult to detect in randomized controlled studies in humans that are often limited in size. In 2017, a Cochrane systematic review on effect of iodine supplement use in pregnancy was published, and the authors concluded "There were insufficient data to reach any meaningful conclusions on the benefits and harms of routine iodine supplementation in women before, during or after pregnancy" (39).

Therefore, although mild-to-moderate ID is highly prevalent in pregnant women, there is still great uncertainty about the potential consequences for child development, about what

represents the optimal range of iodine intake for pregnant women, and whether iodine supplements in pregnancy are beneficial in mild-to-moderate ID.

Figure 3 and **Figure 4** are reprints of results from a study where rats were fed diets containing different amounts of iodine from three months pre-pregnancy and through pregnancy and lactation, and the offspring were tested for multiple measures of brain development (42). Results in this study show that even mildly ID diets resulted in reduced motor performance in the offspring (**Figure 3**), and they also documented changes to cell structures in the brain (**Figure 4**), and to signalling pathways important for motor coordination (42). In this study, they found that iodine supplements given to the pregnant rats from day 0 of gestation effectively prevented negative effects of mild ID (42).

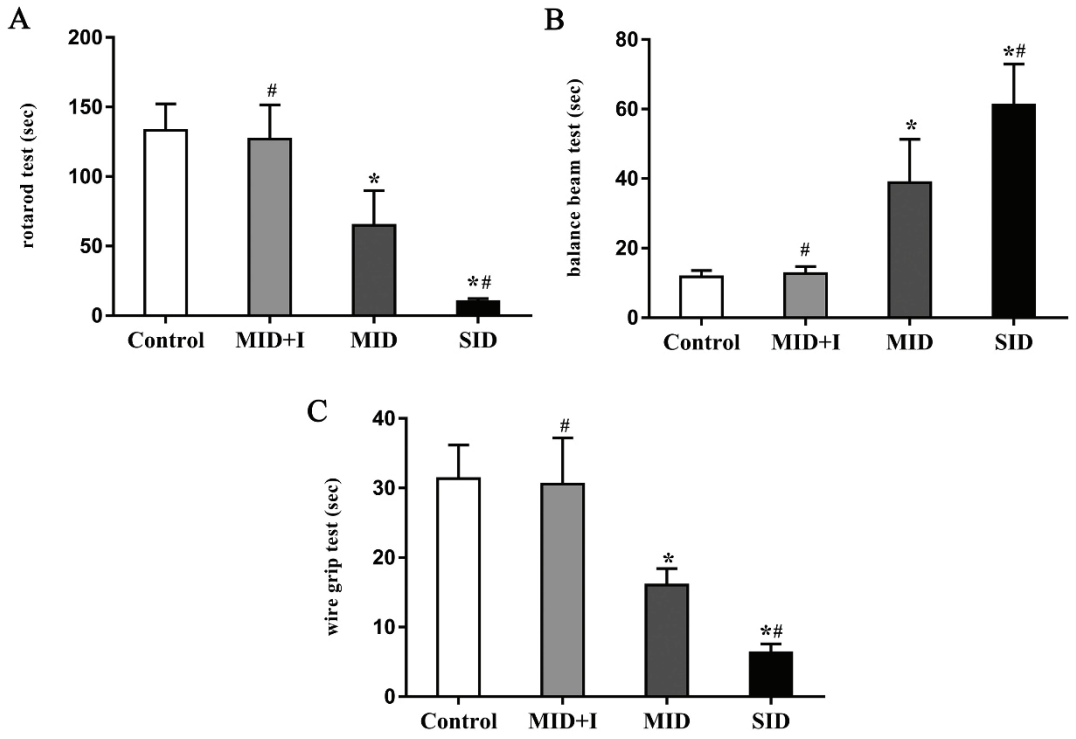

Figure 3 Performance on tests of motor coordination in offspring of rats with different iodine intake during pre-pregnancy, pregnancy and lactation (42). Controls had adequate iodine intake, MID had mildly iodine deficient diets, MID+I had mildly iodine deficient diets but received iodine supplements from conception, SID had severely iodine deficient diets. Offspring of iodine deficient rats (MID and SID) performed poorer on all tests, while offspring of supplemented rats (from day 0 of gestation) performed equally well as the controls. Reprinted from Wang Y *et al*. (2018) J Nutr Biochem 58:80-89, Copyright 2018, with permission from Elsevier.

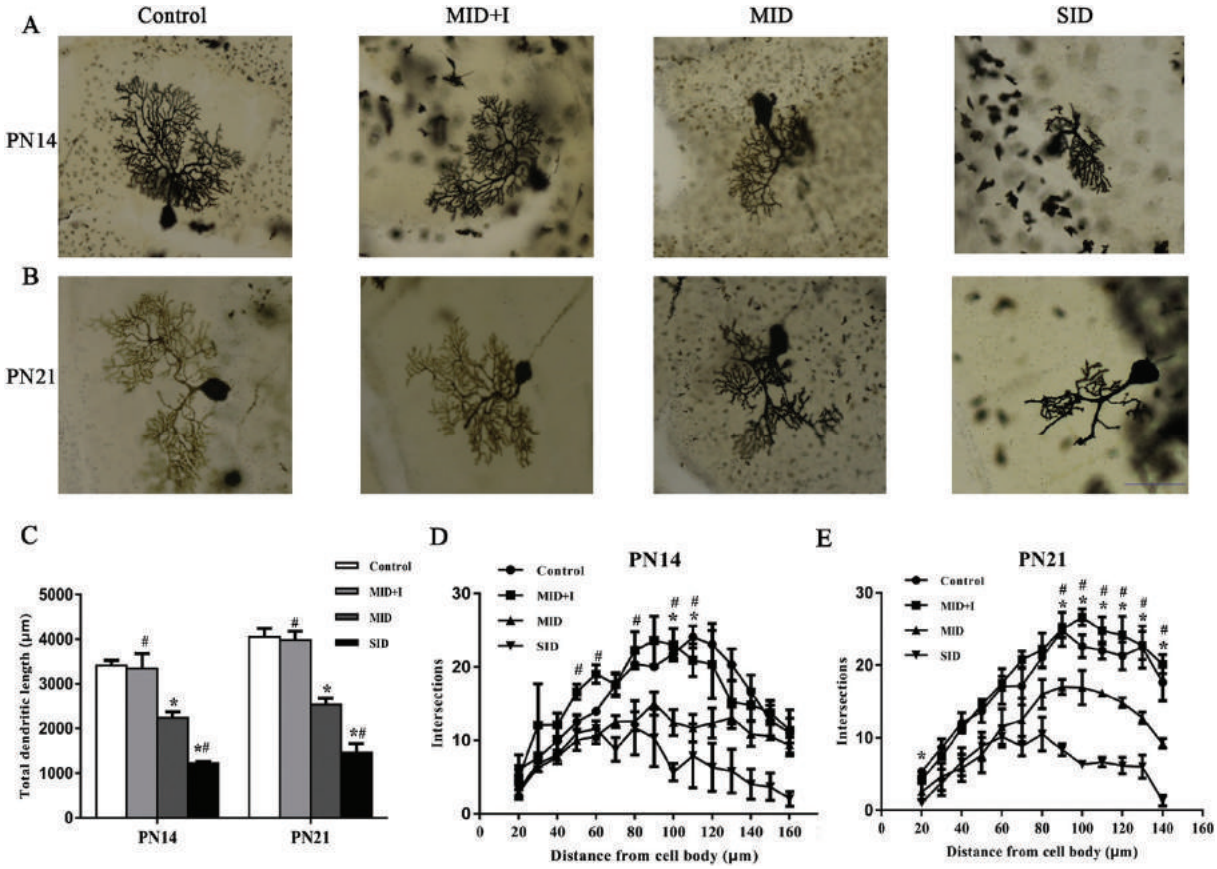

Figure 4 Dendritic branches of Purkinje cells in the cerebella of rat offspring at postnatal day 14 (PN14) and 21 (PN21) when born by rats with different iodine intake during pre-pregnancy, pregnancy and lactation (Control: adequate iodine intake, MID: mildly iodine deficient diet, SID: severely iodine deficient diet, MID+I: mildly iodine deficient diet and iodine supplement from conception) (42). The dendritic tree morphology was altered in offspring of iodine deficient rats, but iodine supplement from gestational day 0 prevented impairments. Reprinted from Wang Y *et al*. (2018) J Nutr Biochem 58:80-89, Copyright 2018, with permission from Elsevier.

Potential interaction with other nutrients or thyroid disrupting substances

Several other nutrients and non-nutrients are also known to affect thyroid hormone signalling. Examples are iron, selenium, zinc, thiocyanate in cigarettes, glucosinolates in cruciferous vegetables, and also a range of pollutants with thyroid-disrupting properties (43-45). It has been hypothesized that deficiencies in other micronutrients may aggravate ID and contribute to alter thyroid function (46). It has also been hypothesized that mild-to-moderate ID might make the developing foetus more vulnerable to thyroid disrupting chemicals which are often halogen-containing organic compounds (i.e. with fluorine, chlorine or bromine) (43). Today, there is an increasing concern about persistent organic pollutants in the environment causing adverse effects on child neurodevelopment, often via effects on thyroid hormone signalling (5).

In the twentieth century there was a substantial increase in population IQ (estimated to ~3 IQ points increase per decade) in both low- and high-income countries, and this phenomenon is often referred to as "the Flynn effect" credited to the researcher Flynn who first summarized the documentation in the 1980ies (47). Currently, newer data indicate that this trend has gone into reverse, and IQ is declining (48). A possible explanation to this "negative Flynn effect" has been suggested to be the increased exposure to thyroid disrupting chemicals in combination with a high prevalence of mild-to-moderate ID in pregnant women (43).

Preventing iodine deficiency – an international effort

In 1917, the first study documenting that iodine supplements could prevent goitre (thyroid enlargement) caused by ID was published (49). Switzerland was the first country in the world to introduce a salt iodization programme in 1922 (50). However, prevention of ID did not gain speed until studies were published in 1970-90 documenting that ID not only cause goitre, but also reduce the cognitive function, and ID was estimated to be the leading cause of preventable mental retardation around the world (50). In 1980, the first global estimate of ID was published by WHO reporting that ~20-60% of the world's population were iodine deficient (50).

Since then, tremendous effort has been made to eradicate ID, and many countries with a history of severe ID are today considered iodine sufficient due to salt iodization. This process has been guided by important international organisations such as WHO and the International Council for Control of Iodine Deficiency Disorders (established in 1986, and today named the Iodine Global Network (IGN)). Surprisingly, while elimination of ID is now an integral part of the nutrition strategies in most developing countries, it has not been prioritized by the health authorities in many industrialized countries (49). Particularly Europe is lagging behind and remains the continent with the highest prevalence of ID. Today, *severe* ID is almost completely eradicated, but mild-to-moderate ID is still prevalent in many countries and especially among pregnant women (33).

Iodine nutrition in Norway

In Norway, the natural iodine content in the soil and drinking water is low, and drinking water contains 0.5-5 µg/L (51). Before 1950, endemic goitre due to ID was documented in several regions of Norway, particularly in inland areas where the consumption of seafood was low (52). Moderate to severe ID affected the health of both humans and livestock, but in 1950 iodine was added to livestock feed to improve animal health. This action consequently and

"accidentally" boosted the iodine content of cow's milk making it the most important dietary source of iodine for milk-drinking Norwegians since then (52). Iodine was also added to table salt at a voluntary basis in 1938, and this salt is still available on the market. However, only 5 µg of iodine was added per gram of salt, so the contribution to the dietary intake was negligible. Based on a few, small, random studies measuring urinary iodine concentrations, the Norwegian population was considered iodine replete from the 1950'ies onwards, and the recent history of endemic ID was more or less forgotten.

In 2013, findings in the Norwegian Mother and Child Cohort Study (MoBa) indicated that ID had re-emerged in pregnant women in Norway (53). This could be explained by trends in the diet characterized by a substantial decrease in milk consumption combined with a low intake of seafood. Due to few food sources, iodine intake was highly dependent on individual food choices and the use of iodine-containing supplements (53). About the same time, it was also discovered that the iodine concentration in Norwegian milk was almost halved since year 2000 (54). This could be attributed to a change in feed composition with more use of rapeseed ingredients containing goitrogens inhibiting iodine uptake (55). Since 2012, the iodine concentration in the feed has been increased and the Norwegian milk now contains ~16 µg/dl. Nevertheless, this does not secure an adequate iodine intake for all women of childbearing age since many women have a low consumption of milk (55). In MoBa, 28% consumed less than 2 dl milk/yoghurt per day (53).

In 2018, the Directorate of Health issued an advise for all women of childbearing age with a low milk intake (i.e. <3 dl milk including yoghurt per day, or <5 dl per day if fish intake is low) to take an iodine supplement (100 µg/day). Pregnant and lactating women with a low milk intake (<6 or 8 dl per day depending on fish intake) are recommended a supplement providing 150 µg iodine per day. However, the knowledge about iodine in the population is very low (56, 57), and these recommendations are currently "well hidden" on a web page and not very actively communicated. More recent studies (published in 2017-18) have confirmed that large groups of the Norwegian population, including women of childbearing age, and pregnant and lactating women, have insufficient iodine intake (57-64). Median UIC of the pregnant women in these studies range from 84 to 92 µg/L, well below the cut-off for median UIC used to define an adequate iodine intake for pregnant women by WHO (i.e. ≥150 µg/L).

Although strongly recommended by WHO, there is still no established routine monitoring of iodine status in Norway, but the Norwegian Scientific Committee for Food Safety are currently assessing different strategies to implement adequate salt iodization to prevent ID.

Table 2 Dietary sources of iodine in the Norwegian diet. Contribution to iodine intake from a standard portion size.

Food item	Iodine µg/100 g [a]	Regular portion size [b]	Iodine µg/portion	Percent of RDI per portion [c]
Iodized salt for home use	500	1 gram	5	3
Milk	15	One glass (2 dl)	30	20
Flavoured yoghurt	13	One unit (150 g)	20	13
White cheese	27	For one slice of bread (20 g)	5	3
Brown cheese	203	For one slice of bread (16 g)	32	22
Mackerel	20	One dinner portion (150 g)	30	20
Cod	279	One dinner portion (200 g)	558	372
Farmed salmon	7	One dinner portion (150 g)	11	7
Caviar	85	For one slice of bread (15 g)	13	9
Mackerel in tomato sauce	15	For one slice of bread (40 g)	6	4
Egg	35	One egg (56 g)	20	13
Tap water	0,2	One glass (2 dl)	0,4	0,3

[a] Data from the Norwegian food composition table (matvaretabellen.no, accessed 1 June 2018)
[b] Standard Norwegian portion sizes (65)
[c] RDI; Recommended daily intake of iodine for adults (150 µg/day) according to WHO (9) and the Nordic Nutrition Recommendations (7)

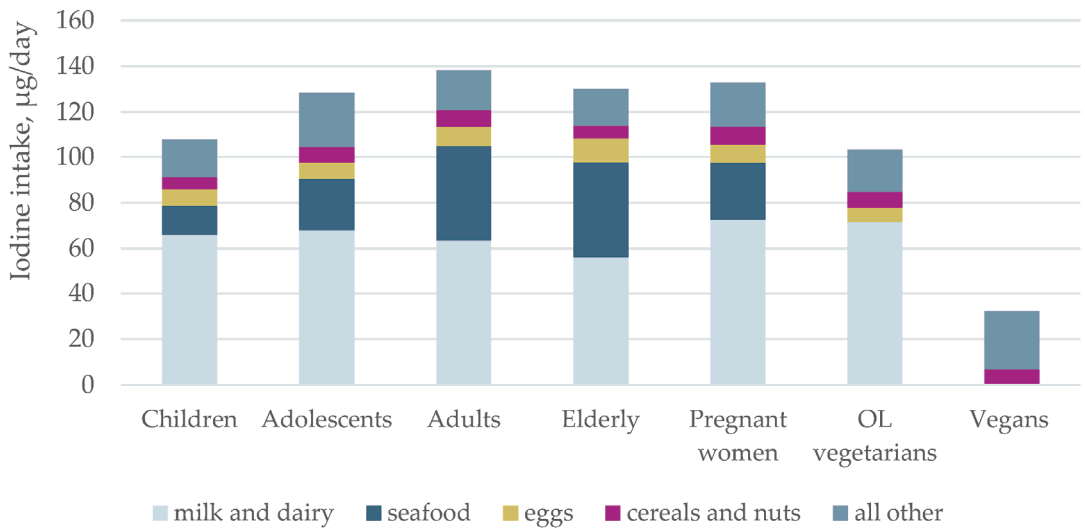

Figure 5 Contribution of different foods to the iodine intake in the Norwegian diet. Study participants were a convenience sample from the Oslo-area (total *n*=276, pregnant *n*=45) and results were based on 2 x 24-h weighed food records (58).
Abbreviation: OL vegetarians: ovo-lacto vegetarians
Reprint from Brantsaeter AL *et al.* Nutrients. 2018;10(2) (Open access, free to share; CC BY 4.0)

MoBa - a unique opportunity to explore impact of prenatal iodine nutrition

To our knowledge, the Norwegian Mother and Child Cohort Study (MoBa) is the world's largest pregnancy cohort in terms of participants (more than 114,000 mother-child pairs and 75,000 fathers) and the extensive collection of data (multiple questionnaires and biological samples) (66). MoBa can also be linked to several national registries through a personal ID-number, like the Norwegian Patient Registry, the Norwegian Prescription Database, and the Norwegian New-born Registry. MoBa is of unique value for research on iodine nutrition in pregnancy because of its size, but also since there is data on habitual iodine intake in the first half of pregnancy based on an extensive and validated MoBa food frequency questionnaire (FFQ) (67, 68). Data on maternal habitual diet in the first half of pregnancy provides an indicator for long-term diet and iodine intake also prior to pregnancy. Mothers reported their use of iodine-containing supplements in the MoBa FFQ, but unfortunately, the question on supplement-use was not clear as to what time period it covered (i.e. current use, average use since becoming pregnant, or use only while using). Thus, data on supplement use is limited to any use/no use in gestational week 0-22, and to timing of initiation of use (this was asked about in other questionnaires).

In MoBa, maternal habitual iodine intake from food did not vary by maternal age, marital status, and income, and varied only to a very small extent (up to 7%) with body mass index

(BMI), parity, education, and smoking (53). The large number of participants makes it possible to explore potentially weak and non-linear associations to multiple outcomes on child neurodevelopment, and biological samples facilitate studying mechanisms, interactions, and mediating factors. Thus, data from MoBa can provide further insight into what is the optimal range of iodine intake for pregnant women, and it can also suggest what the neurodevelopmental consequences of prenatal ID are.

Neurocognitive function and mental health – relevance for society

If strategies to prevent mild-to-moderate ID can prevent loss of cognitive ability and IQ-points, this is important and highly cost-effective both at a societal level, but also at a personal level, since IQ is associated with educational attainment and income, as well as general health and wellbeing (69). A potential loss of 1-3 IQ points due to mild-to-moderate ID (37) may not make a *big* difference to an individual's intellectual capacity, but it can substantially increase the risk of being intellectually disabled (IQ<70) and decrease the chance of being highly intelligent (IQ>130) at a population level. Also, since mild-to-moderate ID affects such large parts of the population, a loss of even just 1 IQ point can have substantial economic consequences (69).

In Norway, an estimated 15-20% of children and adolescents under age 18 years have reduced function due to symptoms of mental disorders such as anxiety, depression and behaviour disorders, and about half of these meet the requirements for a psychiatric diagnosis (70). Attention difficulties, attention-deficit/hyperactivity disorder (ADHD) and behaviour disorders are most common in younger children and in boys, whereas in older children and in girls, anxiety and depression are predominant and the incidence is rising (70). In most children, symptoms are temporary, and surveys indicate that about 1/3 of 16-year-olds have at some stage had enough symptoms to meet the criteria for a psychiatric diagnosis (70). In 2017, the use of ADHD medication in children aged 10-14 years was 1.5% in girls and 4.1% in boys, and in adolescents aged 15-19 years use of antidepressants were 3.1% in girls and 1.2% in boys (71). The worldwide prevalence of ADHD in children and adolescents is estimated to be 5-7% (72).

If mild-to-moderate ID is a causal factor for mental health disorders, then securing adequate iodine nutrition is an important part of preventive medicine. "Mental health and well-being are fundamental to our collective and individual ability as humans to think, emote, interact with each other, earn a living and enjoy life" (WHO, 2013 (73)). According to WHO, mental-,

neurological- and substance use disorders account for nine out of the 20 leading causes of years lived with disability worldwide and 10% of the global burden of disease (73).

Gaps in knowledge

Summing up, the detrimental effects of severe ID are well documented, but there are still important gaps in knowledge when it comes to mild-to-moderate ID in pregnancy. In 2016, Pearce *et al.* (33) highlighted the urgent need to elucidate:

- What are the potential consequences of maternal mild-to-moderate ID in pregnancy on child neurodevelopment
- What are the specific domains in neurodevelopment affected by mild-to-moderate ID
- What is the impact of iodine supplement use in pregnancy in mild-to-moderate ID
- What is the effect of concurrent micronutrient deficiencies, particularly of iron and selenium which are important for thyroid function

In addition, more knowledge is needed regarding what is the optimal range of iodine intake for pregnant women. Today, the recommended iodine intake for pregnant women vary greatly between countries/institutions. Aiming to secure an adequate iodine intake for pregnant women should not put other groups of the population at risk of iodine excess and therefore should not aim higher than necessary.

Aims and research questions

The aim of this project was to make use of the potential in MoBa (within the frames of a Ph.D.-project) to explore the association between maternal iodine intake and child neurodevelopment in a population characterized by mild-to-moderate iodine deficiency in pregnant women.

Main research questions

1. Is maternal habitual iodine intake (calculated by an FFQ covering the first half of pregnancy) in non-users of iodine-containing supplements associated with:
    a. Maternal thyroid function in gestational week 18 (Paper 3)?
    b. Child language skills, motor development, and behaviour problems at ages 3 and 8 years, and school performance at age 8 years (Papers 1, 2, and 4)?
    c. Risk of ADHD diagnosis in the child (Paper 2)?
2. What is the impact of taking an iodine-containing supplement in pregnancy on the outcomes listed above, and are potential effects dependent on timing of initiation (Papers 1-4)?

Other research questions

- What is the iodine status of the MoBa pregnant women (Paper 3)?
- What is the iodine status of 8 year old children in MoBa (Paper 4)?
- Does the MoBa FFQ provide a valid measure of habitual iodine intake (Paper 3)?
- Is maternal spot-UIC in gestational week 18 (measured in a subsample of 2910 participants) associated with maternal thyroid function in gestational week 18 or child language and learning at age 8 years (Papers 3 and 4)?
- Does maternal selenium status, iron status, and/or intake of cruciferous vegetables affect the association between maternal iodine status and thyroid function (Paper 3)?
- Is maternal iodine intake related to loss to follow-up in the 3- and 8 year questionnaires (Papers 1, 2, and 4)?
- Is maternal iodine intake differently associated with neurodevelopment in boys and girls (Papers 1, 2, and 4)?
- Based on the results from MoBa, what is indicated as being the optimal iodine intake in pregnancy (Thesis)?

Subjects and methods

This study is based on data from MoBa and selected sub-studies of MoBa (MoBa eTox and HELIX), and on data on MoBa-participants from the Norwegian Patient Registry (NPR).

Study population

The Norwegian Mother and Child Cohort study (MoBa) – Papers 1-4

MoBa is an ongoing prospective population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health (66, 74). Women pregnant in their first trimester were recruited from all over Norway during the years 1999 to 2008. Participants were recruited to the study by postal invitation before the routine free ultrasound examination at around gestational week 18. The women were asked to provide blood and urine samples at baseline and to answer questionnaires (in Norwegian) at regular intervals during pregnancy and after birth (questionnaires available at http://www.fhi.no/moba). More than 99% of the participants were of Caucasian origin. Pregnancy and birth records from the Medical Birth Registry of Norway are linked to the MoBa database (75).

The women consented to participation in 40.6% of the pregnancies. The cohort now includes 114,500 children, 95,200 mothers and 75,200 fathers. In our project, we used data based on versions 8 (paper 1), 9 (paper 2), and 10 (papers 3 and 4) of the quality-assured data files released for research in 2015-17. The flow chart of inclusion is shown in **Figure 6**, page 25.

The Norwegian Patient Registry (NPR) – Paper 2

The NPR is a nationwide registry on diagnoses assembling data from mandatory registration of diagnoses performed by all government-owned hospitals and outpatient clinics (76). NPR is owned by the Government and administered by SINTEF Health Research, a non-profit research organization. NPR was established in 1997, but started collecting individual-level data in 2008 based on codes defined in the International Classification of Diseases-10 (ICD-10). Healthcare is free of charge for children under the age of 16 years in Norway. There are only few private practitioners, and currently they do not register diagnoses at an individual level (accounts for an estimated 5-10% of the ADHD diagnoses in NPR) (77). In our study, we included data on ADHD diagnosis in MoBa-children registered by Dec. 2015.

The MoBa eTox – Papers 2-4

The MoBa eTox substudy is a biomonitoring study based on a selected sample of n=2999 triads (mother, father, and child) from MoBa, and the aim is to examine the importance of

specific nutrients (vitamins and essential elements) and heavy metals in relation to risk of developing health problems. Inclusion criteria were: available data from the Medical Birth Registry, singleton birth, complete dataset of questionnaires up to child's age 3 years (questionnaires 1-6 and father questionnaire), complete set of biological material from mother, father and child, child alive and living in Norway in 2014, and samples not reserved for a study on autism (which included all children with autism diagnosis or symptoms of autism, including severe language delay). The MoBa eTox study-sample was drawn from *n*=24,264 eligible triads (i.e. 22% of all MoBa pregnancies) and by design comprised highly dedicated families. In MoBa eTox, maternal plasma, whole blood, and urine samples from gestational week 18 (mean: 18.5, SD: 1.3) were analysed for a range of factors including plasma TSH, FT4, FT3, thyroid peroxidase antibodies (TPOAb), thyroglobulin antibodies (TgAb), and ferritin, whole blood selenium, and urinary iodine and creatinine. Our study included *n*=2910 of the MoBa eTox participants, excluding participants with non-valid FFQs (*n*=30) and those who had reported use of thyroid medication in pregnancy (*n*=59) (see flow chart of inclusion in Fig. 1, paper 3).

The Human Early-Life Exposome (HELIX) project – Paper 4

HELIX is a collaborative research project including *n*=32,000 participants from six existing birth cohort studies in Europe of which *n*=8000 are from MoBa (78). The aims of the study are to implement novel exposure assessment and biomarker methods to characterize early-life exposure to multiple environmental factors and associate these with omics biomarkers and child health outcomes, thus characterizing the "early-life exposome". Biomarkers are measured in a subset of *n*=1200 mother-child pairs, and this sample includes ~300 MoBa children living in the Oslo area that were 8-9 years in 2014-15. Originally, we planned to use data from the clinical examination of these children which comprised a computer test measuring a range of neurodevelopmental outcomes, and parent-completed questionnaires on child behaviour. However, with only 300 MoBa participants in HELIX, the statistical power to detect associations with maternal iodine status in pregnancy was too low, and therefore we decided it would not be right to include data on child neurodevelopment from HELIX. We did include data on urinary iodine concentration from the 8-9 year old HELIX-children (*n*=279) analysed in a sample consisting of 50% evening spot urine and 50% following morning spot urine. Except for UIC, no data from HELIX was made available to our project.

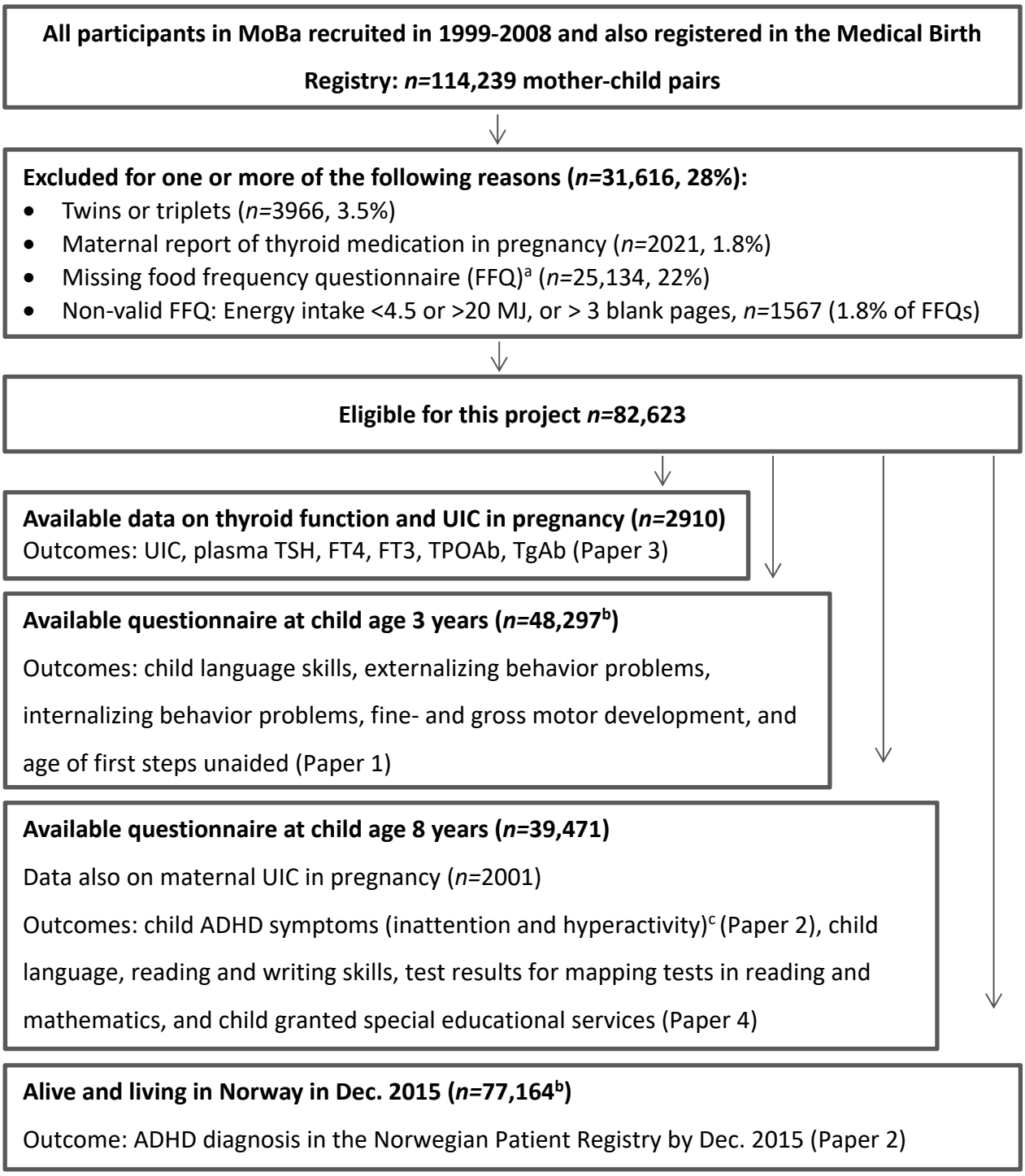

Figure 6 Flow chart of inclusion

[a] The food frequency questionnaire was in use in MoBa from 2002

[b] Only complete cases (participants with no missing covariates) were included in the analyses in papers 1 and 2 (data from 3 years and on ADHD). Missing values on covariates were imputed for papers 3 and 4. About 4% had missing on one or more covariate.

[c] Child ADHD symptoms were analysed based on version 9 of the MoBa data files when the collection of data at child age 8 years was not yet complete (*n*=27,945).

Exposure variables

Around gestational week 22, mothers were asked to complete an extensive food frequency questionnaire (FFQ) specifically designed for MoBa (79). The MoBa FFQ was introduced in MoBa in 2002, and therefore participants recruited before 2002 are not included in our study sample. The FFQ asks about the habitual (average) intake of food and supplements after becoming pregnant (i.e. gestational week 0-22) and contains 340 questions (67). The participants reported the frequencies of consumption of 255 different foods and dishes, and nutrient intake was calculated based on standard Norwegian portion sizes, the Norwegian food composition table, published analyses of Norwegian milk and food samples (51, 80), and data on >1000 food supplements collected from suppliers (81). The MoBa FFQ has previously been validated (68, 82), and results showed that the FFQ provides a realistic estimate of habitual intake of energy, nutrients, foods, and of iodine. The reference methods for iodine intake in the validation study were a 4 day weighed food diary and 24 hour urinary iodine excretion. Iodine supplement use was also reported in the general questionnaires (completed in GW 18 and 30) for specific time intervals (use/no use, no data on dosage).

Iodine from food in non-users of iodine-containing supplements

Originally, we planned to use *total* iodine intake (from food and supplements) as our primary exposure. This was changed when we discovered from the literature that the effect of habitual/long-term iodine intake might be different from short-term supplement use (40). Also, the questions on supplement use in the MoBa FFQ could easily be misinterpreted, so we were not sure if the women had reported their current intake of supplements (in GW 22), their average use since becoming pregnant (GW 0-22), or their use only in periods they were using.

To isolate the effect of habitual iodine intake, we thus decided to explore associations between iodine intake from food and outcomes in non-users of iodine-containing supplements.

Iodine supplement use (dosage, timing, current use)

Since we were unsure of what time period the mothers had reported supplement use for in the FFQ, we decided to explore iodine supplement dosage in categories (no use, 1-200 µg/day, and >200 µg/day). If the mother had reported taking >200 µg/day, this would represent a high intake regardless of what time period she had in mind when completing the FFQ (i.e. her current use, average use in GW 0-22, or use only while using).

About 2/3 of the mothers who had reported use of iodine-containing supplements in the FFQ had also reported use in one or more time interval in the general questionnaires. We generated a variable for the first reported use (no use, initiated use 0-26 weeks before conception, initiated use in GW 0-12, or in GW>12) to explore whether timing of initiation was associated with outcomes.

Some mothers had reported use of iodine-containing supplements in the general questionnaires, but not in the FFQ and vice versa. For papers 3 and 4, all these participants were defined as iodine supplement users, but then, no data was available on dosage (only use/no use in GW 0-22).

Urinary iodine concentration (UIC)

UIC was available for a subsample of MoBa women (*n*=2910) participating in the MoBa eTox study. UIC was measured in the urine sample collected at the routine ultrasound examination offered in gestational week 18 (mean GW: 18.5, SD: 1.3). UIC was measured per litre and per gram creatinine. Method for analyses are described in Paper 3.

Outcome variables

In MoBa, a range of measures on neurodevelopment are included in the questionnaires at different ages. The measures are in most cases based on international standard instruments like for example the Ages and Stages Questionnaire (83) and the Child Behaviour Checklist (84). We decided to have a broad approach and include outcomes that provide valid measures of cognitive ability (language/communication, reading, writing, and mathematics), motor development (fine and gross), and of behaviour problems (externalizing, internalizing, and ADHD). The 3-year questionnaire was selected because it had a high participation rate, the data collection was completed, and it included measures of language, communication, fine- and gross motor skills, and on behaviour problems. The 8-year questionnaire was included because the data collection would be completed by 2017, and it contained questions about school performance and special educational services in addition to a language scale and ADHD-symptoms.

Measures of maternal thyroid function in GW 18 was included to study mechanisms by which ID could affect neurodevelopment. The following parameters were measured in plasma in the women in MoBa eTox (*n*=2910): TSH, FT4, FT3, TPOAb, and TgAb.

An overview and brief description of all outcomes are provided in **Table 3**. For a more detailed description of the outcomes, readers are referred to the original papers attached.

Changes from the original protocol

We originally planned to include one more substudy in MoBa, the ADHD Study, but this was abandoned due to several reasons, most importantly because of issues regarding lack of statistical power and selection bias (see **Appendix 1**).

Also, we originally planned to explore the iodine intake of the MoBa children as an additional exposure. We later decided not to include this exposure variable since the data on food intake of the children was very limited, and also since the iodine status of children in Norway was probably adequate (at a group level) (51, 55, 85, 86). We finally concluded that the publications would be too complicated and comprehensive if we also included this dimension in the analyses, and we decided to focus on prenatal exposure to ID in this project.

Table 3 A brief description of the outcomes included in papers 1-4

Outcome variables	Time	Measure	n	Description
Measures of maternal thyroid function (Paper 3)				
Urinary iodine concentration	GW 18	µg/L	2910	Spot-urine sample, mean GW 18.5 (SD: 1.3)
Plasma TSH	GW 18	mU/L	2901	Blood sample, mean GW 18.5 (SD: 1.3)
Plasma FT4	GW 18	pmol/L	2900	Blood sample, mean GW 18.5 (SD: 1.3)
Plasma FT3	GW 18	pmol/L	2901	Blood sample, mean GW 18.5 (SD: 1.3)
Plasma TPOAb	GW 18	>6.6 IU/ml	2900	Blood sample, mean GW 18.5 (SD: 1.3)
Plasma TgAb	GW 18	>7.0 IU/ml	2900	Blood sample, mean GW 18.5 (SD: 1.3)
Child development at age 3 years (Paper 1)				
Language delay	3 years	yes/no	48.020	Based on a question on typical sentence complexity (89)
Severe language delay	3 years	yes/no	48.020	Based on a question on typical sentence complexity (89)
Communication skills	3 years	z-score	48.161	6 items from ASQ validated in (90)
Externalizing behaviour problems	3 years	z-score/ >1.5 SD	47.977	11 items from CBCL (88) covering aggressive behaviour (7 items) and attention problems (4 items)
Internalizing behaviour problems	3 years	z-score/ >1.5 SD	47.978	9 items from CBCL (88) covering anxiety/depression (3 items), emotionally reactive behaviour (2 items), and somatic complaints (4 items)
Fine motor skills	3 years	z-score	47.792	2 items from ASQ validated in (90)
Gross motor skills	3 years	z-score	48.087	2 items from ASQ validated in (90)
Motor milestone – first steps unaided	Up to 3 years	>17 months	60.318	Maternally reported age at first steps unaided in questionnaires at child age 18 and 36 months
Child development at age 8 years (Paper 4)				
Language skills	8 years	z-score	39.229	13 items from CCC-S (91) covering speech, vocabulary, grammar and discourse.
Reading skills	8 years	z-score	31.822[a]	3 items on reading skills the Vineland Adaptive Behaviour Scale-II (92)
Writing skills	8 years	z-score	31.812[a]	2 items on writing skills from the Vineland Adaptive Behaviour Scale-II (92)
Mapping test in mathematics	8 years	ordinal	37.433	Mothers reported teachers feedback on test-results on a national, mandatory mapping test: “mastered subject well”, “must work more but teacher is not concerned”, or “teacher is concerned”
Mapping test in reading	8 years	ordinal	38.619	Mothers reported teachers feedback on test-results on a national, mandatory mapping test: “mastered subject well”, “must work more but teacher is not concerned”, or “teacher is concerned”
Special educational services	8 years	yes/no	39.471	The child granted any special educational services at school (yes)
Risk of ADHD (Paper 2)				
Diagnosis in the Norwegian Patient Registry	By Dec. 2015	yes/no	77.164	ADHD diagnosis registered in the Norwegian Patient Registry by Dec. 2015 (child age 9.9 years (range: 6.4-13.8). ADHD was defined for ICD-10-diagnosis of hyperkinetic disorder coded as F90.0, F90.1, F90.8, or F90.9
Maternally reported ADHD symptoms	8 years	z-score	23.804[b]	Four-point Likert scales (never/rarely, sometimes, often, or very often) covering inattention problems (9 items) and hyperactivity/impulsivity (9 items) from the ADHD Rating Scale (93)

[a] The items were not included in the first version of the MoBa 8 year questionnaire.

[b] For ADHD symptoms, the sample was based on version 9 of the MoBa files when the collection of data at age 8 years was not yet complete.

Abbreviations: ASQ: Ages and Stages Questionnaire, CBCL: Child Behaviour Checklist, CCC-S: Children’s Communication Checklist – Short Version

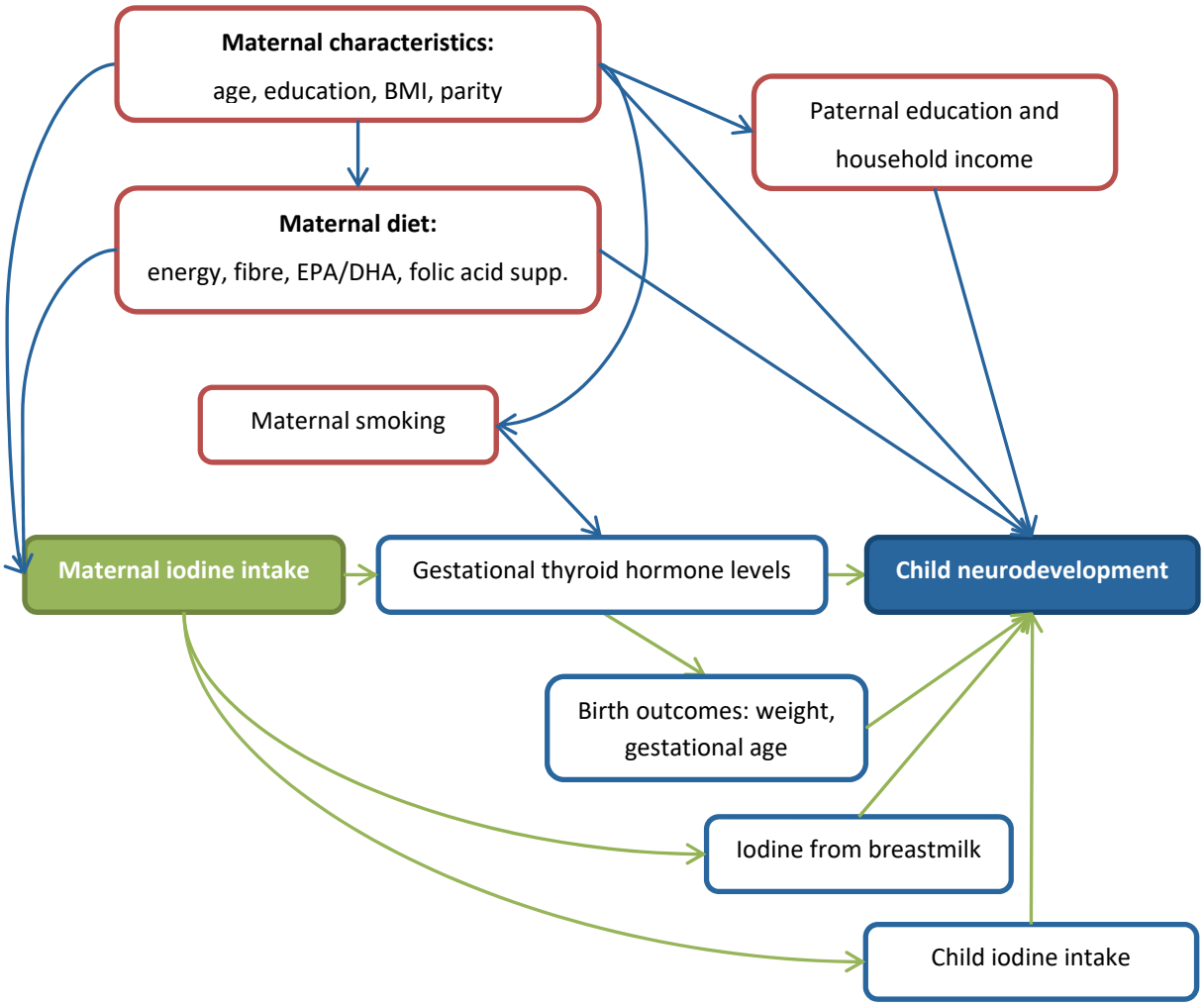

Figure 7 Simplified directed acyclic graph (DAG) illustrating the association between maternal iodine intake in pregnancy and child neurodevelopment, confounding factors (red boxes) and mediators (blue boxes). Green lines illustrate potential causal pathways. Potential effect modifications by maternal intake of cruciferous vegetables and selenium- or iron status are not included in the DAG.

Covariates

Covariates were included in the models based on knowledge from previous literature and are visualized for child neurodevelopmental outcomes in a simplified directed acyclic graph (DAG) in **Figure 7**, page 30.

All adjusted models included the following covariates (self-reported unless other is specified):

- Maternal age at time of birth (continuous measure, years, Medical Birth Registry)
- Maternal pre-pregnancy BMI (continuous measure, kg/m^2)
- Maternal education (four categories: ≤12, 13-16, 17+ years)
- Parity (previous pregnancies ≥22 weeks, three categories: 0, 1, ≥2)
- Marital status reported in pregnancy (married/cohabitant: yes/no)
- Fibre intake calculated based on the FFQ (marker of a healthy diet, continuous: g/day)
- Smoking in pregnancy (three categories: i) no reported smoking in pregnancy, ii) reported occasional smoking or stopped smoking before week 12, iii) reported daily smoking at any time in pregnancy and hadn't stopped smoking before week 12)

Models that included iodine from food as the exposure or in interaction-terms:

- Maternal energy intake calculated based on the FFQ (continuous: MJ/day)

Models exploring impact of iodine supplements:

- Chronic illness before or during pregnancy (asthma, diabetes, inflammatory bowel disease, rheumatic disease, epilepsy, multiple sclerosis or cancer before or during pregnancy (yes/no))
- Any use of folic acid supplement in the time period from 4 weeks before to 8 weeks after conception (yes/no)
- Total intake of the long chained marine omega 3 fatty acids eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) contributed by food and supplements in GW 0-22 (expressed as g/day)

Models with creatinine-adjusted UIC as exposure:

- Urinary creatinine concentration (g/L)

For models on specific outcomes:

- Continuous outcomes on child development: child sex (an important determinant of the outcomes) (not included in paper 1)
- Language and communication: bilingual mother and/or father (yes/no)
- ADHD (continuous symptom score): birth season (important determinant of ADHD)
- Reading and writing skills: maternal history of reading and writing difficulties (yes/no)
- Plasma values of TSH, T4, T3: gestational age at sampling (days), iron status (plasma ferritin <12, 12-30, <30), and selenium status (whole blood selenium <80, ≥80 µg/L)
- Plasma TPOAb, TgAb: iron status (plasma ferritin <12, 12-30, <30), and selenium status (whole blood selenium <80, ≥80 µg/L)

Statistical analysis

Statistical analyses were performed in STATA (versions 14 or 15; Stata Corp., College Station, TX).

In papers 1 and 2, only complete cases were included in the analyses. For papers 3 and 4, we decided to use multiple imputation as a technique to handle missing information on covariates, but not on exposures or outcomes. Four percent of the women had missing data on one or more covariate, mainly on pre-pregnancy BMI and maternal education. To maximize the use of available data, minimize bias, and to obtain more appropriate estimates of precision, we imputed missing values in STATA by multiple imputation by chained equations (MICE) and generated 20 imputed datasets for analyses.

Associations between the exposure iodine from food (or UIC) and outcomes were explored by multivariable regression analyses using flexible modelling techniques for the potential non-linear associations (restricted cubic splines, 3-4 knots). Overall associations were tested by testing the betas of all spline transformations equal to zero (testing H0: no association). Non-linearity was tested by testing the second and higher spline transformations equal to zero.

Covariates were included in the models based on DAGs (see **Figure 7**, p. 30 and Online Supplemental Material for papers 2-4). We explored potential non-linearity in associations between continuous covariates and outcomes while controlling for all variables included in the final models, and if non-linearity was detected, the covariates were included as restricted cubic spline variables. Since some mothers participated in MoBa with more than one

pregnancy, we controlled for random effect of sibling clusters (mother ID clusters) in all models and reported robust confidence intervals for all results.

In papers 1 and 2, iodine from supplements was categorized by dosage (no use, 1-200 µg/day, >200 µg/day), and by timing of first reported use (no use, 0-26 weeks before conception, GW 0-12, and GW>12). Impact of supplement use (dosage or timing) was explored by strata of iodine intake from food (above/below the estimated average requirement) by including interaction terms between iodine supplement-categories and iodine from food-categories in the regression models.

In papers 3 and 4, impact of any iodine supplement use in GW 0-22 (yes/no) and timing of first reported use (same definition as in papers 1 and 2) was explored by including interaction terms with iodine from food (as restricted cubic spline variables with 3 knots). If the interaction terms were not significant ($p<0.05$), iodine from food was excluded from the models and results reported by supplement category (by use/no use and timing of initiation).

In paper 1 we calculated attributable risk fraction for the dichotomous outcomes, i.e. the percentage of the outcome that could be attributed to having a low iodine intake compared to a thought scenario of having an iodine intake of 160 µg/day. This measure was used to translate the complex results from the flexible models into more meaningful summary measures.

A p-value<0.05 was considered statistically significant. The issue of multiple testing was considered and is further elaborated in the discussion.

Sensitivity analyses were performed for the main models in each paper and are described in the respective papers. The sensitivity analyses were conducted to test the robustness of the results in the presence of uncertainty. Examples are:

- Repeating the tests in a subsample with calculated energy intake by the FFQ close to the mean value (±1 SD) since this subpopulation presumably had lower measurement error of the exposure variable
- Check if iodine from milk/yoghurt and iodine from fish exhibits similar associations to outcomes as total iodine intake from food
- Check if allowing even more flexibility in the associations (by applying a higher number of knots) change the shape of the association curves and if results from models with a categorized exposure (e.g. quintiles) are similar

- Exclude/include outlying values of the outcome
- Check if associations are similar in different strata (by child sex, maternal education and maternal smoking in pregnancy)

Ethics

MoBa is conducted according to the guidelines laid down in the Declaration of Helsinki and written informed consent was obtained from all participants. MoBa has obtained a license from the Norwegian Data Inspectorate. The current study was approved by The Regional Committee for Medical Research Ethics South East Norway 2014/2211. Our project is based on already existing data.

Literature search strategy

In order to be updated on relevant literature, I have since September 2014 subscribed to weekly e-mail updates from PubMed on new literature matching the following search criteria:

1) To monitor relevant publications on iodine: (((((iodine pregnancy) OR iodine deficiency) OR iodine neurodevelopment) OR iodine cognitive) OR iodine IQ) OR iodine supplement
2) To monitor new publications from MoBa: (Norwegian mother and child cohort study)

In 2014, I also scanned all titles/abstracts matching the search criteria listed above for the time period before 2014. The searches were broad, and all titles/abstracts were read to identify relevant papers. These references were imported into EndNote and categorized by topic. In addition, I also found relevant papers, reports, and books from reference lists, in newsletters from WHO and the Iodine Global Network (http://www.ign.org/), and from colleagues.

Summary of results

Iodine status of the MoBa population

Maternal iodine intake from food in the first half of pregnancy is shown in **Figure 8**, p. 36. Median calculated iodine intake from food was 121 µg/day (IQR: 89, 161 µg/day, range: 9-792 µg/day, 90 percent range: 54-245 µg/day), and it was not different in users and non-users of iodine-containing supplements (difference in mean: 0.2 µg/day, p=0.70) or in responders and non-responders to the questionnaires at child age 3 and 8 years (**Table 4**, p. 38). The distribution reveals a large variation in iodine intake, and a high proportion of women had an intake below the recommended intake (**Figure 8**). The dietary sources of iodine by level of iodine intake from food are shown in **Figure 9**.

In women who did not use iodine-containing supplements, the prevalence of insufficient iodine intake was high. In 95%, the calculated habitual intake was below the WHO recommendation of 250 µg/day for pregnant women (13), 80% had an intake below the Nordic recommendation of 175 µg/day (7), and 74% had below the estimated average requirement by the U.S. Institute of Medicine (16)).

Based on reporting in the FFQ, the prevalence of use of iodine-containing supplements in GW 0-22 was 31% (difined as supplement users in papers 1 and 2), and the distribution of calculated dosage is shown in **Figure 10**, p. 39. When including also iodine supplement use reported in the general questionnaires covering the same time period, the prevalence was 37% (used in papers 3 and 4 when defining iodine supplement users).

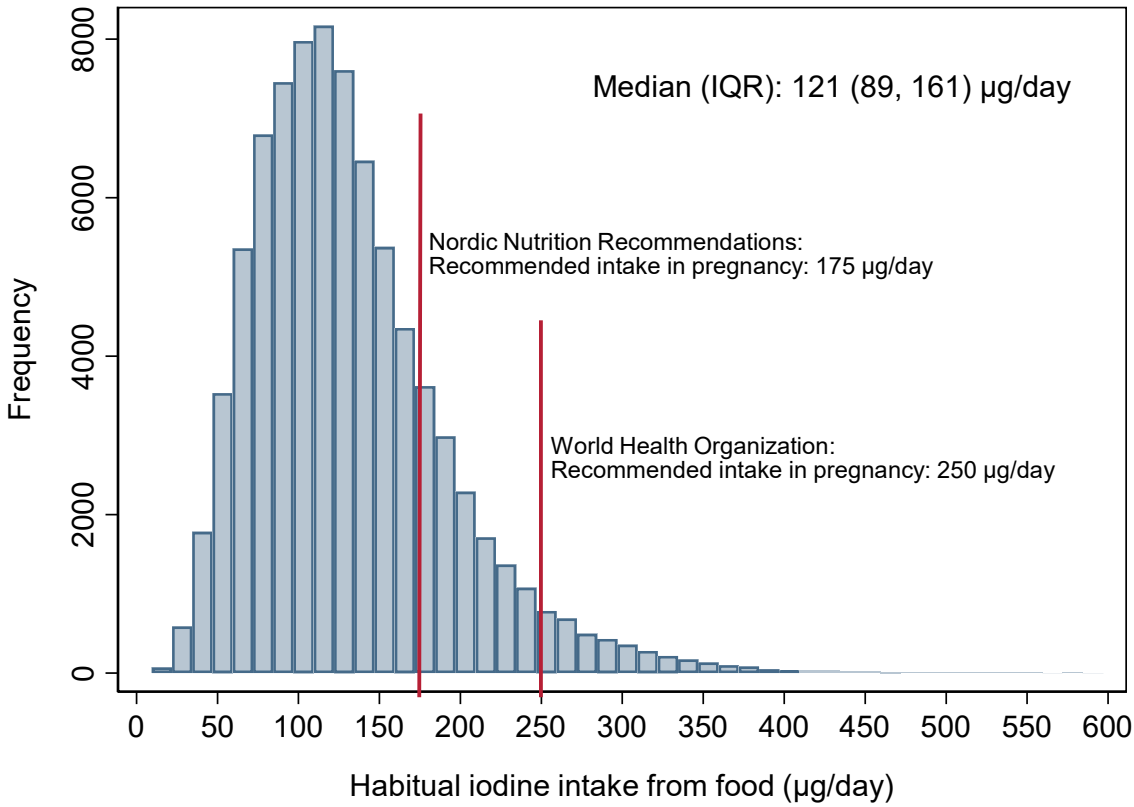

Figure 8 Maternal habitual iodine intake from food (*n*=82,623). The sample consists of participants eligible for our iodine project (singleton pregnancies, available food frequency questionnaire (FFQ), no reported use of thyroid medication in pregnancy, and recruited in pregnancy in 2002-08). Iodine intake was calculated based on the MoBa FFQ. Participants with iodine intake from food >600 µg/day (*n*=11) are not shown. The range of iodine intake from food was 9-792 µg/day.

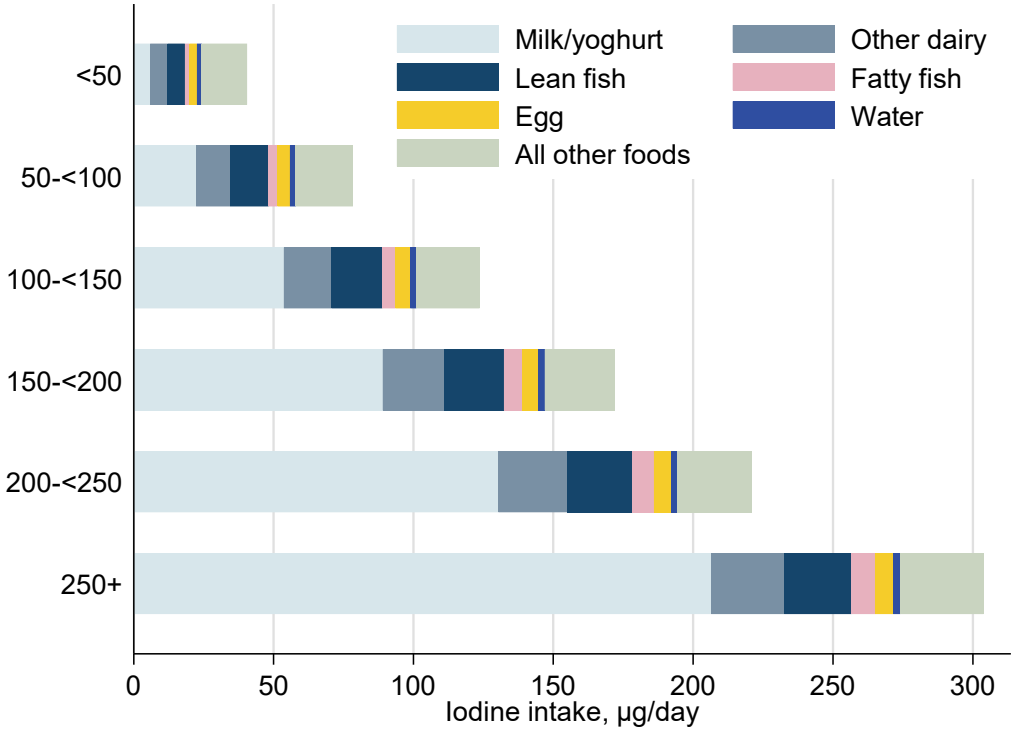

Figure 9 The mean contribution to iodine intake (µg/day) from different food groups by exposure level (i.e. groups defined by calculated total iodine intake from food based on the MoBa food frequency questionnaire, µg/day). In the total sample (*n*=82,623 pregnant women), the mean contribution was 47% for milk/yoghurt, 13% for other dairy products, 13% for lean fish, 4% for fatty fish, 4% for egg, 2% for drinking water, and 17% for all other foods.

Table 4 Characteristics of participants in different study samples.

	MoBa [a]	3 years [b]	8 years	MoBa eTox
		Paper 1	**Paper 4**	**Paper 3**
Study sample, n (%)	82,623 (100)	48,282 (58)	39,471 (48)	2910 (3.5)
Maternal age at delivery, mean (SD), years	30.2 (4.6)	30.4 (4.4)	30.6 (4.4)	30.3 (4.2)
Pre-pregnancy BMI, mean (SD), kg/m^2	24.0 (4.3)	23.9 (4.1)	23.8 (4.1)	23.9 (4.0)
Parity, %				
0	47	50	47	52
1	35	34	35	33
2 or more	17	16	17	14
missing	0.7	-	0.3	-
Maternal education, %				
≤12 y	30	27	24	26
13-16 y	42	45	45	47
>16 y	26	28	29	25
Other/missing	2.8	-	2.1	2.1
Married/cohabitant, %	95.8	97.2	96.8	98.4
Smoking in pregnancy, %				
Occasionally	15	14	14	13
Daily	6.1	4.9	4.4	4.4
Chronic illness, %	10.1	10.0	9.9	8.9
Household income, %				
Low	29	25	24	26
Medium	41	41	42	43
High	29	32	32	29
Missing	3.5	2.2	2.3	1.9
Iodine from food, median (IQR), µg/day	**121 (89, 161)**	**122 (89, 161)**	**122 (89, 161)**	**121 (90, 160)**
Iodine supplement GW 0-22 [c], %	**37**	**37**	**37**	**40**

[a] Singleton pregnancies, no thyroid medication, and available food frequency questionnaire data
[b] Only complete cases with data on all covariates were included at child age 3 years (Paper 1)
[c] Any reported use of iodine-containing supplements in the FFQ or in the general questionnaires

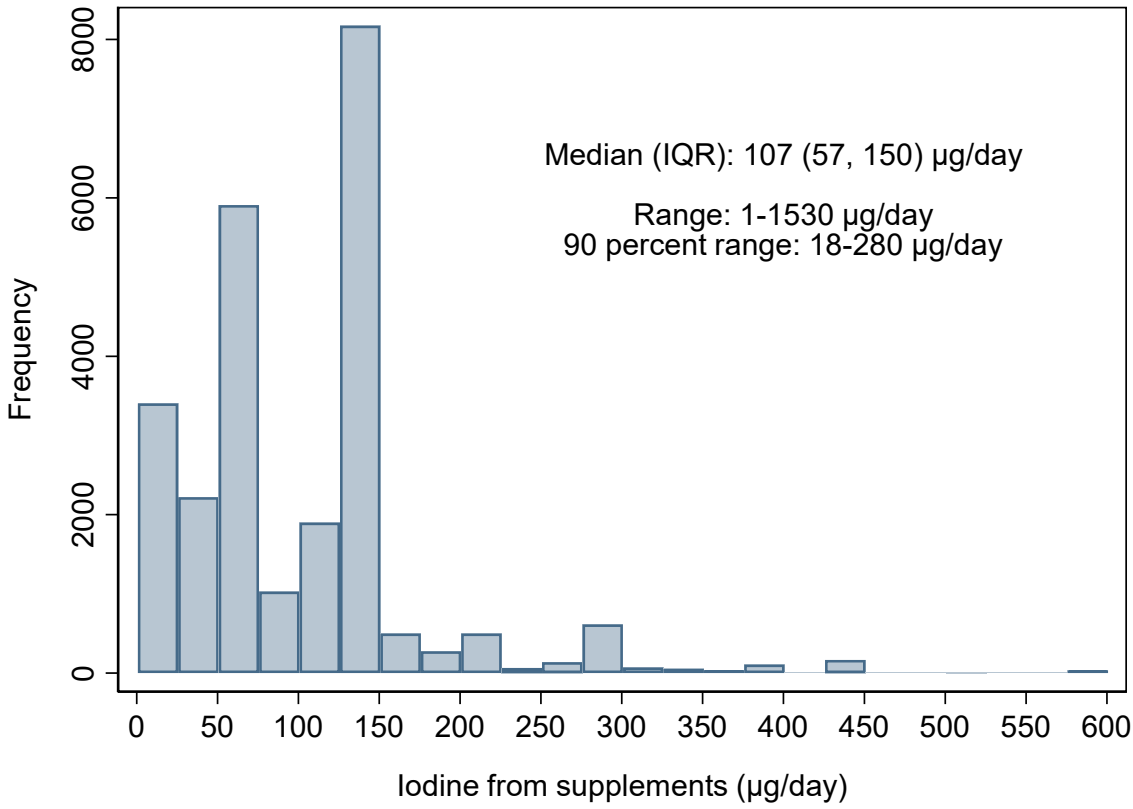

Figure 10 Iodine from supplements in pregnancy in participants reporting use in the MoBa food frequency questionnaire (FFQ) (*n*=25,401). Supplement-users with iodine from supplements ≥600 µg/day (*n*=141, 0.6%) are not shown. From the FFQ, it was not clear if the question asked about the average intake in GW 0-22, the current intake when completing the questionnaire in GW 22, or the intake only when using the supplement.

Median UIC in mean gestational week 18.5 (SD: 1.3) in the subsample of MoBa pregnant women participating in the MoBa eTox study (n=2910) was 68 µg/L (IQR: 35, 116 µg/L), and when restricting to the non-users of iodine containing supplements (n=1738), median UIC was 59 µg/L (IQR: 32, 101 µg/L). The prevalence of UIC<50 µg/L was 37% in the whole sample, and 43% when restricting to non-supplement users. Median UIC in non-supplement users with a total milk and yoghurt intake <3 dl/day (n=697) was 49 µg/L. The distribution of UIC in non-supplement users and current supplement users at the time of UIC sampling is illustrated in **Figure 11**.

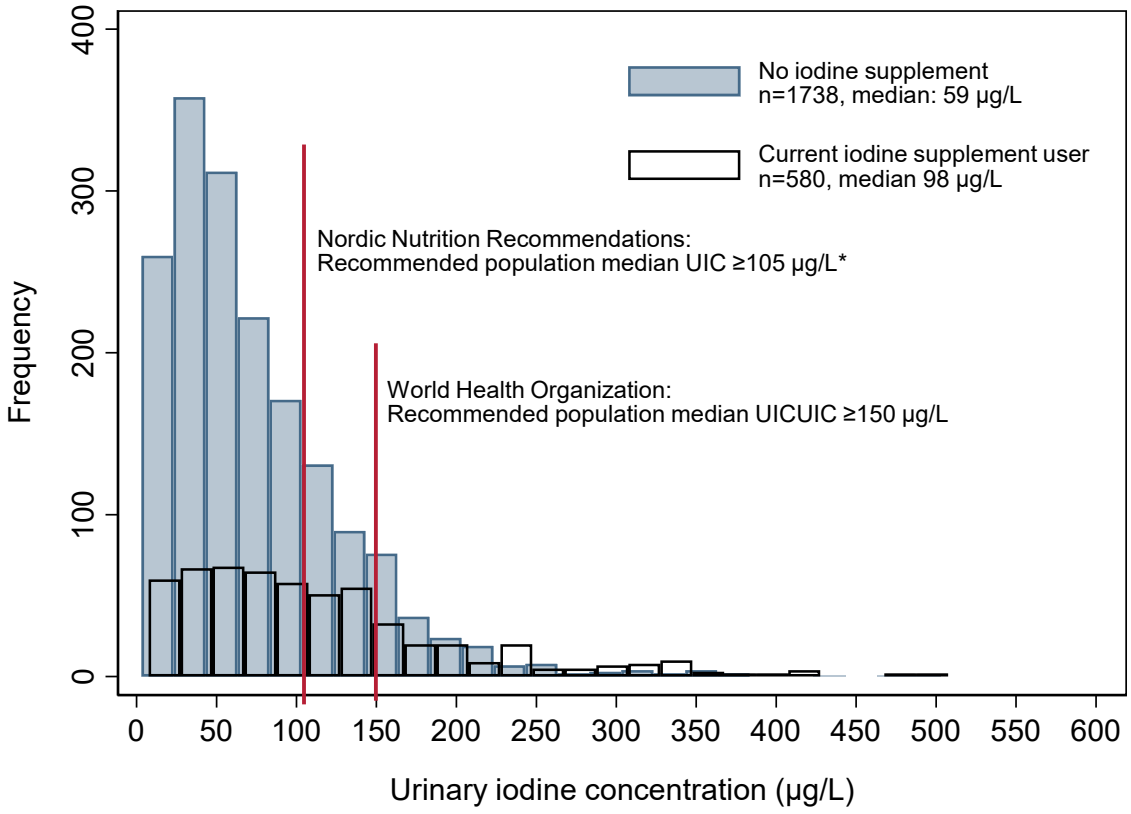

Figure 11 Urinary iodine concentration (UIC) in mean gestational week 18.5 (SD: 1.3). The two histograms illustrate the distributions of UIC in non-supplement users (not reporting use either in the food frequency questionnaire or in the general questionnaires) and in current supplement users (reporting use in gestational weeks 17-20 in questionnaire 3).
* Recommended dietary intake in pregnancy is ≥175 µg/day (7) which corresponds to a UIC of ≥105 µg/day assuming that 90% of ingested iodine is excreted in the urine and the 24-h urine volume is 1.5 L.

All in all, there was a high prevalence of insufficient iodine intake in the MoBa-population. Even the women using iodine-containing supplements had a median UIC well below 150 µg/L, the recommended cut-off for defining iodine sufficiency in pregnancy by WHO (13).

Paper 1: Suboptimal maternal iodine intake is associated with impaired child neurodevelopment at 3 years of age in the Norwegian Mother and Child Cohort Study

The associations between maternal iodine intake in pregnancy (from food and supplements) and child neurodevelopment at age 3 years were explored in n=48,297 mother-child pairs.

In 33,047 non-users of iodine-containing supplements, we found that a low iodine intake from food (i.e. below the estimated average requirement for iodine in pregnancy (160 µg/day), comprising 74% of the included mothers) was associated with more language delay (p = 0.024), more externalizing and internalizing behaviour problems (both $p < 0.001$), and reduced fine motor skills (p = 0.002). We found no association with child gross motor skills or with risk of not walking unaided at age 17 months. In the participants with a low iodine intake (<160 µg/day), suboptimal iodine intake was estimated to account for 5% of cases of language delay (21% of cases with severe language delay) and 16% of cases of behavioural problems (score on behaviour problems >1.5 SD).

We also investigated maternal use of iodine-containing supplements in gestational week 0-22 (no use vs. 0-200 µg/day, and >200 µg/day), and timing of first reported iodine supplement use (no use vs. before pregnancy (0-6 months), first trimester, or later) in mothers with iodine intake from food above/below 160 µg/day. Supplement use was not associated with any beneficial effects on the measures of child neurodevelopment, but was associated with more behaviour problems when initiated in the first trimester in mothers with a low iodine intake from food.

Paper 2: Maternal iodine intake and offspring attention-deficit/hyperactivity disorder: Results from a large prospective cohort study

Data from MoBa were merged with data from the Norwegian Patient Registry (NPR) on registered child ADHD diagnoses by Dec. 2015. The analyses included 77,164 mother-child pairs, and the MoBa children had mean age 9.9 years (range: 6.4-13.8). Outcomes were child ADHD diagnosis and also maternally reported child ADHD symptom score based on 18 items from the ADHD symptoms checklist from the 8 year questionnaire (available for 27,945 children).

In non-supplement users, a low maternal iodine intake from food (<200 µg/day) was associated with more ADHD symptoms (adjusted difference in score up to 0.08 SD, $p < 0.001$, n = 19,086), but not with risk of ADHD diagnosis.

Maternal initiation of iodine supplement use in the first trimester was associated with a ~50% increased risk of child ADHD diagnosis and a higher ADHD symptom score. The estimates were attenuated when restricting the control group to more matched controls who also used vitamin- and mineral supplements other than the standard recommended ones (but without iodine). The estimated increased risk of ADHD diagnosis was then ~30%.

Paper 3: Iodine intake is associated with thyroid function in mild- to moderately iodine deficient pregnant women

In this paper, we explored associations between maternal iodine intake and maternal thyroid function in mid pregnancy (mean gestational week 18.5, SD: 1.3) in 2910 women with available blood and urine measurements of thyroid function parameters and UIC.

Iodine intake from food was associated with UIC ($p<0.001$), but explained only 4% of the variation in UIC. Median UIC was 68 µg/L (59 µg/L in non-supplement users), and it increased with calculated iodine intake by the FFQ, but remained <100 µg/L also for the highest calculated iodine intakes by the FFQ. In participants reporting supplement use at the time of sampling, UIC was 98 µg/L.

Iodine intake from food (by the FFQ) was not significantly associated to the outcome measures, but jointly, the curve shapes indicated that autoregulatory mechanisms, characterized by an increase in FT3 and lowering of FT4, were active when habitual iodine intake from food was below ~150 µg/day.

UIC was inversely associated with FT3 ($p=0.002$) and FT4 ($p<0.001$). A recent introduction of an iodine containing supplement (after gestational week 12) was associated with indications of lower thyroid hormone production (lower FT4, $p=0.027$, and non-significantly lower FT3, $p=0.17$). Combined, these results indicate that an acute high iodine intake inhibits FT4 production and/or release temporarily in mothers with mild-to-moderate ID.

We also explored whether reference ranges (i.e. the 2.5 and 97.5 percentile) for TSH, FT4, and FT3 differ with iodine intake and with UIC in TPOAb-negative women. There was no difference, and thus our results suggest that normal reference ranges can be determined based on data also from mildly iodine deficient populations, contrary to the current guidelines.

However, an important limitation of the study of reference ranges was that there were few women with iodine intake in what is considered the optimal range, and thus there was limited power to compare a low intake with an adequate.

Paper 4: Language delay and poorer school performance in children of mothers with inadequate iodine intake in pregnancy - results from follow up at 8 years in the Norwegian Mother and Child Cohort Study

This paper is based on 39,471 mother-child pairs with available data on maternally reported child development at age 8 years.

In participants who had not reported use of iodine supplements in pregnancy, a low maternal iodine intake from food (lower than ~150 µg/day) was associated with poorer child language skills (p=0.013), reading skills (p=0.019) and writing skills (p=0.004), poorer school test result in reading (p<0.001), and increased likelihood of the child receiving special educational services (p=0.042). Differences were generally small (up to 0.10-0.15 SD for the continuous outcomes). Use of iodine-containing supplements in pregnancy was not significantly associated with the outcomes.

UIC in a subsample of 8 year old children (n=279) indicated adequate iodine status in the children (i.e. median UIC ≥100 µg/L; median UIC was 110 µg/L, IQR: 79, 155 µg/L).

Discussion

Methodological considerations

Study sample and potential for selection bias

The participation rate in MoBa is 40.6% of all invited pregnant women (66). Compared to the total population of women that gave birth in Norway during the years of recruitment, MoBa participants comprise fewer young women (<25 years), fewer mothers with more than two previous pregnancies, fewer women with previous stillbirths and neonatal deaths, fewer women living alone, and fewer smokers (87). Also, the MoBa participants were higher educated and more often supplement users (folic acid and multi-supplements) (87). The only exclusion criteria was that the questionnaires were only available in Norwegian, so the study participants had to be able to read Norwegian.

Although self-selection to the study is likely to affect the prevalence of exposures and outcomes, it does not necessarily introduce bias in estimates of exposure-outcome associations. This has previously been documented both in MoBa (87, 88) and in a large birth cohort in Denmark (89). However, if there are some underlying factors causally related with both participation, the exposure of interest, and the outcome under study, the self-selection to the study might also bias associations. We believe that this is not likely to be a substantial source of bias when exploring iodine intake in MoBa, as we have documented that iodine intake and iodine supplement-use varied only to a very limited degree with maternal background characteristics (see Supplementary Table S1, Paper 4). Actually, the fact that MoBa-participants are probably more equal to each other compared to the whole group of invited women, might make it easier to adequately adjust for confounding factors and help to reduce the potential for residual confounding (87). However, we cannot exclude the possibility that maternal iodine status in itself could have affected participation, for example by affecting maternal mental health. If this is the case, then this would most likely result in attenuation of the associations we have studied.

In our study, loss to follow-up is probably of greater significance as a potential source of selection bias. In MoBa, the response rate to the questionnaires were 52% at 3 years and 38% at 8 years (**Table 5**, p. 45). Interestingly, neither iodine intake from food (by the FFQ) nor reported use of iodine-containing supplements were different in responders compared to eligible non-responders (**Table 4**, p. 38). However, the prevalence of children with

difficulties/poorer scores might have been different. Indeed, the likelihood of ADHD diagnosis in children of responders to the 8 year questionnaire was lower than the likelihood in age-matched eligible non-responders (i.e. born the same years) (OR: 0.75 (95% CI: 0.67, 0.83), $p<0.001$)[3]. If children with questionnaire data had less difficulties in general, this selection bias would most likely attenuate associations studied. It would however, not change the *shape* of the association curves.

Participants in MoBa eTox (Paper 3) were a selected sample of highly dedicated participants in MoBa with complete data-collection of questionnaires and biological samples from mother, father and child up to child age 3 years. Also, participants with symptoms of autism (including severe language delay) were not included, but were reserved for another study. In this group of well-functioning children, we could not replicate the finding of an association between iodine intake (or UIC) and language and learning at 8 years (Paper 4), and we suspect that this might be explained by selection bias in this subgroup.

Table 5 Overview of invited and participating pregnancies and mother-child pairs in MoBa version 10 (released for research in 2017).

	N	**Response rate**
Invited to participate in MoBa (pregnancies)	277,702	
Recruited (pregnancies)	112,908	41% of invited
Participating mother-child pairs ver. 10	114,239	
Questionnaire	**Mother-child pairs**	
GW 17	102,229	89%
GW 22 (MoBa food frequency questionnaire)[a]	87,743 [a]	77% [a] (85% after 2002)
GW 30	94,205	82%
6 months	89,680	79%
18 months	76,409	67%
3 years	58,838	52%
8 years	43,254	38%

[a] The MoBa food frequency questionnaire was included in MoBa in March 2002.

[3] OR was calculated restricted to children born 2003-06 for which the data collection was complete in version 9 of the MoBa data-files, and where we also have information on ADHD-diagnosis from the patient registry.

The Norwegian population is generally well nourished, highly educated, there is a relatively low variability in socioeconomic status, and high living standards. If there are other factors that are important for the vulnerability to mild-to-moderate ID, like smoking, iron status, selenium status, exposure to thyroid disrupting chemicals etc., the result from our study might not be directly generalizable to populations with different exposure to these factors. However, in our study we did not detect any significant effect modification by any of these factors (although we did not explore exposure to the chemicals) on maternal thyroid function (see Paper 3).

Quality of the data

Iodine intake calculated by the FFQ

The use of a self-administered FFQ in MoBa allowed for the collection of data on the habitual intake of food and nutrients from the large group of pregnant mothers included the study. As for all dietary survey methods, there are several sources of bias with an FFQ. In fact, an FFQ is not regarded as suitable for measuring habitual iodine intake in regions were salt is the major contributor to iodine in the diet because it is difficult to estimate salt intake, and also because the concentration of iodine in the salt often vary (90). However, in Norway both salt and drinking water contribute with only negligible amounts of iodine, and milk and fish are the main dietary sources (51), thus an FFQ can be of great value.

Although extensive with its 14 pages and 340 questions, the MoBa FFQ did not cover the *whole* range of food items and dishes in the Norwegian diet, and only frequencies were reported, not portion sizes (with a few exceptions) . Also, the iodine content of foods is often highly variable, including in milk (55) and in seafood (91). The use of fixed values for iodine content of the foods, often based on limited data on the actual content of iodine in each food/drink on the market in Norway, might have introduced both systematic and random errors in calculated iodine intake. For example, the validation study showed that there was significant seasonal variations in UIC, most likely caused by a lower concentration of iodine in milk during the summer months when the cows were outdoors and were given less iodized feed (82).

The FFQ covers the first half of pregnancy, and recollection bias, as well as over- and underreporting, might affect the calculated intakes (67). Still, the MoBa FFQ has been shown to produce realistic estimates of food and nutrient intake at an individual level in a validation study, including also a validation done specifically for iodine (68, 81, 82). Since there are few

food sources of iodine in Norway, and these foods are often consumed in a regular pattern, iodine intake might be measured somewhat more accurately than other nutrients by the FFQ. Indeed, this was indicated in the validation study where iodine was among the nutrients with the highest correlation to the nutrient intakes calculated based on a 4 day weighed food diary (Spearman r=0.46) (68).

Accuracy when completing the FFQ might also depend on maternal personality and her literacy skills. In fact, we observed a highly significant U-shaped association between calculated energy intake by the FFQ and maternal self-reported history of reading or writing difficulties reported in the 8 year questionnaire (results not shown since exploring this association was not included in our application to MoBa). This can probably be explained by an increased likelihood of over- and underreporting in the FFQ if maternal literacy skills were low. Since calculated iodine intake is moderately correlated with calculated energy intake (Pearson r=0.58), this illustrates the importance of carefully adjusting for energy intake to control for this dimension in the exposure variable.

To exclude participants with unrealistic FFQs, we applied the energy-filter commonly used in MoBa (i.e. excluding participants with energy intake: <4.5 or >20 MJ) (67). In addition, we decided to exclude participants with more than three blank pages in the FFQ. While it could be realistic to have three blank pages if one did not eat or drink certain food/drink-categories, it was not likely to have four blank pages for this reason. Participants with three blank pages had an average energy intake that was only 9% lower compared to the participants with no missing pages, while those with four blank pages had 24% lower calculated energy intake which clearly indicated underreporting.

Figure 12 and **Figure 13** show the association between iodine intake by the FFQ and UIC in non-supplement users (n=1738) in the MoBa eTox study. Iodine intake by the FFQ explained only 4% of the variation in UIC, but median UIC increased with increasing habitual iodine intake (**Figure 13**). Also, as habitual iodine intake by the FFQ increased, so did the variance of UIC. Our results thus agree with the results of the validation study documenting that the FFQ provides a realistic estimate of the habitual iodine intake. However, the curve for the median UIC was somewhat less steep than expected. Possible explanations are:

- As the variance in UIC increases with calculated habitual iodine intake, the median UIC naturally has a less steep curve than the mean due to the skewed distribution of UIC

- The FFQ might tend to underestimate low intakes and overestimate high intakes (regression to the mean)
- At a very low habitual iodine intake, the basal loss of iodine in urine might cause a negative iodine balance and depletion of iodine stores (i.e. lower intake than excretion)
- All possible sources of iodine in the diet is not covered by the FFQ
- Milk iodine concentration might have varied during the study period contributing to measurement error and attenuation of the association

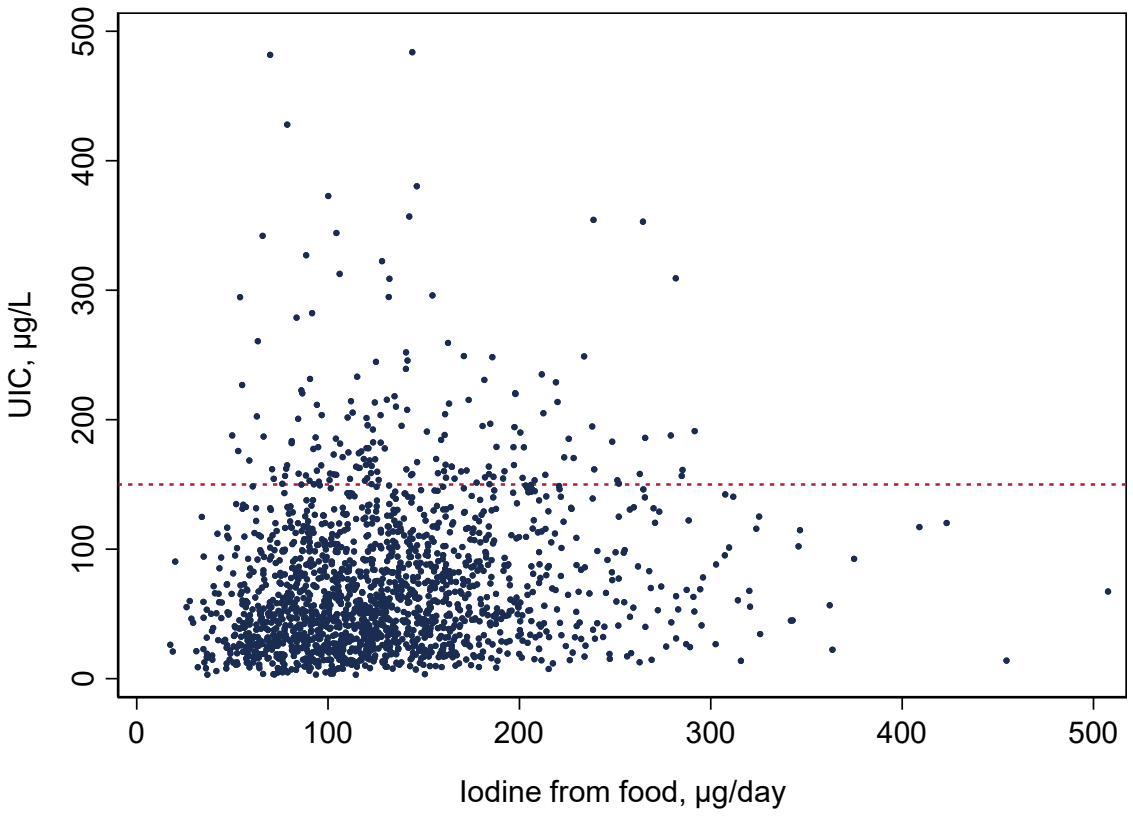

Figure 12 Scatterplot of iodine intake from food (by the MoBa food frequency questionnaire (FFQ)) and urinary iodine concentration (UIC) in a subsample of *n*=1738 non-users of iodine containing supplements. Observations with UIC>500 µg/L (*n*=3) are not shown. The red (dotted) line illustrates the recommended minimum median UIC in pregnant women by WHO (13). UIC and iodine intake by the FFQ were weakly correlated (Spearman *r*=0.20, *p*<0.001), but the correlation was slightly higher when UIC was divided by urinary creatinine concentration (Spearman *r*=0.30, *p*<0.001).

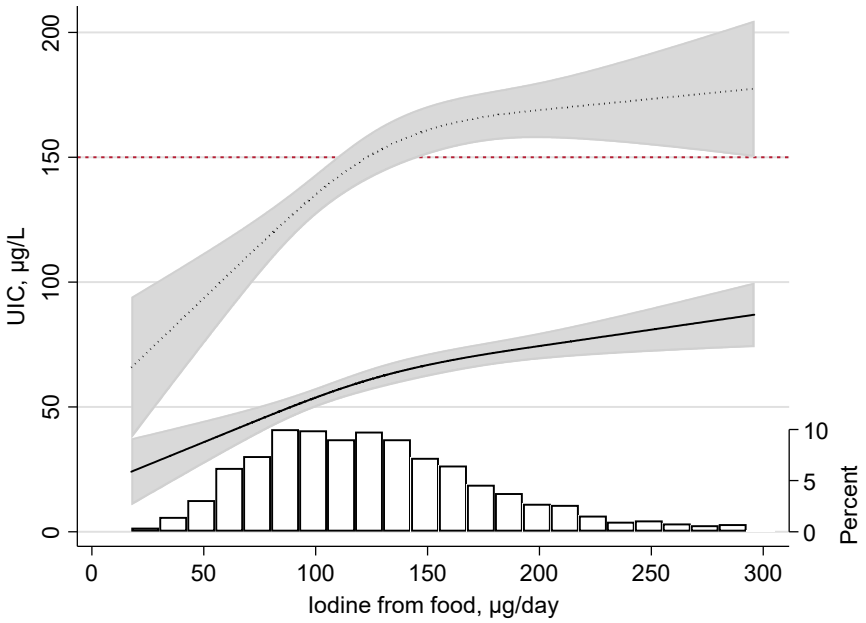

Figure 13 Association between calculated iodine intake (by the MoBa food frequency questionnaire) and urinary iodine concentration (UIC) in *n*=1738 non-supplement users. The graphs illustrate the median (solid line) and the 90 percentile (black dotted line), and the shaded areas are the 95% CIs. The red dotted line illustrates the recommended minimum median UIC in pregnant women by WHO (13). Associations are estimated by quantile regression using restricted cubic splines (3 knots) adjusting for energy intake.

Reported supplement use

Regarding supplement use – the wording of the question on supplement-use in the FFQ was "Do you use or have you used supplements during this pregnancy", with the answer alternatives "yes" or "no". "If yes, we ask you to name and quantify the supplements you have used/are using", with open blank spaces to fill in name (and brand), frequency and amount. However, the question comprise both previous and present use. This was different from the wording of the food questions, which asked respondents to average their intake since getting pregnant. Therefore, we expect that some women have reported their average use of supplements in GW 0-22, some have reported their current intake in GW 22, and some have reported their use only while using. Thus, we have limited information on dosage and frequency of use. There were also questions on iodine supplement use (yes/no) during specific time periods in the general questionnaires (at GW 17 and GW 30), and we combined the data from all three questionnaires to obtain information of use, and of timing of use.

In MoBa eTox, UIC was 66% higher in participants who reported current supplement use in GW 17-20 compared to non-supplement users (**Figure 14**). Explanations for UIC in

supplement users not being even higher might be that not all participants took the supplements daily, and that some of the supplemental iodine might have been trapped in the thyroid since this was an ID population. Evidence supporting the latter is reported in a cluster-randomized controlled study in Bangladesh including pregnant women with UIC comparable to the MoBa women (median UIC at baseline (GW ≤20): 50 µg/L vs. median in non-supplemented women in MoBa in GW 18: 59 µg/L). The pregnant women were given a daily oral supplement of 250 µg iodine (*n*=477). The UIC was re-measured in GW 36 and did not differ from non-supplemented controls (*n*=394) (92).

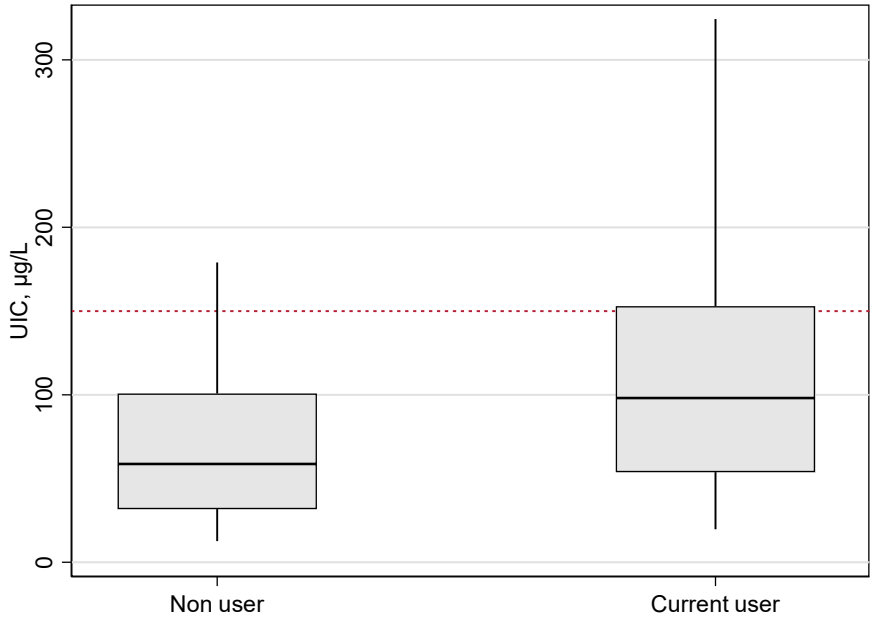

Figure 14 Boxplot showing urinary iodine concentration (UIC) by reported use of iodine-containing supplements. The boxes represents non-users (no reported use in GW 0-22, *n*=1738, median 59 µg/L) and current users at the time of UIC sampling (GW 17-20, *n*=580, median 98 µg/L). Boxes show the 25th and 75th percentiles, and the whiskers illustrate the 90 percent range. The red (dotted) line is the recommended minimum median UIC in pregnant women according to WHO (13).

Urinary iodine concentration (UIC)

Both UIC and urinary creatinine concentration were measured in MoBa eTox in GW 18 (*n*=2910). UIC is an excellent and objective biomarker of recent iodine intake since >90% of dietary iodine is excreted in the urine within 24-48 hours (90). However, since a person's iodine intake varies a lot from day to day, UIC is not a sensitive biomarker of iodine status at an individual level although it is accurate for evaluating iodine status at a group level (93).

Much of the variation in UIC can be explained by hydration status at the time of sampling, and to obtain a measure that is more representative of a person's iodine status, it is common to adjust UIC for urinary creatinine to remove variation in UIC caused by hydration status (see association between urinary creatinine and UIC in **Figure 15**). Creatinine is excreted at a fairly constant rate and has much less inter-individual variation than UIC. This can be done in several ways (94), but the most commonly used method in iodine research is simply to divide UIC by urinary creatinine. In our dataset, dividing by creatinine increased the correlation between iodine intake by the FFQ and UIC as expected (Spearman r=0.20 for UIC in µg/L and r=0.30 for UIC in µg/g creatinine in non-supplement users). **Figure 16** shows the association between the two UIC measures. We decided to use a slightly different method to adjust for creatinine (in paper 3), a method commonly used in nutritional epidemiology to adjust for energy intake. We obtained a creatinine-adjusted UIC (UIC~Cr) by using the residual method (95) (i.e. residuals after regressing log UIC on log urinary creatinine plus the predicted UIC at the median creatinine concentration). UIC~Cr was highly correlated with UIC in µg/g creatinine (Spearman r=0.93). UIC~Cr was chosen because it is on the same scale as crude UIC (µg/L) and could be interpreted as UIC at a median hydration state.

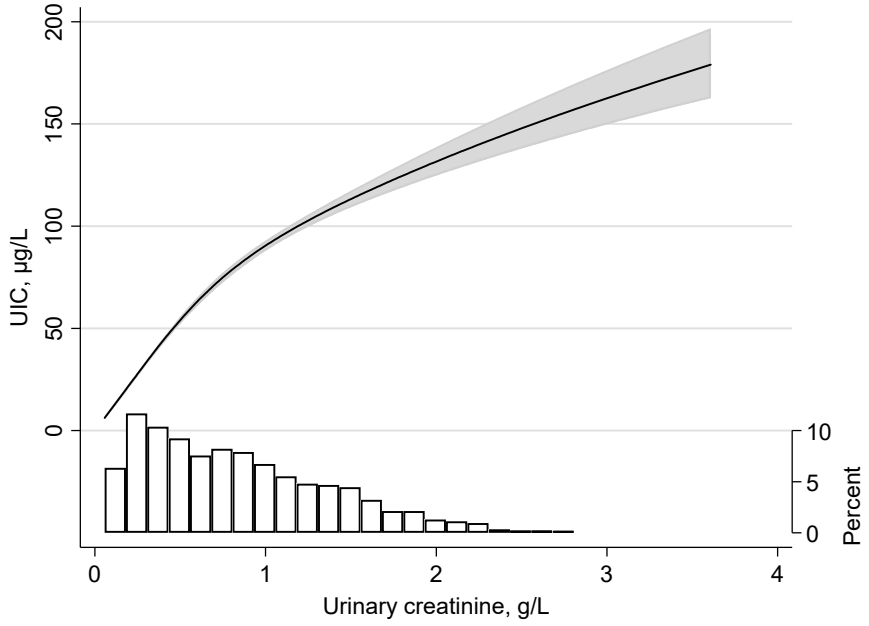

Figure 15 Association between urinary creatinine and UIC in pregnant women in MoBa eTox (*n*=2910). The line illustrates the estimated geometric mean of UIC, and the shaded area represent the 95% confidence interval. The association was modelled using restricted cubic splines with 3 knots modelling log UIC on log urinary creatinine. Urinary creatinine explained 50% of the variation in UIC.

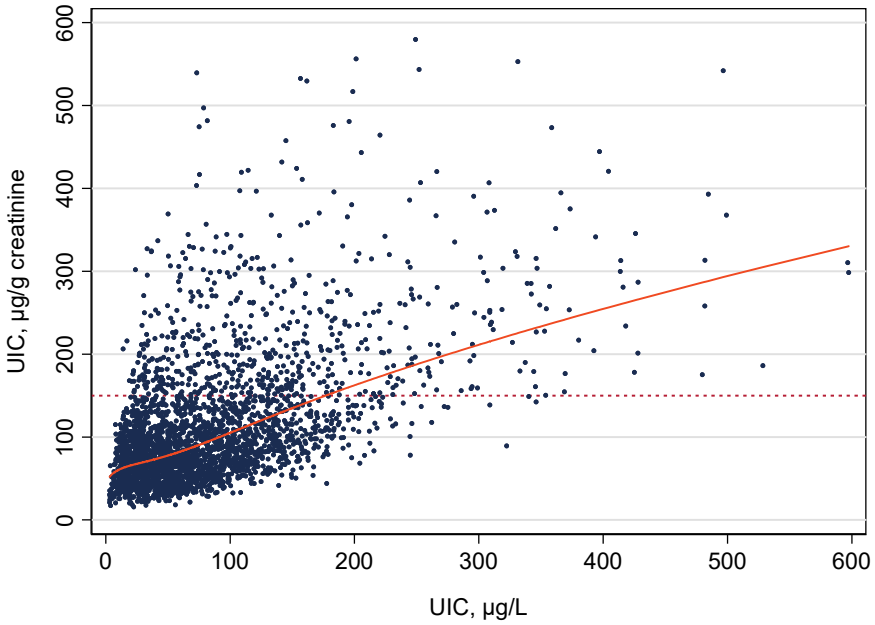

Figure 16 Urinary iodine concentration and urinary creatinine concentration in *n*=2908 MoBa-women. Participants with values above 600 µg/L and/or 600 µg/g creatinine are not shown (*n*=17). The scatterplot illustrates the high correlation between these measures (Spearman correlation coefficient: *r*=0.67, *p*<0.001) and the line shows the close to linear association between the geometric means* (estimated by regressing the log-transformed UIC measures using restricted cubic splines, 3 knots).
* The geometric mean is very close to the median and can be interpreted as such.

Importantly, although adjusting for creatinine provides a measure that better reflects an individual's iodine status, this at the same time can introduce other dimensions in the UIC variable that may bias associations between UIC and outcomes (see examples in **Figure 17**). Thus, it is not necessarily a superior measure over crude UIC when studying associations with health outcomes. In paper 3, we therefore decided to present results using both crude UIC and UIC~Cr. In paper 4, we only reported results for UIC in µg/L when we explored associations with child outcomes. However, using UIC~Cr in paper 4 did not change the null-findings (also stated in the paper, but results were not shown).

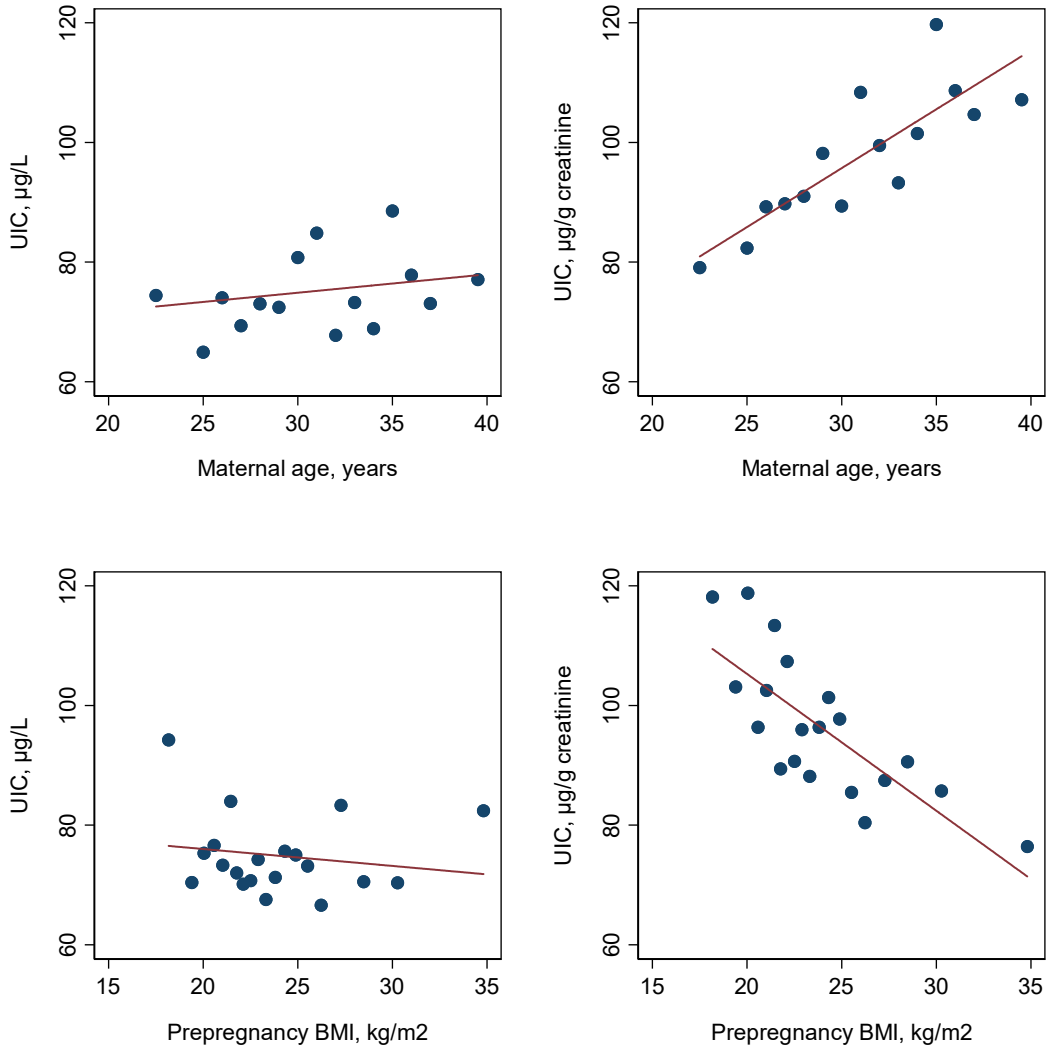

Figure 17 Graphs showing the associations between maternal age, pre-pregnancy BMI and different measures of UIC (µg/L in the left column, and µg/g creatinine in the right column) in *n*=1738 non-users of iodine-containing supplements. Dots in the scatter plots represent local mean values of UIC (in *n*=20 bins), and the lines represent the estimated regression line modelling the mean UIC (crude models). The graphs demonstrate that when you adjust UIC for hydration level by dividing by creatinine (or by calculating the residuals), you at the same time introduce other dimensions in your exposure variable (i.e. UIC) that are associated with urinary creatinine. Examples shown are age and BMI, but also unmeasured factors might be important (e.g. muscle mass/fitness level) that can be related not only to the unmeasured factor, but also to your outcome of interest (e.g. maternal thyroid function or child neurodevelopment).

Validity of outcomes

In papers 1-4 the analyses comprise a range of different outcomes. Some were subjective, like maternally-reported child skills and behaviour, and some were more objective, like measures

of maternal thyroid function, child ADHD-diagnosis, child performance on school mapping tests in reading and mathematics, and child being granted special educational services in school. All outcomes on child development have measurement error, but the sources of error might be different for different outcomes. Details regarding the validity of each separate outcome are presented in the respective papers and will not be repeated here. Consistent results over these sometimes very different measures of child cognitive development and behaviour problems, would indicate a robust association between maternal iodine nutrition and child neurodevelopment in MoBa.

Selection of covariates

In MoBa, the extensive data-collection allowed us to adjust for a range of covariates. We selected covariates carefully based on previous knowledge and on plotting potential confounders in a directed acyclic graph (DAG). A simplified version of the DAG is displayed in **Figure 7**, page 30. Below is an overview of covariates that were considered for inclusion and a brief description of the rationale for including them or not.

Adjusting for energy and fibre intake

There was a moderate positive correlation between iodine intake from food and energy intake calculated based on the FFQ (Pearson r=0.58, see **Table 6**, p. 55). Consequently, iodine intake correlated positively with all other nutrients, and for some nutrients there was a strong correlation (calcium and B_2 had a correlation coefficient >0.7) which could mainly be explained by milk being an important source. Interestingly, intake of the omega 3 fatty acids EPA and DHA from food was only weakly associated with iodine intake (r=0.27) demonstrating that fatty fish was not an important source of iodine.

To disentangle the effect of iodine from the other nutrients, we included energy intake and fibre intake as covariates in the models. In MoBa, dietary fibre intake reflects intake of fruits, vegetables, and whole grains and is a good marker of a healthy dietary pattern in the pregnant women (96, 97). Dietary fibre has thus been used as a proxy of a healthy diet in several previous MoBa publications (98-100). **Table 6** shows that when you remove variation in nutrient intake caused by energy intake (i.e. obtain residuals after regressing nutrient intakes on energy intake), energy-adjusted iodine intake is no longer associated with many energy-adjusted nutrients, but association to a few nutrients remains moderate to high (calcium, B_2 and B_{12} remains >0.50). This can be explained by milk intake as milk is an important source of these nutrients and of iodine.

Table 6 Correlations (Pearson) between food intake of iodine intake and other nutrients calculated based on the MoBa food frequency questionnaire

	Calculated intakes	**Residual adjusted [a]**	**Calculated intakes**
	From food only	**From food only**	**From supplements only**
	n=82,021	*n*=82,021	*n*=25,236 [b]
	Pearson's *rho*	Pearson's *rho*	Pearson's *rho*
Iodine	1	1	1
Energy intake	**0.58**	0	0.01 (from food)
Fibre intake	0.36	-0.11	0.02 (from food)
EPA+DHA	0.27	0.19	0.16
Selenium	**0.57**	0.32	**0.73**
Zinc	**0.69**	0.47	**0.92**
Iron	0.41	-0.09	**0.51**
Calcium	**0.90**	**0.84**	0.22
Vitamin C	0.20	-0.08	0.47
Vitamin D	0.38	0.22	0.35
Vitamin A	0.33	0.09	**0.58**
Vitamin E	0.30	-0.18	0.36
Vitamin B_1	**0.66**	0.38	0.25
Vitamin B_2	**0.89**	**0.84**	0.29
Vitamin B_6	**0.58**	0.29	0.32
Vitamin B_{12}	**0.66**	**0.53**	0.05
Folate	0.49	0.15	**0.58**

Correlations higher than 0.5 are highlighted (in bold)
[a] Correlations are between residuals of nutrient intakes after regressing nutrient intake on energy intake.
[b] Includes only participants who reported use of iodine-containing supplements in the FFQ.

Adjusting for energy intake may also to some extent adjust for maternal "questionnaire personality", i.e. the general tendency of over-reporting or underreporting when completing questionnaires (101, p. 278). We observed that calculated energy intake per se was associated with most of the outcomes in our study, and we suspect that this might, at least partly, be due to the maternal self-reporting in both the FFQ and for outcomes.

Other covariates

We also included variables on maternal baseline characteristics as they could be associated with both exposure and outcome (age, pre-pregnancy BMI, maternal education, previous pregnancies ≥22 weeks, maternal smoking in pregnancy). For smoking, we also explored potential interaction effects, but since the interaction terms with smoking were not statistically significant for any of the outcomes in the study, we included it only as a separate covariate as an indicator of both socioeconomic status and healthy lifestyle.

Bilingualism was reported for mother and/or father in about 11% of pregnancies. This covariate was included in all models on child language outcomes since bilingualism was associated both with a slightly lower maternal iodine intake i pregnancy (mean difference: -4 µg/day, $p<0.001$), and also an increased risk of child language delay (for example increased risk for having a typical sentence complexity of no more than 2-3 words at 3 years of age (OR: 2.0, 95% CI: 1.8, 2.2)).

In models with iodine supplement use as exposure and child neurodevelopment outcomes we additionally adjusted for use of folic acid supplement (use within the interval from 4 weeks before to 8 weeks after conception) and total intake of the long chained marine omega 3 fatty acids (EPA+DHA from food and supplements). This was because the use of different supplements were correlated, and both folic acid and EPA and DHA are important nutrients in foetal brain development (102-104).

For some continuous outcomes we also included important determinants of the outcome to improve the precision of the estimates (e.g. gestational age at sampling of blood and urine, or child sex). This was not done for dichotomous outcomes since it has the opposite effect and reduces precision in logistic regression (105).

Biological measures of maternal iron- and selenium status were associated with iodine intake by the FFQ and/or UIC and was included in models that were based on MoBa eTox data (where these measures were available). Both iron and selenium are important for thyroid function (45). The variables were only included as single covariates since no evidence of interactions was detected.

Covariates that were not included

Maternal mental health was not included in any of the models. Iodine nutrition is linked to risk of thyroid disorders, which in turn are associated with maternal mental health (106). Anxiety, depression, difficulty sleeping, fatigue, nervousness, irritability and depression are all among the listed symptoms of hyper- and/or hypothyroidism according to the American Thyroid Association (107). There are also studies that have described an association between TPOAb positivity and depression (108), and the current guidelines of the American Thyroid Association includes this recommendation: «All patients with depression, including postpartum depression, should be screened for thyroid dysfunction» (107). Even in the absence of overt thyroid disease, we cannot rule out that small changes in thyroid hormone levels may affect maternal mental health. The evidence for this is not strong, but an

association has been documented in a study on elderly people (109). Maternal depression could theoretically affect iodine intake since depression may lead to alterations in diet quality. We have made efforts to block this pathway (in the regression models) by adjusting for maternal fibre intake since fibre is a good indicator of a healthy diet (96, 97). Maternal mental health in pregnancy may not be a strong mediator by itself, but it can represent an indicator of iodine status and thyroid function in pregnancy. Since thyroid function is on the main causal pathway, we believe that adjusting for maternal mental health would introduce bias.

Pregnancy- and birth outcomes such as gestational length and birth weight were not included in the models or in the selection criteria for the study population. These factors may represent indicators of the exposure or mediators in the exposure-outcome associations, and including them might also introduce risk of residual confounding by unmeasured factors that are causally related to birthweight or gestational age and the outcome. Thus, conditioning on them either by adjusting for them or selecting based on them (for example by excluding low birthweight children) can introduce bias (110, 111). Since the exposures are measured *before* the pregnancy and birth-outcomes, they by cannot be confounders by definition since it is not possible for these factors to affect the exposures in our study.

Postnatal factors such as breastfeeding, child diet, daycare, and parental style were not included since these factors could not be causally related to the exposure (as they did not precede exposure in time). Also, they could potentially be affected by maternal mental health and/or by child functioning and thus represent indicators of the exposures and/or outcomes.

Potential for residual confounding

Since MoBa is an observational study, we cannot rule out the possibility of residual confounding. Notably, maternal iodine intake was *not* strongly associated with any of the maternal background characteristics (Supplementary Table S1, Paper 4), but unmeasured confounders could still be present. Due to collinearity, we were not able to adjust for milk/yoghurt intake (the correlation between milk/yoghurt intake and iodine intake was high (Spearman r=0.86)). Thus, factors associated with milk/yoghurt intake might potentially have confounded the associations. Examples could be other nutrients or unmeasured maternal characteristics that are determinants of milk intake. For instance, we observed that iodine intake was weakly, but negatively associated with iron status (Spearman r= -0.06, p=0.014 in non-supplement users in MoBa eTox) which might be explained by the low iron content of milk. However, a higher iron status in iodine deficient women would probably tend to attenuate the associations we explored in our project since iron is also an important nutrient in

brain development, and since women in MoBa had a high prevalence of iron deficiency (i.e. 44% had low or empty iron stores in MoBa eTox (i.e. plasma ferritin <30 ng/ml)).

Also, when exploring iodine from supplements, other nutrients from the multi-supplements or factors associated with the maternal behaviour of taking a supplement could potentially confound the effect estimates. However, we tried to adjust for these factors in Paper 2 where we found that iodine from supplements was associated with increased risk of ADHD. We used a more matched control group who also reported use of multi-supplements, but not containing iodine.

Ideally, we would control for genetic predisposition for the outcomes, but there were limited data on this available. In Paper 4, we controlled for maternal history of reading/writing difficulties for outcomes on child reading and writing, but this did not change the results.

Statistical methods

In the models exploring associations with outcomes, we decided to model continuous exposures (e.g. iodine intake or UIC) by flexible modelling techniques for two reasons. Firstly, we wanted to explore the shape of the curves to see which range of iodine intake that was associated with the lowest risk of impairments. Linear associations could not be expected since a U-shaped relationship between iodine status and thyroid function has been reported in the literature (34, 35). Secondly, this allowed us to take full advantage of all the information in the exposure variables, thus preserving the statistical power of the data when estimating associations with outcomes. In iodine research, continuous measures of UIC are often modelled as categorized variables (e.g. UIC above or below 150 µg/L). Although it might be easier for the reader to interpret the summary statistics and to compare results between studies, categorization leads to a loss of power and a high risk of misclassification (112, p. 308). Also, if the aim is to explore UIC as a measure of iodine status, broad exposure categories may contain both levels associated with low and increased risks causing attenuation of the results, and identifying inflection points on the associations (i.e. where the risk starts to increase) is not possible unless one divide into many categories. By using restricted cubic splines, we could test the overall association (testing H0: no association), and evidence of non-linearity, and it also allowed us to report specific estimates with confidence intervals for any chosen value of the exposure, not only for ranges (113).

When using calculated nutrient intakes based on an FFQ as exposures, it is important to carefully adjust for energy intake, and there are several methods commonly used in nutritional

epidemiology for this purpose (101). After much consideration whether to use energy as a separate covariate in the models, to use a nutrient to energy ratio, or perform an energy-adjustment of iodine intake by the residual method, we decided to just include energy intake as a separate variable in the models. We found that the other methods of energy-adjustment introduced bias at high and low calculated iodine intakes. Also, for iodine, the most relevant exposure is the absolute iodine intake and not iodine intake relative to energy intake.

Although we explored multiple associations in our project, we decided not to lower the *p*-value used to define statistical significance to adjust for multiple comparison. Some researchers argue that with a *p*-value of 0.05 there is an increased risk of reporting significant associations due to chance. On the other hand, according to Professor Walter Willett, author of one of the most influential books in nutritional epidemiology, the general consensus in nutritional epidemiology is that adjusting the *p*-value for multiple comparison will unduly reduce power and increase the risk of "false negatives" (112, p. 312). It is, however, important to present results from all analyses (i.e. include also the non-significant findings) so that the readers can evaluate for themselves the consistency of the results with other results in the study and in the literature (112). In our study, we had predefined all associations that were to be explored, and all results are reported in the papers.

The most important strengths of the study

MoBa is a population-based, large prospective study with statistical power to detect even weak and potentially non-linear associations between exposures and outcomes. The extensive data collection includes data on iodine intake shown to represent a valid measure of iodine intake at an individual level (68). A high proportion of women had a low iodine intake in pregnancy (as was also confirmed by measurement of UIC in a subsample), while iodine intake in the children was probably sufficient after weaning (2, 55, 58).

Socioeconomic status and other maternal characteristics were not important determinants of iodine intake (53). In Norway, there are relatively small differences in socioeconomic status, and the living standards are high. There is free health-care for pregnant women and children, and people are generally well nourished. Altogether, this may reduce the risk for residual confounding (although not eliminate).

The major limitations

There was great variability in iodine intake, but few mothers had an adequate habitual iodine intake by WHO definition. For example, only 5% had a calculated iodine intake from food

>250 µg/day, and even iodine supplement users (who reported current use) had a median UIC indicating insufficient intake (the median was 98 µg/L, and the recommended cut-off defining adequacy is 150 µg/L). As a consequence, almost all participants in MoBa were exposed to what is commonly defined as mild-to-moderate ID, and there is a lack of participants with what is defined as an adequate iodine intake in pregnancy for comparison.

As indicators of habitual iodine intake, both iodine intake by the FFQ and the UIC have relatively large measurement errors. However, both variables *are* indicators of the actual iodine intake, and the large number of participants combined with the flexible modelling techniques allowed us to "separate the signal from the noise" given that the measurement errors were mainly random and not systematic. While random measurement error may not affect the shape of the curves when exploring associations with the outcomes, it will most often tend to attenuate estimates towards a null-finding (114, p. 289). Thus, had we been able to accurately measure the true long-term/habitual iodine intake, the estimated association between maternal ID and child neurodevelopment would probably have been stronger. But again, the observational design means we cannot rule out the possibility of residual confounding in MoBa and conclude on causality.

Ethical considerations

MoBa is conducted according to the guidelines laid down in the Declaration of Helsinki and a written informed consent was obtained from all participants (66). The current study was approved by The Regional Committee for Medical Research Ethics South East Norway 2014/2211. All data for this project was already collected.

Being the world's largest study of its kind, MoBa represents a unique opportunity to study an almost infinite number of associations between exposures and outcomes. However, the huge amount of data also leads to risk of misreporting. Examples include not taking full advantage of the opportunities in the data related to your research questions, using suboptimal or wrong statistical methods, or to selectively publish or highlight significant findings (115). Publications based on MoBa are often regarded as important because of the large number of participants and its population-based, prospective design. In our study, we tried to minimize the risk of misreporting by including experts from different fields of research as co-authors (i.e. nutritional epidemiology, mental health, statistics, and endocrinology), by predefining all associations we were to explore in detailed protocols for each paper, and by publishing results for all analyses regardless of the findings.

A potential conflict of interest in this project is that the PhD-student is employed by a dairy company (TINE SA), and the PhD-project is partially funded by this company. To minimize the risk of bias, the collaboration between NIPH, OsloMet, and TINE SA was formalized before start-up in a contract stating the purpose of the project, the responsibilities of each party, and that all results were to be published regardless of the findings. Only the PhD-student outside NIPH has access to the MoBa data, and the main supervisor at NIPH has the primary responsibility for the quality of all published results. Ideally, nutrition research should be financed by public funding sources to minimize the risk of bias caused by financial interests (116). However, there are limited resources available for research in nutritional epidemiology, and this project proposal had already been turned down by the Research Council of Norway several times (in the program for independent basic research projects - Medicine, Health Sciences and Biology (FRIMEDBIO)). TINE decided to support the project to raise awareness on ID, to increase the knowledge on potential consequences of ID in young women, and to have one more employee with a PhD. Industrial PhD-projects are partially funded by the Research Council of Norway, and the threshold for receiving partial public funding is much lower than for complete funding (e.g. in FRIMEDBIO). The role of TINE has been transparently communicated whenever results from our project have been presented.

Discussion of the main findings

Iodine status of the participants in MoBa

Both the FFQ-data and the measured UICs documented that ID was highly prevalent in MoBa pregnant women, and that only few had a calculated habitual iodine intake above the recommended levels. Forty percent of the MoBa women had an intake of milk and yoghurt of <300 g per day (equals up to ~3 dl), and results from the MoBa eTox sub-study showed that these women had a median UIC of 49 µg/L if they did not use iodine-containing supplements. In mothers who reported not using dairy products (2.4%), median UIC was 39 µg/L. This documents that our study sample were not only mildly-to-moderately ID, but that quite a large proportion actually had moderate-to-severe ID (9, 117). The low iodine status of pregnant women in Norway has later been confirmed in other studies in pregnant women (58, 59, 61, 63) and also in women of childbearing age (57), but the median UICs were slightly higher in these studies compared to ours (75-92 µg/L versus 68 µg/L in MoBa eTox). This can probably be explained by an increase in Norwegian milk-iodine concentration in 2012 after it was discovered that it had decreased by ~40% compared to what was measured in 2000 (54). More iodine was then added to the feed of the cows to compensate. The lower concentration

was later discovered to be caused by an increased use of rapeseed ingredients in the feed from year 2000 which has an inhibitory effect on iodine uptake and excretion in the milk (55). However, iodine concentration in milk was probably fairly constant during the years of data-collection by the MoBa FFQ as we found no evidence of differences in UIC by year of birth in MoBa eTox after controlling for maternal milk/yoghurt intake and supplement use (**Figure 18**).

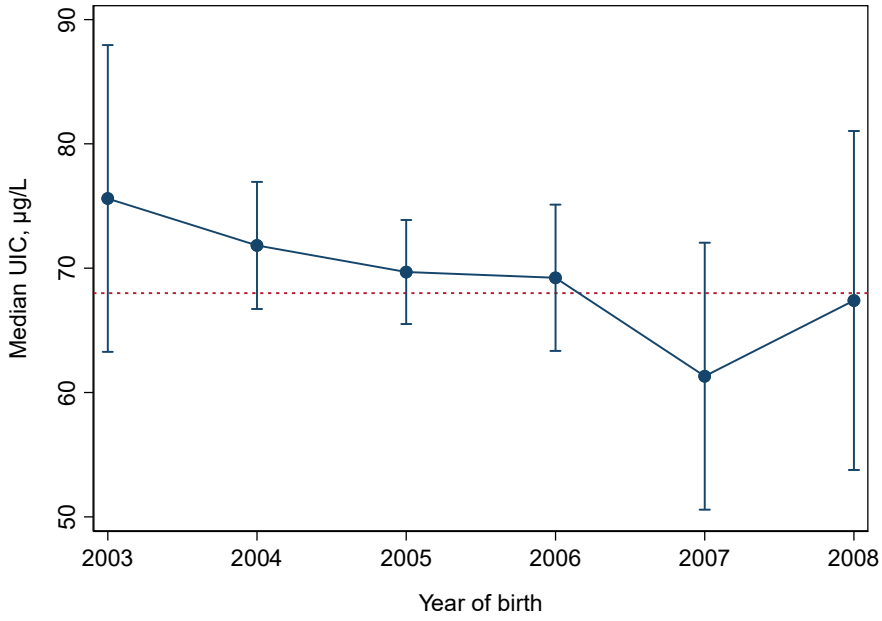

Figure 18 Median urinary iodine concentration by year of birth adjusted for use of iodine-containing supplements and intake of milk/yoghurt (*n*=2910 in MoBa eTox). The plot illustrates the estimated medians and 95% confidence intervals obtained by quantile regression. There were no significant differences in median UIC by year of birth. The red (dotted) line illustrate the median UIC in MoBa eTox (i.e. 68 µg/L).

Compared to other large pregnancy cohorts in areas with mild-to-moderate ID where UIC has been measured, iodine status was lower in MoBa (median UIC: 68 µg/L). In ALSPAC (UK, *n*=1040) median UIC was 91 µg/L (37), in the Gestational Iodine Cohort (Australia, *n*=228) it was 81 µg/L (38, 118), and in the INMA cohort (Spain, *n*=1522) it was 137 µg/L (119). In the Generation-R cohort (the Netherlands, *n*=1156) the median UIC indicated adequate iodine intake (203 µg/L) (120).

In women with a low iodine intake from food, breast milk iodine concentration is also low (60). In MoBa, >80% of women initiated breastfeeding, and the mean duration of breastfeeding was 9 months (121). A recent study from Oslo, Norway documented that the median iodine concentration of breastmilk samples from *n*=175 women was 68 µg/L (60).

Although there is not yet an established cut-off for defining an adequate level in breastmilk, a median of ≥100 µg/L is often regarded as an indicator for adequacy. Children of iodine deficient pregnant mothers were thus likely exposed to ID until complementary feeding was introduced. We did not measure iodine in breastmilk in MoBa, therefore we cannot explore from our data *when* the potential negative effects of maternal ID occur. However, other studies point towards the first half of pregnancy as being the time when the foetus is most vulnerable to maternal iodine status and low maternal T4 (24, 122).

In Norway, the few studies available on iodine intake in infants and children indicate that ID is not prevalent in children after introduction of iodine-fortified infant foods and milk (55, 58, 123). This is supported in our findings in the subgroup of 8 year old children from MoBa where UIC indicated an adequate intake at the group level (Paper 4).

Iodine intake and thyroid function in pregnancy

When maternal habitual iodine intake fell below ~150 µg/day (corresponding to a UIC~Cr below ~100 µg/L) we saw indications of the autoregulatory mechanisms being activated to preserve iodine (Paper 3). We observed an increased level of FT3, and a trend towards a decrease in FT4 to FT3 ratio. There was no change in TSH, providing further evidence for an autoregulatory adaptation independent of TSH-signalling (23). For FT4, we saw that FT4 started to decrease below an habitual iodine intake of ~150 µg/day, but the change was far from significant (p=0.82). In contrast, association between UIC and FT4 was highly significant but it was *inverse*. This finding was somewhat unexpected. We hypothesize that in mild-to-moderate ID, the thyroid might be extra sensitive to an acute higher availability of iodine causing a transient inhibition of thyroid hormone production and release. This effect is well known to occur at acute high iodine availability to prevent an overproduction of thyroid hormones and is named "The Wolff-Chaikoff effect". But, to our knowledge this effect has not been documented at iodine intakes at these low levels that are even below the recommended intake for pregnant women by WHO. However, maternal hypothyroxinaemia is known to occur more often in pregnant women that have insufficient iodine (24, 124, 125).

A recent introduction of iodine supplement use (within the last 5 weeks) was associated with lower FT4, whereas a more long-term use was not (Paper 3). This provides further support to our theory that thyroid function is transiently inhibited when iodine availability increases in mild-to-moderate ID. Since the foetus is particularly vulnerable to a low maternal T4 in the first trimester, this inhibition might mediate the negative effect of mild-to-moderate ID on

child neurodevelopment, and might explain why we see an increased risk of child behaviour problems when the mother initiated iodine supplement use in the first trimester (Papers 1 and 2). In rat-studies, inducing a mild and transient hypothyroxinaemia for as short as three days early in gestation has been documented to cause irreversible impairments to the brain of the offspring (126, 127).

Maternal iodine intake and child neurodevelopment

In our study, we explored associations between maternal habitual iodine intake and multiple outcomes on brain development. Combined, the results indicate that a maternal habitual iodine intake from food below ~150 µg/day is associated with lower child cognitive performance, more behaviour problems, and poorer fine motor skills, but not gross motor skills (Papers 1, 2, and 4). The result for ADHD diagnosis was not significant, but this might be because only 2% of the MoBa children were diagnosed with ADHD causing a lack of statistical power to detect an association (Paper 2). Also, many other factors are important determinants of a child being diagnosed with ADHD, and not all children with "true" ADHD are diagnosed (77). The alternative outcome, ADHD symptom score based on 18 items in the 8 year questionnaire, provided an alternative continuous outcome measure, and the association was significant for this outcome. We reported in Paper 2 that the association with ADHD symptoms of inattention was significant whereas for symptoms of hyperactivity, it was not (adjusted model: p=0.16). In the published paper only 71% of the 8-year questionnaires were included. However, when reanalysing the data based on the complete dataset in version 10, associations to both subscales of ADHD were significant. Also, at 3-years we reported that habitual iodine intake was associated with externalizing and internalizing behaviour problems (Paper 1). The results were consistent for all the subscales that were included in the externalizing- (aggressive behaviour and attention problems) and internalizing scores (anxious/depressed, emotionally reactive, and somatic complaints) (see **Figure 19**, p. 65, these results were not included in the paper).

For all outcomes in our study, effect sizes were relatively small, but as discussed earlier, the associations may have been attenuated by measurement error of the exposures and outcomes (114). Even *if* effects of mild-to-moderate ID are small, we would still argue that they are important due to the high prevalence of ID. Also, we observed the highest increased risk for the outcome severe language delay (at 3 years, Paper 1) which may indicate that mild-to-moderate ID can be a risk factor also for more severe brain damage. This will be further followed-up in MoBa since a separate publication on iodine and autism is currently drafted.

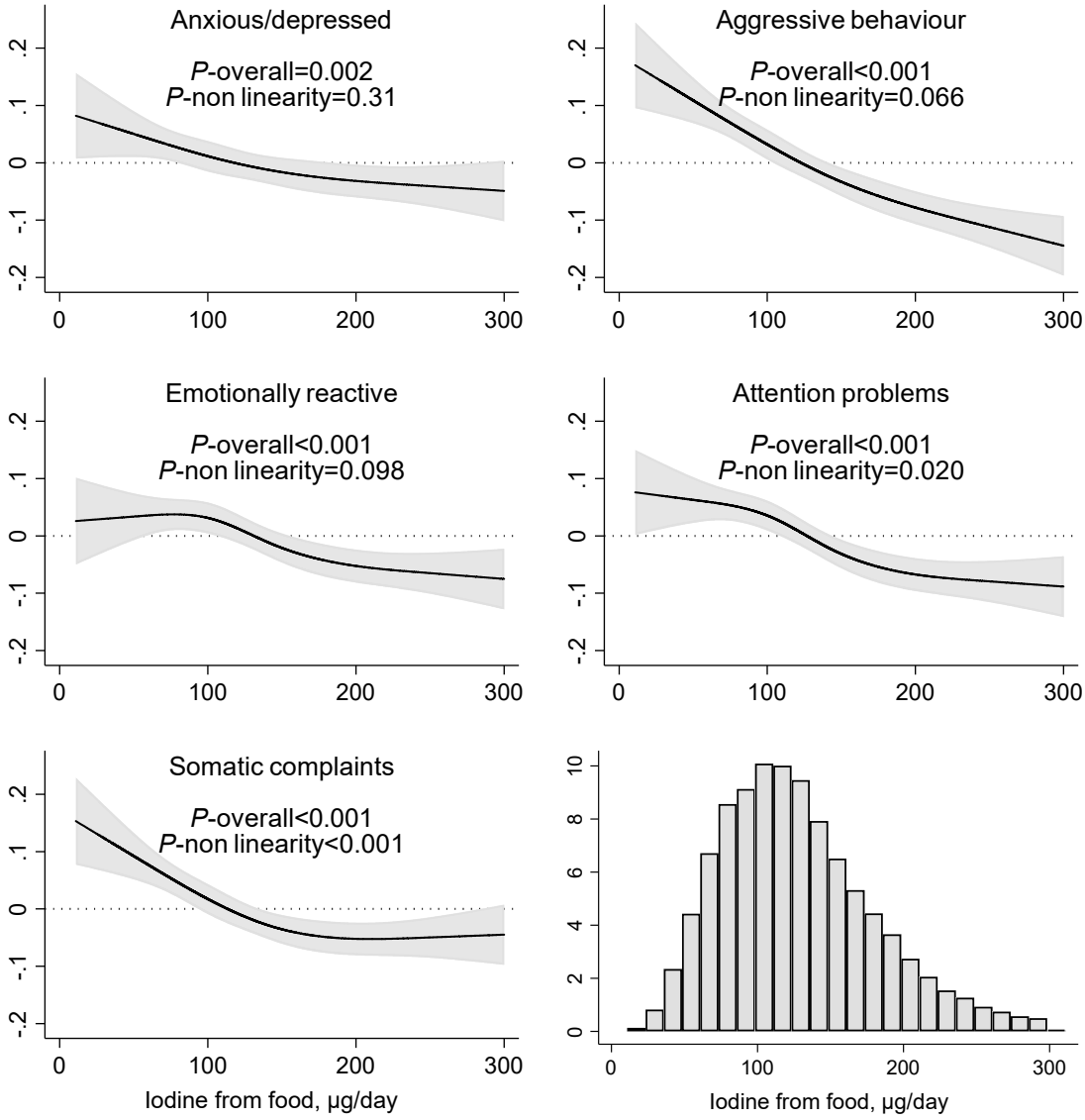

Figure 19 Maternal habitual iodine intake (non-supplement users) and child behaviour problems (mean z-scores) at 3 years of age (n~33.000 mother-child pairs), adjusted models. The left column shows subscales of internalizing behaviour problems, and the right shows externalizing. The shaded areas represent robust 95% confidence intervals. Models are adjusted for maternal age, pre-pregnancy bmi, education, parity, smoking in pregnancy, energy intake, fibre intake, and for random effects of sibling clusters. The histogram illustrates the distribution (in percent) of iodine intake from food in the FFQ.

Previous studies have reported increased risk of lower child performance in children of mothers with a spot UIC in pregnancy <150 µg/L (or µg/g creatinine) which approximately corresponds to an iodine intake <250 µg/day (37, 38, 118). Our results indicate that the optimal range of intake might extend lower (from ~150-200 µg/day), but the evidence is weak since there were only few participants with higher intakes in MoBa for comparison. However,

reviewing the literature to date, we cannot see that there is any evidence this far to support the increased recommended iodine intake for pregnant women compared to adults. A median UIC in the range between 100 and 150 µg/L in pregnant women is today considered insufficient, but may not represent true ID. It is likely that a habitual iodine intake from food >150 µg/day prior to pregnancy secures an adequate iodine store to cover the needs in pregnancy. Importantly, the knowledge on mild-to-moderate ID in pregnancy is still scarce (24, 33, 128), and more research is needed. An overview of observational studies reporting associations between prenatal mild- to moderate ID and child neurodevelopment is provided in **Appendix 2**.

Maternal use of iodine supplements and maternal- and child outcomes

Of all outcomes studied, we found no indication of beneficial effects of maternal use of iodine-containing supplements, regardless of timing of initiation and of maternal habitual iodine intake from food. In fact, initiating supplement use in the first trimester was associated with more child behaviour problems and ADHD (Papers 1 and 2). These findings might be due to chance and explained by multiple testing, however, for many of the other outcomes studied there was a small, non-significant decrease in child performance when supplement use had been initiated in the first trimester (Papers 1 and 4). Also, we saw indications of reduced maternal thyroid function when mothers had initiated supplement use within the last 5 weeks (Paper 3). If an abrupt increase in iodine intake causes a temporary inhibition of thyroid function, this could explain negative effects on child neurodevelopment, particularly if it happens during the most vulnerable period in brain development in the first trimester (122).

Other studies have also failed to show beneficial effects of iodine supplement use on maternal thyroid function or child neurodevelopment in areas with mild-to-moderate ID (39-41, 129, 130). There has been several review papers on the topic (24, 131-133), including a Cochrane review (39), but they all conclude that more research is needed in areas with mild-to-moderate ID. Still, iodine supplements are often recommended for pregnant women in areas where pregnant women have insufficient iodine intake by WHO criteria (median UIC<150 µg/L) or where subgroups of pregnant women might have ID, like in the Netherlands (134) and the U.S. (135). The WHO only recommend iodine supplements for women of childbearing age, pregnant and lactating, in areas where the iodine status of the general population is not adequate (i.e. median UIC in school aged children (or non-pregnant adults) <100 µg/L) or has not been adequate the last two years (9).

All in all, studies to date, including our publications, indicate that initiating use of iodine supplements *in* pregnancy is probably too late to benefit child neurodevelopment and might also transiently inhibit maternal thyroid function. Thus, in our opinion, there is little or no evidence supporting either an increased recommended iodine intake for pregnant women, or recommending iodine supplements for pregnant women in countries where UIC in pregnant women indicate mild-to-moderate ID by WHO criteria (UIC<150 µg/L). Rigorous RCTs that are sufficiently powered to detect differences are needed to further elucidate this.

Why do we not observe consistent results when using UIC as exposure?

Since we had data on UIC in almost 3000 pregnancies, this could have provided a great opportunity to replicate our findings with an alternative, and more objective, exposure measure. Consistent findings would have increased the likelihood of the associations observed being related to iodine intake since the sources of measurement error are different for the FFQ compared to the UIC. As discussed previously, we suspect that the null-findings in exploring UIC and outcomes on child neurodevelopment might primarily be caused by selection bias. This is supported by the finding that associations between iodine intake from food and outcomes in this subgroup (i.e. the MoBa eTox-population) were not consistent with the associations seen in the whole MoBa, but were largely consistent with the associations of UIC and outcomes (Paper 4). Additionally, since this was an ID population and the median UIC in non-supplement users was only 59 µg/L, a high spot-UIC in an individual might much more often represent a short term high iodine intake than a long-term adequate intake. Thus, UIC will not to the same extent as the FFQ capture the habitual iodine intake although some of the variation in UIC is explained by habitual iodine intake. No level of UIC would thus represent an optimal intake.

On the other hand, when we explored maternal thyroid function, the UIC in MoBa eTox provided a very relevant measure of short-term iodine availability whereas the FFQ data provided a measure the habitual iodine intake. Results in Paper 3 indicate that habitual iodine intake has an opposite effect on levels of FT4 to acute iodine availability (measured by UIC). Based on these results, we hypothesized that mild-to-moderate ID may increase the sensitivity of the thyroid for being temporary inhibited by short-term higher availability of iodine, resulting in reduced levels of maternal FT4 and FT3 (Paper 3) as discussed above. This needs to be further studied in other study designs using repeated measurements of UIC and of maternal thyroid function.

Conclusion

Overall, the results from MoBa indicate that mild-to-moderately insufficient iodine intake in pregnancy (less than ~150 µg/day) was associated with changes in maternal thyroid function, poorer child cognitive development, and more child behaviour problems. There was no evidence of beneficial effects of maternal use of iodine-containing supplements. Initiating iodine supplement use might lead to a temporary inhibition of thyroid hormone production/release in mild-to-moderate ID which potentially can affect neurodevelopment negatively. This was indicated for child behaviour problems, including ADHD, when supplement use was initiated in the first trimester.

Answers to the specific research questions

What is the iodine status of the MoBa pregnant women and their 8 year old children?
Both calculated iodine intake based on the MoBa FFQ and measurements of spot-UIC in a subsample of women documented a high prevalence of insufficient iodine intake in the MoBa pregnant women. UIC measured in a subsample of MoBa children aged 8 years indicated a sufficient iodine intake in the children at a group level.

Does the MoBa FFQ provide a valid measure of habitual iodine intake?
Our study supports previous findings (68, 82) that the MoBa FFQ provides a valid measure of habitual iodine intake. We did, however, observe some indication of underreporting at low calculated iodine intakes and over-reporting at high calculated intakes. Even at calculated habitual intakes above the WHO recommendation (i.e. >250 µg/day), median UIC remained <100 µg/L indicating iodine insufficiency (i.e. median UIC<150 µg/L).

Is maternal iodine intake related to loss to follow-up in the 3- and 8 year questionnaires?
There was no difference in either the habitual iodine intake calculated based on the MoBa FFQ or the reported use of iodine-containing supplements between responders and non-responders to the questionnaires at child age 3- and 8 years.

Main research question 1: *Is maternal habitual iodine intake (calculated by an FFQ covering the first half of pregnancy) in non-users of iodine-containing supplements associated with maternal thyroid function in gestational week 18, child language skills, motor development, and behaviour problems at ages 3 and 8 years, school performance at age 8 years, and ADHD diagnosis (by Dec. 2015)?*
Altogether, the results in papers 1-4 indicate that mild-to-moderate ID in pregnancy, characterized by a habitual iodine intake from food below ~150 µg/day or by UIC below

~100 µg/L, is associated with changes in maternal thyroid function and impaired child neurodevelopment. Except from gross motor development, maternal mild-to-moderate ID was associated with all domains studied, i.e. cognitive performance, fine motor skills, and behaviour problems, including ADHD-symptoms, but not ADHD diagnosis.

In MoBa, very few of the participants had a habitual iodine intake above the recommended intake for pregnant women by WHO. Thus, no conclusions can be drawn regarding the potential impacts of high intakes.

Main research question 2: *What is the impact of taking an iodine-containing supplement in pregnancy on the outcomes listed above, and are potential effects dependent on timing of initiation?*

We found no evidence of any beneficial effects of maternal use of iodine-containing supplements, neither on maternal thyroid function, nor on child neurodevelopment. On the contrary, there were some indications of a temporary inhibition of maternal thyroid function by a recent introduction of an iodine-containing supplement (within the last 5 weeks), and introducing use in the first trimester was associated with more child behaviour problems at 3 years, ADHD symptoms at 8 years, and ADHD diagnosis in the patient registry (NPR).

Is maternal spot-UIC in gestational week 18 (measured in a subsample of 2910 participants) associated with maternal thyroid function in gestational week 18 or child language and learning at child age 8 years?

A low maternal spot-UIC (lower than ~100 µg/L) was associated with changes in maternal thyroid hormone levels, but not with child outcomes at age 8 years. However, the mother-child pairs with available UIC-measurements (n=2910) were a highly selected group of dedicated and well-functioning MoBa participants which might explain, at least partly, the null-findings on child outcomes.

Does maternal selenium status, iron status, and/or intake of cruciferous vegetables affect the association between maternal iodine status and thyroid function?

The prevalence of having a low iron status was high, but few had a low selenium status, and the intake of cruciferous vegetables was generally low. We found no evidence that either selenium status, iron status, or intake of cruciferous vegetables affected the associations between the measures of maternal iodine status and measures of thyroid function in pregnancy.

Is maternal iodine intake differently associated with neurodevelopment in boys and girls?
There was no evidence that the associations between exposures and outcomes studied in this project were different by child sex.

Overall, what is indicated as being the optimal iodine intake in pregnancy?
In sum, the results from our project indicate that a maternal habitual iodine intake below ~150 µg/day (equalling a UIC below ~100 µg/L) is suboptimal. The MoBa study cannot be used to study the impact of high intakes since the iodine intake of the MoBa women was generally low, and in those with a high calculated iodine intake there was evidence of over-reporting (i.e. median UIC was lower than expected).

Future perspectives

Implications of the study findings

This is, by far, the largest study to date documenting the potential negative impact of mild-to-moderate ID on child neurodevelopment. The results from MoBa provide an important contribution to the knowledge on ID, and hopefully they will contribute to highlight the acute need for actions to prevent ID in countries where ID still exists. Indeed, they already have in Norway where many researchers are now working in iodine-related projects, and the health authorities are currently implementing strategies to increase the iodine intake in the population. The results of our study indicate that up to 2/3 of children born in Norway today may not reach their full developmental potential due to maternal ID (i.e. mothers having a habitual iodine intake lower than 150 µg/day). They may also have more behaviour problems, including symptoms of ADHD, anxiety and depression.

Although milk and seafood are high in iodine, these food sources do not secure an adequate iodine intake in a country where the contribution of iodine from drinking water and from iodized salt is negligible. Today, a particularly vulnerable group for ID in Norway are women of childbearing age with a habitual intake of <3 dl milk/yoghurt per day.

Results from our study indicate that iodine supplement use initiated in pregnancy is not beneficial and might even be harmful for the developing foetus if the mother has mild-to-moderate ID and initiates iodine supplement use in the first trimester. These results are important since most multivitamin/-mineral supplements, including supplements that are marketed for pregnant women, contain iodine and are frequently used. Iodine supplements are even recommended for *all* pregnant women in many countries (like in the U.S. (135)). However, the potential impact of taking an iodine-containing supplement in pregnancy needs further investigation in studies with other designs, such as RCTs, since one cannot conclude based on the data available to date. Also, there is a need for more studies investigating the hypothesized "stunning effect" of the thyroid function when iodine intake is abruptly increased in mild-to-moderate ID in the first trimester. This can be done by performing repeated measurements of thyroid hormones before and after initiating iodine supplement use in an RCT. Although more data is needed to conclude on the potential benefits and safety of iodine supplements, there is increasing evidence that the optimal strategy to secure an adequate iodine status during neurodevelopment is to make sure *all women of childbearing age* have an adequate iodine status *before* conception. Securing an adequate long-term iodine

intake in women of childbearing age should probably be the focus of prevention, and supplements should perhaps not be recommended for all pregnant women unless ID is more severe.

Overall, our findings imply that the recommended intake for pregnant women by WHO (≥250 µg/day) might be unnecessary high. The results from MoBa indicate that a habitual iodine intake from food ≥150 µg/day represent an optimal intake for pregnant women. We found little evidence for benefits of higher intakes from food and/or supplements. According to WHO, median UIC in pregnant women should be ≥150 µg/L to reflect iodine adequacy, and this corresponds to an iodine intake of ~250 µg/day (13). To secure such a high intake in pregnant women based on food sources (including iodized salt), other groups of the population will consequently have significantly higher intakes than their recommended minimum. A study published in 2018 by Dold *et al.* (136), the SIMPLIFY study, showed that in populations where UIC in pregnant women was ≥150 µg/L, UIC in other population groups were well above the threshold of 100 µg/L (~200 µg/L in schoolchildren and ~160-200 µg/L in women of childbearing age). In our opinion, the increased recommendation for pregnant women is not supported in the literature and could increase the risk of iodine excess in pregnant women and other population groups, and especially in people taking iodine-containing supplements. This has been documented in China where salt iodization is securing an adequate intake for pregnant women as recommended by WHO, but at the same time introduces risk of iodine excess in children (137). Aiming at a median UIC of ≥100 µg/L for women of childbearing age will most likely secure an adequate iodine reserve to cover the needs in pregnancy. Whether this also applies to exclusively breastfed infants is uncertain since the optimal level of iodine in breastmilk is not yet established.

The first paper from this project, published 17 May 2017 has already reached 32 citations in peer-reviewed journal articles by 30 August 2018 (listed at https://scholar.google.com/). This shows that results from our study are already being discussed and followed up by iodine researchers internationally.

Implications of our findings for design in future studies

- Our results imply that it is likely important to separate long-term/habitual iodine intake and short-term supplement use when studying the associations between iodine intake and outcomes.

- Since the effect of supplement use might be dependent on timing of initiation, it is important to take this into account when designing a study.
- Results from MoBa and other observational studies indicate that the effects of mild-to-moderate ID and supplement use on child development are most likely small, thus large studies are needed to be sufficiently powered to document effects. RCTs exploring supplement use are needed to establish causal effects.
- When studying mild-to-moderate ID, it is key that the women are truly iodine deficient, and based on the result from MoBa data the median UIC should be <100 µg/L.
- There is a need for longitudinal follow up of maternal thyroid hormone levels after initiation of iodine supplementation in pregnancy to verify the hypothesized temporary "stunning effect" on thyroid function when abruptly increasing iodine intake in mild-to-moderate ID.

Thoughts for further research in MoBa

MoBa still offers great opportunities to further explore iodine nutrition. Some ideas are listed below:

- Other relevant outcomes: Time to conception[4], pregnancy and birth outcomes (e.g. intrauterine death[4], preeclampsia[4], gestational length[4], birth weight[4], and new-born TSH), maternal postnatal depression, child growth, autism[4], CP, medication for anxiety and depression in adolescence, maternal risk of high blood pressure and cardiovascular disease 10 years after pregnancy.
- Explore potential effect modification by maternal iodine status on the impact of thyroid disrupting chemicals on maternal thyroid function and on child neurodevelopment.
- Measurement of UIC and thyroglobulin (Tg) in a *random* sample of pregnant women in MoBa and explore associations to child outcomes.

Ideas for further research on iodine

- A prospective study of iodine status and time to conception/fertility.
- A research-based iodine monitoring programme in Norway with aim to document effects of salt iodization programme on iodine intake/status, prevalence of thyroid disorders, new-born TSH etc. An example of such a monitoring programme is DanThyr in Denmark (138).

[4] Publications are already planned on these outcomes.

- Additional mechanistic studies, both animal- and cell studies, exploring potential interaction effects between mild-to-moderate ID and exposure to thyroid disrupting chemicals.

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Papers 1-4

PAPER 1

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Nutritional Epidemiology

Suboptimal Maternal Iodine Intake Is Associated with Impaired Child Neurodevelopment at 3 Years of Age in the Norwegian Mother and Child Cohort Study

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Abstract

Background: Severe iodine deficiency in pregnancy has major effects on child neurodevelopment, but less is known about the potential consequences of mild-to-moderate deficiency and iodine supplement use.

Objective: We explored the associations between maternal iodine intake and child neurodevelopment at 3 y of age and the potential impact of maternal intake of iodine from supplements on the same outcomes.

Methods: This population-based prospective observational study included 48,297 mother-child pairs recruited during pregnancy from 2002 to 2008. Maternal iodine intake was calculated based on a validated food-frequency questionnaire answered during midpregnancy that covered mean intake since the beginning of pregnancy. Associations between iodine intake and maternal-reported child language and motor development and behavior problems were explored by multivariable regression analyses.

Results: In 33,047 mother-child pairs, excluding iodine supplement users, maternal iodine intake was associated with child language delay ($P = 0.024$), externalizing and internalizing behavior problems (both $P < 0.001$), and fine motor skills ($P = 0.002$) but not gross motor skills or the risk of not walking unaided at 17 mo of age. In 74% of the participants who had an iodine intake <160 μg/d (Estimated Average Requirement), suboptimal iodine intake was estimated to account for ~5% (95% CI: −5%, 14%) of the cases of language delay, 16% (95% CI: 0%, 21%) of the cases of externalizing behavior problems >1.5 SD, and 16% (95% CI: 10%, 21%) of the cases of internalizing behavior problems >1.5 SD. In 48,297 mother-child pairs, including iodine supplement users, we found no protective effects of supplemental iodine during pregnancy on neurodevelopment.

Conclusions: Maternal iodine intake below the Estimated Average Requirement during pregnancy was associated with symptoms of child language delay, behavior problems, and reduced fine motor skills at 3 y of age. The results showed no evidence of a protective effect of iodine supplementation during pregnancy. *J Nutr* doi: 10.3945/jn.117.250456

Keywords: iodine, dietary supplements, pregnancy, neurodevelopment, Norwegian Mother and Child Cohort Study, MoBa

Introduction

Iodine deficiency (ID) is one of the most common micronutrient deficiencies worldwide (1). Iodine is required for the production of thyroid hormones, which in turn are essential for brain development in fetal and postnatal life. ID is recognized globally as the main cause of potentially preventable brain damage (1). A recent systematic review estimated that introducing salt iodization in areas of chronic ID may increase mean intelligence quotients (IQs) by 8–10 points (2). Although the consequences of severe ID have been thoroughly investigated, less is known about the potential effects of mild-to-moderate ID during pregnancy (3). Results from 2 observational studies indicate that it might affect cognitive development negatively (4, 5).

Iodine requirements are higher during pregnancy because of the increased production of maternal thyroid hormones, transfer of iodine to the fetus, and increased renal clearance of iodine (6). In 2001, the Institute of Medicine established an Estimated

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Average Requirement (EAR) for pregnant women of 160 μg/d (7), a value also recommended as a cutoff for estimating the prevalence of inadequate iodine intake (8).

In countries in which the population is at risk of ID, the WHO recommends iodine supplementation to secure adequate iodine intake in pregnancy (9). There is, however, a lack of evidence to support the recommendation for iodine supplements during pregnancy in areas with mild-to-moderate ID, and some studies have even indicated that supplemental iodine is associated with adverse effects on child neurodevelopment (10, 11).

The Norwegian population has been considered iodine-replete since iodine was added to cow fodder in the early 1950s, but trends in food consumption over the last decades, characterized by decreases in milk and fish intake, have led to the reappearance of insufficient iodine intakes (12, 13). In Norway, the contribution of iodine from iodized salt for home use (0–5 μg I/g NaCl) and drinking water (~2 μg/L) are negligible (14); thus, iodine intake depends on individual food choices. In Norway, there is no official recommendation for iodine supplement use. Estimated iodine intake based on a validated FFQ in 61,904 pregnant women in the Norwegian Mother and Child Cohort Study (MoBA) revealed a large variation in iodine intake and high prevalence of inadequate intakes (median: 166 μg/d; interdecile range: 71, 369 μg/d) (13). Thirty-two percent of the women reported taking supplements containing iodine. MoBa is one of the world's largest pregnancy cohorts, and it is also the largest study to our knowledge to include data on iodine intake during pregnancy. It thus provides a unique opportunity for studying the impact of inadequate maternal iodine intake and prenatal supplement use on developmental outcomes in children.

The primary aim of this study was to explore associations between iodine intake from food during pregnancy and measures of child neurodevelopment (language, communication, motor development, and behavior problems) at 3 y of age. A second aim was to explore the potential impact of prenatal iodine supplement use, both the dosage and timing of introduction, on the same outcome measures.

Methods

Subjects and design

This study was based on data from MoBa, a prospective population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health (15). Participants were recruited from all over Norway from 1999 to 2008 and were asked to answer questionnaires (in Norwegian) at regular intervals during pregnancy and after birth. In total, >99% of the participants were Caucasian. Pregnancy and birth records from the Medical Birth Registry of Norway are linked to the MoBa database (16). The women consented to participation in 41% of the pregnancies. The cohort now includes 114,500 children, 95,200 mothers, and 75,200 fathers. This study was based on version 8 of the quality-assured data files released for research in 2015 and restricted to participants recruited from 2002 to 2008 because the FFQ was included in the study from March 2002.

A total of 48,297 mother-child pairs were included in this study (60,318 for first steps unaided) (**Figure 1**). For the main analysis, which was restricted to participants who did not report the use of supplements containing iodine during pregnancy, 33,047 mother-child pairs were included (41,245 for first steps unaided). To be eligible for inclusion, participants had to have responded to *1*) a general questionnaire around gestational week 17, *2*) an FFQ around gestational week 22, and *3*) a questionnaire when the child was 3 y of age (or alternatively, for the first steps unaided outcome, a questionnaire at 18 mo of age). Only singleton pregnancies were included. Mothers who reported the use of thyroid medication at any time during pregnancy were excluded from the study. Only participants with information in all covariates were included in the analysis because of the large sample size and low rates of missing values. FFQs with >3 blank pages or with calculated energy intakes <4.5 or >20 MJ/d were excluded (17).

Exposure variables: iodine intake from food and supplements

The FFQ was specifically designed for MoBa (18) and was introduced in March 2002. It is a semiquantitative questionnaire designed to capture dietary habits and the use of dietary supplements during the first half of pregnancy and included questions about the intake of 255 food items or dishes (17). The intake of specific foods and nutrients were calculated based on standard Norwegian portion sizes, the Norwegian food composition table, an analysis of Norwegian milk and food samples (14, 19), and data on the content of >1000 food supplements collected from suppliers (20).

A validation study of 119 women in MoBa recruited 24 ± 12 d (mean ± SD) after the completion of the MoBa FFQ showed that, relative to a dietary reference method (4-d weighed food diary) and several biological markers, the MoBa FFQ produces a realistic estimate of habitual intake and is a valid tool for ranking pregnant women according to high and low intakes of energy, nutrients, and foods (21). The relative validity of total iodine intake from food and supplements and the intake of specific food groups such as dairy products and seafood were evaluated separately (22). The total iodine intake calculated from the FFQ correlated well with the iodine intake reported from the 4-d food diaries at midpregnancy (r = 0.48; 95% CI: 0.33, 0.61) and with 24-h urinary iodine excretion data (r = 0.42; 95% CI: 0.26, 0.56). The triangular validity coefficient for total iodine intake by the FFQ was (r = 0.62; 95% CI: 0.46, 0.77). However, the methods covered somewhat different time periods and the reported supplement use varied between the periods, and large day-to-day within-person variation in iodine intake (reflected in urinary iodine excretion) could be expected. In fact, the FFQ correlation coefficients for the calculated iodine intake and major iodine food sources were higher than for most other foods and nutrients, indicating a regular consumption pattern of food items containing iodine (21). In nonusers of iodine supplements, the estimated median iodine intake from food was 122 μg/d from the FFQ, 120 μg/d from the 4-d food diary, and 122 μg/d based on 24-h urinary iodine excretion data (assuming that 90% is excreted in the urine) (22, 23).

We divided iodine contributed from supplements into 3 categories (0, 1–200, and >200 μg/d). Supplemental iodine >200 μg/d (reported by 2.4% of the women) was defined as a separate category because higher intakes might increase the risk of iodine excess. The timing of the first reported use of supplements containing iodine up to gestational week 22 was reported in the general questionnaires at weeks 17 and (if available) 30 and was coded in 4 categories (never, 0–26 wk before pregnancy, gestational weeks 0–12, and gestational week ≥13).

Neurodevelopmental outcomes

Mothers' reports on child development and behavior provided the basis for the outcome variables. The assessment tools in MoBa are based on standardized and validated scales constructed to identify difficulties within each developmental domain (24–27). Included items were selected from full scales and represented key developmental domains (**Supplemental Methods**). Data on all outcomes were coded as missing when the age of the child at the time of the report was ≥3.5 y, except for the motor milestone.

Language delay. The child's typical level of sentence completeness at 3 y of age was reported by the mothers on a scale developed by Dale et al. (24). The mother classified her child's language competence according to 6 different categories: *1*) not yet talking, *2*) talking but incomprehensibly, *3*) talking in one-word utterances, *4*) talking in 2- to 3-word phrases, *5*) talking in fairly complete sentences, and *6*) talking in long and complicated sentences. The validity of the language and grammar scale has been evaluated by Roth et al. (28). We defined options 5 and 6 as normal language development, options 1–4 as language delay (including severe language delay), and options 1–3 as severe language delay.

Communication skills. Six items from the validated Norwegian version of the Ages and Stages Questionnaire (ASQ) on communication skills

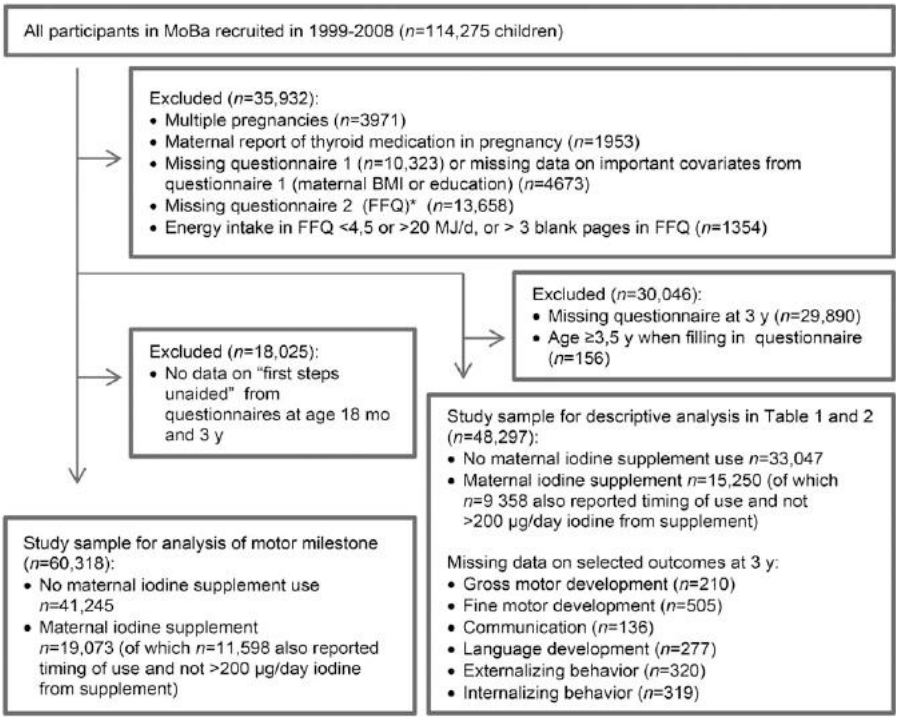

FIGURE 1 Flowchart of inclusion. The general questionnaire was answered around gestational week 17, and the FFQ was answered around gestational week 22. The FFQ was included in the MoBa from 2002. MoBa, the Norwegian Mother and Child Cohort Study.

were included in the questionnaire at 3 y of age (29). Mean scores were calculated and standardized. Good reliability of the scale in MoBa was previously demonstrated with the use of a 2-parameter item response theory analysis (mean factor loading: 0.82) (30).

Motor milestone. The age when the child started walking unaided was reported by the mother when the child was aged 18 mo and/or 3 y. The report on this motor milestone has been shown to be highly reliable (31). Still not walking at 17 mo, corresponding to the 95th percentile, was used as a cutoff for delay.

Motor skills at age 3 y of age. Four items from the ASQ provided the basis for a previously validated score on motor skills—2 on gross motor development and 2 on fine motor development (32). Mean scores were calculated and standardized.

Behavior problems. Externalizing and internalizing behaviors were measured with the use of the child behavior checklist (33). The selected 20 items represented subscales of the internalizing domain (emotionally reactive, anxious and/or depressed, and somatic complaints) and subscales of the externalizing domain (attention problems and aggressive behavior). Mean scores were calculated and standardized. Previous studies reported adequate reliability for the externalizing and internalizing behavior scales (30). The subset of items used in MoBa has been found to be representative (34). A cutoff for child behavior checklist domain scores ≥1.5 SD was chosen to recognize children with high levels of behavior problems.

Covariates

Maternal age was obtained from the Medical Birth Registry of Norway. Prepregnancy BMI, educational status, parity, and parental bilingualism were obtained from the first general questionnaire. Furthermore, because of previous reports of associations between folic acid supplement use and developmental outcomes in MoBa (28, 35), we included a variable that reflected the use of folic acid supplements within the interval from 4 wk before to 8 wk after conception. Maternal energy intake, fiber intake (as a marker of a healthy dietary pattern), and total EPA and DHA intake (from food and dietary supplements) were calculated from the MoBa FFQ. Smoking during pregnancy was collected from questionnaires at gestational weeks 17 and (if available) 30 and when the child was aged 6 mo. Smoking during pregnancy was coded in 3 categories: no reported smoking during pregnancy, reported occasional smoking or stopped smoking before gestational week 12, and reported daily smoking (at any time during pregnancy and had not stopped smoking before gestational week 12).

Other potential covariates or effect modifiers that were explored but not included in the final analysis because they did not change the effect estimates were maternal intake of alcohol and fish, child's sex, year of birth, marital status, paternal educational status, parents' income, maternal chronic illness, and child's age at assessment. Total intake of the n–3 FAs EPA and DHA was only included as a covariate in the analysis of supplemental iodine because it did not change the effect estimates of iodine from food.

Ethics

MoBa was conducted according to Declaration of Helsinki guidelines, and written informed consent was obtained from all participants. This study was approved by the Regional Committee for Medical Research Ethics (Oslo, Norway).

Statistics

We estimated the associations between exposures and neurodevelopmental outcomes with multivariable regression models. Dichotomous outcomes were analyzed with the use of logistic regression, and continuous outcomes were analyzed with the use of generalized linear models with the distribution family that provided the best fit (γ or Gaussian) and identity link. Because some mothers participated in MoBa with >1 pregnancy, all models were adjusted for random effects of sibling clusters.

Analyses of associations between iodine intake from food and neurodevelopmental outcomes were restricted to nonusers of iodine supplements to isolate the effect of long-term iodine intake (as a proxy of iodine status). Iodine intake was modeled with the use of restricted cubic splines with 4 knots at the 5th, 35th, 65th, and 95th percentiles that corresponded to estimated iodine intakes of 54, 102, 142 and 243 µg/d, respectively. All regression models (including crude models) were adjusted for energy intake to control for measurement error. We used 2 different methods to control for energy intake: *1*) the residual method with an energy adjustment of the exposure variable and *2*) the addition of energy intake as a separate covariate. We compared the results to models based on a subsample with a highly restricted energy filter (8–11 MJ/d). The residual method introduced errors at the high and

low levels of exposure, so the second strategy was chosen. Energy intake was modeled with the use of piecewise linear splines (knots at 8.5 and 11 MJ/d) in models in which energy intake was not linearly related to the outcomes when adjusting for all other covariates. Adjusted models also included the following baseline characteristics: maternal age, educational status, parity, prepregnancy BMI, fiber intake, and smoking during pregnancy. For language and communication outcomes, parental bilingualism and folic acid supplement use within the interval from 4 wk before to 8 wk after conception were also included in the adjusted models. Possible interaction effects were explored for BMI, age, educational status, smoking during pregnancy, child's sex, and parity.

The reference value of iodine intake was set at 160 μg/d, which corresponds to the EAR for iodine during pregnancy established by the Institute of Medicine (7). Results are reported as ORs or standardized β coefficients with 95% CIs, and *P* values are reported for overall associations between exposure and outcome and for evidence of nonlinearity in the associations. The tests for overall associations were performed by testing the coefficients of all 3 spline transformations of iodine intake equal to zero and for nonlinearity by testing the coefficients of the second and third spline transformation equal to zero. Tabular results were calculated based on the spline models. Associations were also explored with iodine intakes categorized in 6 categories, and the results were in agreement with results from the flexible spline models (data not shown).

Attributable risk fractions were estimated for *1*) all participants with iodine intakes <160 μg/d (corresponding to the EAR) and *2*) restricted to iodine intakes <100 μg/d. The estimated risks attributed to low iodine intake were calculated based on the models described previously by comparing an ideal situation of women having an iodine intake of 160 μg/d (scenario 1) to the actual situation of reported intakes (scenario 0).

The impact of the amount of iodine from supplements was explored with the use of multivariable regression, including interaction terms between supplemental iodine (no supplement, 1–200, and >200 μg/d) and iodine from food (<160 and ≥160 μg/d). Crude models and adjusted models were adjusted with the use of the same covariates described previously, including maternal folic acid supplement within the interval from 4 wk before to 8 wk after conception and total EPA/DHA intake in the adjusted models.

TABLE 1 Maternal and child characteristics by maternal iodine intake from food (in micrograms per day) during the first half of pregnancy (Norwegian Mother and Child Cohort Study)[1]

		Iodine intake from food[2]					
	All	<50	50–99.9	100–149.9	150–199.9	200–250	>250
Mother-child pairs, *n* (%)	48,297 (100)	1779 (3.7)	14,127 (29.3)	17,698 (36.6)	9133 (18.9)	3465 (7.2)	2095 (4.3)
Maternal age at delivery, y	30.4 ± 4.4[3]	29.8 ± 4.5	30.4 ± 4.4	30.6 ± 4.3	30.4 ± 4.4	30.2 ± 4.5	29.7 ± 4.7
<25	8.9	12.8	8.8	7.7	9.2	9.9	13.8
25–34	73.3	71.3	73.9	73.8	72.9	72.6	71.0
≥35	17.7	16.0	17.3	18.5	17.9	17.5	15.2
Parity							
0	49.6	54.2	52.5	48.5	47.2	47.4	50.3
1	34.4	33.4	33.4	35.3	35.4	34.3	31.3
≥2	15.9	12.4	14.1	16.3	17.3	18.3	18.5
Maternal education, y							
≤12	26.7	36.1	25.9	24.5	26.5	30.5	36.3
13–16	44.9	43.5	44.2	45.2	46.0	45.3	42.7
>16	28.5	20.5	29.9	30.3	27.4	24.3	21.0
Married/cohabitant	96.8	95.8	96.8	97.1	96.9	96.5	95.0
Prepregnancy BMI, kg/m^2	23.9 ± 4.1	24.8 ± 4.7	24.0 ± 4.2	23.8 ± 4.1	23.8 ± 4.1	23.9 ± 4.1	24.3 ± 4.5
<18.5	2.9	3.4	2.8	2.8	2.9	3.1	2.7
18.5–24.9	66.9	55.4	66.7	68.3	68.2	65.9	62.8
25–30	21.4	27.7	21.5	20.7	20.7	22.7	23.8
>30	8.8	13.5	9.1	8.2	8.3	8.3	10.7
Smoking during pregnancy							
No	80.9	76.1	80.6	82.0	81.5	79.8	77.9
Occasionally or quit before GW 12[4]	14.1	15.8	14.8	13.5	13.6	14.1	15.7
Daily	4.9	8.1	4.6	4.5	4.9	6.1	6.3
Alcohol during pregnancy (first half)							
No	89.1	90.9	88.4	88.4	89.9	90.1	92.6
Yes	10.9	9.1	11.6	11.6	10.1	9.9	7.4
Chronic illness	10.0	13.7	11.0	9.4	8.9	9.3	10.0
Parents' income							
Low	25.0	28.3	23.4	23.8	26.4	28.6	30.9
Medium	41.3	41.7	40.2	40.9	42.4	43.6	43.0
High	31.5	27.3	34.4	33.2	29.1	25.0	23.0
Missing	2.2	2.8	2.0	2.1	2.1	2.8	3.2
Child's sex							
Male	51.0	48.9	51.3	50.9	51.1	50.4	51.8
Female	49.0	51.1	48.7	49.1	48.9	49.6	48.2
Bilingual parent(s)	9.7	10.1	10.3	10.1	8.8	8.1	9.1

[1] All values are percentages unless otherwise indicated. GW, gestational week.
[2] Excludes iodine from supplements.
[3] Mean ± SD (all such values).
[4] Mothers who reported daily smoking early during pregnancy but no smoking after GW 11.

The impact of the timing of the first reported use of supplements containing iodine (0–26 wk before pregnancy, gestational weeks 0–12, or gestational week ≥13) was explored in participants who reported an intake of 1–200 μg supplemental iodine/d in the FFQ and who also had provided information on the timing of use in the general questionnaires. Timing was explored in the same way as dosage, including an interaction term with iodine from food (>160 or <160 μg/d), and adjusted for the same covariates. *P* values for tests of associations between iodine supplement use and the outcomes were reported separately in participants with a low (<160 μg/d) and high (≥160 μg/d) intake of iodine from food.

Iodine intake from food and the reported use of supplements containing iodine were explored in participants and eligible nonparticipants with missing data on outcomes and/or covariates. A paired-sample *t* test was conducted to compare iodine intake from food (log-transformed), and a chi-square test was used to determine whether the use of supplements was different in the 2 groups.

Statistical analyses were performed with the use of Stata version 14.0 (StataCorp), including a package for calculating tabular estimates based on the models (package xblc) (36) and another for estimating attributable risk fraction (package punaf) (37). $P < 0.05$ was regarded as statistically significant to avoid unduly reducing the power (38). We also report the significance after Bonferroni correction for multiple comparisons ($P < 0.002$).

Results

Background characteristics. The estimated iodine intake from food (not supplements) during the first half of pregnancy ranged from 9 to 678 μg/d (median: 122 μg/d; IQR: 89, 175 μg/d), and 74% had an estimated intake from food lower than the EAR during pregnancy (160 μg/d). Maternal and child characteristics by iodine intake from food (not including iodine from supplements) are shown in **Table 1**. There were only minor differences in background characteristics by dietary iodine intake level.

Maternal dietary characteristics during pregnancy are shown in **Table 2**. The iodine intake from food was positively correlated with the consumption of known iodine sources (milk and yogurt, fish, eggs) and with total energy and nutrient intakes. The Pearson's correlation coefficient (*r*) between energy and iodine intake was 0.57 ($P < 0.001$). Iodine from supplements did not correlate with iodine intake from food ($r = 0.01$).

Participants compared with nonparticipants. The estimated maternal iodine intake from diet did not differ between our study population (n = 48,297) and participants with dietary information who were excluded because of missing data on outcomes and/or covariates (n = 34,355) (mean difference: 0.9 μg/d; $P = 0.87$). Reported iodine supplement use in the FFQ was slightly higher in participants than in nonparticipants (31.6% compared with 29.6%; $P < 0.001$).

Iodine from food and neurodevelopment. Associations between maternal iodine intake from food and neurodevelopmental

TABLE 2 Maternal dietary characteristics and supplement use by maternal iodine intake from food (in micrograms per day) during the first half of pregnancy (Norwegian Mother and Child Cohort Study)[1]

		Iodine intake from food[2]					
	All	<50	50–99.9	100–149.9	150–199.9	200–250	>250
Mother-child pairs, *n* (%)	48,297 (100)	1779 (3.7)	14,127 (29.3)	17,698 (36.6)	9133 (18.9)	3465 (7.2)	2095 (4.3)
Reported energy intake, MJ/d	9.6 ± 2.5[3]	7.1 ± 1.7	8.4 ± 1.9	9.5 ± 2.0	10.8 ± 2.2	11.8 ± 2.4	13.4 ± 2.6
Food intake, g/d							
Milk/yogurt	445 ± 355	54 ± 60	175 ± 122	392 ± 159	637 ± 206	918 ± 232	1426 ± 422
Lean fish	21 ± 13	8 ± 8	17 ± 11	21 ± 12	25 ± 14	27 ± 15	28 ± 18
Fatty fish	12 ± 14	5 ± 6	9 ± 9	12 ± 12	15 ± 16	17 ± 19	19 ± 23
Eggs	11 ± 12	6 ± 6	9 ± 9	11 ± 12	13 ± 14	13 ± 14	14 ± 17
Fruits and vegetables	441 ± 245	297 ± 181	398 ± 214	443 ± 236	482 ± 254	505 ± 287	549 ± 328
Nutrient intake, g/d							
Protein	87 ± 21	60 ± 12	73 ± 13	85 ± 13	98 ± 15	111 ± 15	131 ± 20
Sugar	61 ± 37	52 ± 42	54 ± 34	59 ± 34	66 ± 37	71 ± 41	82 ± 47
Fiber	31 ± 10	22 ± 8	27 ± 8	31 ± 9	34 ± 10	36 ± 11	39 ± 13
Alcohol	0.1 ± 0.7	0.1 ± 0.4	0.1 ± 0.7	0.1 ± 0.8	0.1 ± 0.5	0.1 ± 0.3	0.1 ± 0.8
Iodine source, μg/d							
Milk/yogurt	61 ± 51	6 ± 6	22 ± 16	54 ± 22	89 ± 29	131 ± 33	205 ± 61
Fish	23 ± 16	8 ± 7	17 ± 11	23 ± 14	28 ± 17	32 ± 20	35 ± 26
Eggs	5 ± 5	3 ± 3	4 ± 4	5 ± 5	6 ± 6	6 ± 6	6 ± 7
Supplements	36 ± 72	33 ± 65	35 ± 71	36 ± 73	35 ± 70	36 ± 74	36 ± 73
Supplements (users only)	113 ± 88	105 ± 76	111 ± 88	114 ± 89	113 ± 84	114 ± 91	117 ± 87
Iodine supplement							
No	68.4	68.6	68.3	68.2	68.9	68.2	69.1
1–99 μg/d	15.7	16.4	16.2	15.7	14.8	16.2	14.8
100–199 μg/d	13.5	12.7	13.0	13.8	14.0	13.0	13.5
≥200 μg/d	2.4	2.4	2.5	2.3	2.2	2.6	2.6
n–3 FA supplement	70.1	58.4	68.8	71.1	72.0	72.0	69.3
Folic acid supplement[4]	75.2	72.3	76.8	76.0	74.1	72.1	69.0
Any supplement (in FFQ)	87.3	81.0	86.7	88.1	88.2	87.8	85.9

[1] Values are percentages unless otherwise indicated.
[2] Excludes iodine from supplements.
[3] Mean ± SD (all such values).
[4] Any reported use of folic acid supplements from 4 wk before to 8 wk after conception reported in the general questionnaire (not in the FFQ).

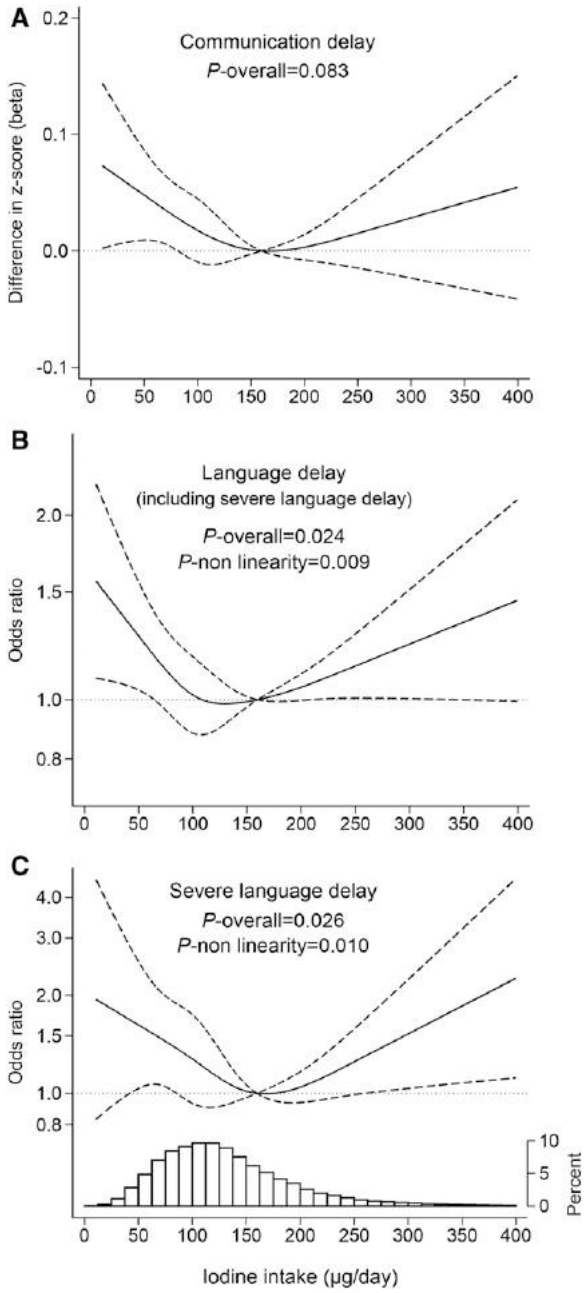

FIGURE 2 Associations between maternal iodine intake from food in pregnancy and child communication (A) and language (B, C) delay at the age of 3 y in the Norwegian Mother and Child Cohort Study. Results are from multivariable regression analyses and restricted to nonusers of iodine supplements during the first half of pregnancy (n = ~33,000 mother-child pairs). Iodine intake was modeled with the use of restricted cubic splines (4 knots), and the reference level was set at 160 µg/d. Dashed lines represent 95% CIs. The histogram in panel C illustrates the distribution of iodine intake. The models were adjusted for maternal age, parity, educational status, BMI, smoking during pregnancy, parent bilingualism, folic acid supplement within the interval from 4 wk before to 8 wk after conception, energy intake, fiber intake, and random effects of sibling clusters. The vertical axes for panels B and C are on a log scale.

outcomes are illustrated in **Figures 2–4.** Low iodine intake was associated with an increased risk of language delay (P-overall = 0.024) and with language delay when restricted to severe delay (P-overall = 0.026). A similar trend was indicated for an association with communication skills (P-overall = 0.083) (Figure 2). The curve shapes for these language outcomes were nonlinear and U-shaped, and the lowest risk of delay was indicated at intakes of ~150–200 µg/d. The group of children characterized with language delay overlapped to a large degree with children who scored low on communication skills [43% of children with language delay and 93% of children with severe language delay also scored low (+2 SD) on communication skills].

Low maternal iodine intake was also associated with more externalizing and internalizing behavior problems (P-overall <0.001) that also remained significant after Bonferroni adjustment for multiple comparisons (Figure 3). The curves displayed similar shapes for continuous outcomes (standardized problem score) and dichotomous outcomes (odds of scoring above the cutoff of +1.5 SD). Maternal iodine intake below ~200 µg/d was associated with an increased risk of both types of behavior problems. For internalizing behavior the curve plateaued when iodine intake reached ~200 µg/d, whereas for externalizing behavior no plateau was observed. Correlations between the 2 behavior scores were r = 0.45. Low maternal iodine intake was also associated with lower fine motor skills (P-overall = 0.002) but not gross motor skills at the age of 3 y or no steps unaided at the age of 17 mo (Figure 4).

Tabular results from unadjusted and adjusted analyses are provided in **Supplemental Tables 1** and **2**.

Significant interaction effects were not detected for iodine with BMI, educational status, parity, smoking during pregnancy, and child's sex. The associations between maternal iodine intake from food and neurodevelopmental outcomes by child's sex are presented in **Supplemental Figures 1–3**. We also explored associations between total iodine intake (including iodine from supplements) and the outcomes, and the associations were attenuated, indicating a differential impact of iodine from food and supplements (data not shown). Venn diagrams illustrating the overlap between the different neurodevelopmental outcomes are presented in **Supplemental Figures 4–8**.

Attributable risk fraction. Attributable risk fraction was calculated for all significant associations between iodine intake from food and dichotomous neurodevelopment outcomes based on the adjusted models described previously, and the reference level was set at 160 µg/d (corresponding to the EAR). In the 74% of participants who had an iodine intake <160 µg/d (median: 105 µg/d; IQR: 80, 129 µg/d) in this sample of nonusers of iodine supplements, the low iodine intake was estimated to account for ~5% (95% CI: −5%, 14%) of cases of language delay (including severe language delay); 21% (95% CI: 0%, 37%) of cases of severe language delay; 16% (95% CI: 10%, 21%) of cases of externalizing behavior problems >1.5 SD; and 16% (95% CI: 10%, 21%) of cases of internalizing behavior problems >1.5 SD.

In the 33% of participants who had an estimated iodine intake from diet of <100 µg/d (median: 77 µg/d; IQR: 62, 89 µg/d) within this subsample, inadequate iodine intake was estimated to account for ~12% (95% CI: −2%, 23%) of cases of language delay; 31% (95% CI: 5%, 50%) of cases of severe language delay; 24% (95% CI: 17%, 31%) of cases of externalizing behavior problems >1.5 SD; and 24% (95% CI: 17%, 31%) of cases of internalizing behavior problems >1.5 SD.

Iodine from supplements and neurodevelopment. The use of supplements containing iodine during the first half of pregnancy was reported by 32% of the mothers in the FFQ. The median contribution of iodine from supplements in this group was 107 µg/d (IQR: 64, 150 µg/d). Only 2.4% of the

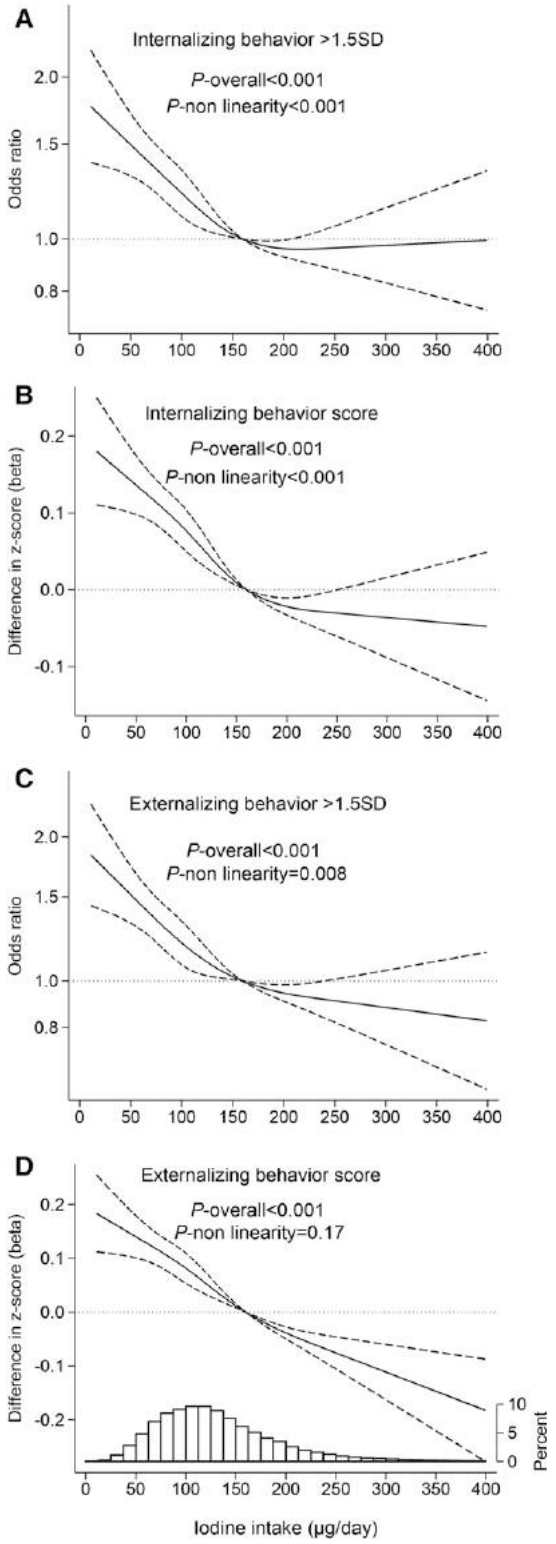

FIGURE 3 Associations between maternal iodine intake from food in pregnancy and internalizing (A, B) and externalizing (C, D) child behavior problems at the age of 3 y in the Norwegian Mother and Child Cohort Study. Results are from multivariable regression analyses and restricted to nonusers of iodine supplements during the first half of pregnancy (n = ~33,000 mother-child pairs). Iodine intake was modeled with the use of restricted cubic splines (4 knots), and the reference level was set at 160 µg/d. Dashed lines represent 95% CIs. The histogram in panel D illustrates the distribution of iodine intake. The models were adjusted for maternal age, parity, educational status, BMI, smoking during pregnancy, energy intake, fiber intake, and random effects of sibling clusters. The vertical axes for panels A and C are on a log scale.

mothers reported taking >200 µg/d. Nine women took single iodine supplements, whereas the remaining women (n = 15,241) reported taking multisupplements containing iodine.

Of the mothers who reported the use of supplements containing iodine in the FFQ, 66% also provided information on the timing of use in the general questionnaires. Among these women, 40% used it before pregnancy (0–6 mo before conception), 29% reported first use in gestational weeks 0–12, and 31% reported first use in gestational week ≥13.

The potential impact of iodine from supplements was explored in 2 groups defined by maternal iodine intake from food (<160 or >160 µg I/d from food), and the results from the multivariable regression analysis are shown in **Table 3**. We found no evidence of beneficial effects on the outcomes for supplemental iodine during pregnancy. Most of the estimates pointed toward small negative effects of supplemental iodine when iodine intake from food was <160 µg/d, but the association was statistically significant only for internalizing behavior problems (P < 0.001), which also remained significant with the Bonferroni correction. Results from crude models are shown in **Supplemental Table 3**.

The impact of the timing of the first report of iodine supplement use was explored in women reporting the use of supplemental iodine (maximum of 200 µg/d) in the FFQ (n = 9358) and compared with the reference group of nonsupplement users (n = 33,047) (**Supplemental Tables 4** and **5**). In women with iodine intake from food >160 µg/d, iodine supplement use was not significantly associated with the outcomes regardless of timing. In women with an intake from food <160 µg/d who reported taking supplements containing iodine before pregnancy (dosage before pregnancy unknown), supplement use was not significantly related to any of the outcomes. However, in those reporting first use in gestational weeks 0–12, supplement use was associated with an increased risk of externalizing behavior problems (adjusted OR of scoring >1.5 SD: 1.28; 95% CI: 1.09, 1.49), and the introduction of supplements in gestational week ≥13 was associated with an increased risk of internalizing behavior problems (adjusted OR of scoring >1.5 SD: 1.27; 95% CI: 1.10, 1.46). These results did not remain statistically significant after adjusting for multiple comparisons.

Discussion

The main finding from this study was that an iodine intake below the EAR value of 160 µg/d was associated with an increased risk of language delay, behavior problems, and fine motor development in children aged 3 y but not with measures of gross motor development (Figures 2–4). We found no evidence of a beneficial effect of supplemental iodine in pregnancy, and there were some indications of negative effects of supplements on behavior outcomes in children of mothers with a low iodine intake from food (<160 µg/d). To our knowledge, this is the most extensive study to date on the potential consequences of maternal iodine intake on child neurodevelopment.

Iodine from food and neurodevelopment. To isolate the effect of long-term iodine intake, we restricted these analyses to participants who did not report the use of supplements containing iodine in the FFQ. Previous studies have indicated that long-term iodine intake might be more important for thyroid function during pregnancy than recent supplement use (39). Indeed, we also found that the associations between iodine

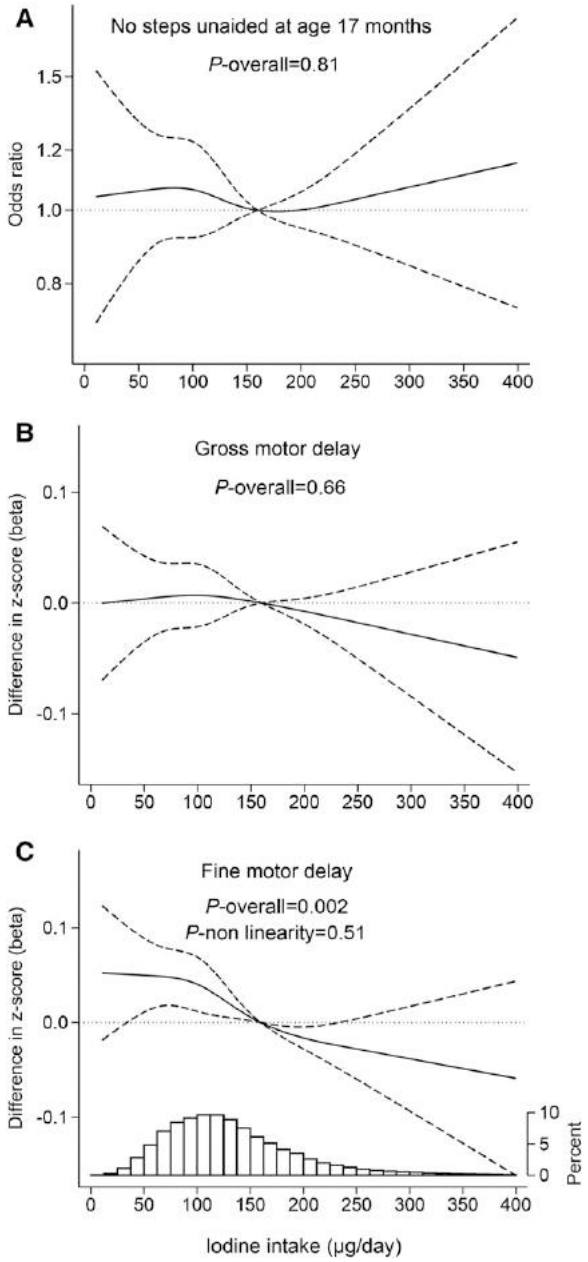

FIGURE 4 Associations between maternal iodine intake from food in pregnancy and child motor development by the age of 3 y in the Norwegian Mother and Child Cohort Study. Results are from multivariable regression analyses and restricted to nonusers of iodine supplements during the first half of pregnancy [n = 41,245 mother-child pairs for first steps (A) and n = ~33,000 for motor scores (B, C)]. Iodine intake was modeled with the use of restricted cubic splines (4 knots), and the reference level was set at 160 µg/d. Dashed lines represent 95% CIs. The histogram in panel C illustrates the distribution of iodine intake. The models were adjusted for maternal age, parity, educational status, BMI, smoking during pregnancy, energy intake, fiber intake, and random effects of sibling clusters. The vertical axis for panel A is on a log scale.

intake and outcomes were attenuated when we included iodine supplement users and modeled the estimated total iodine intake and outcomes.

Results from several studies suggest that maternal general thyroid dysfunction may start to develop when urinary iodine concentration (UIC) in pregnancy is below ~50 µg/L (corresponding to an estimated iodine intake of ~83 µg/d assuming 90% recovery in the urine and a mean urine volume of 1.5 L/d) (40). Shi et al. (41) described a U-shaped relation between urinary iodine excretion and the prevalence of thyroid disorders in a study that included 7190 pregnant women in China, and the risks were lowest in the group of women with a UIC of 150–249 µg/L. This corresponds to a regular iodine intake of ~250–415 µg/d. In our study, the shapes of the association curves indicate that an intake <160 µg/d from food was associated with an increased risk of negative outcomes, in line with the findings of Shi et al. (41). For intakes ≥200 µg/d, our results were not consistent. We found an increased risk of language delay, a reduced risk of behavior problems, and no change in fine motor skills. At 200 µg/d, the intake is still below the recommended intake by the WHO (250 µg/d) (9) and Institute of Medicine (220 µg/d) (7) and well below the upper intake level of 500 µg/d generally regarded as safe (9). Therefore, our study does not consistently indicate an optimal intake level. Caution must be made when interpreting the results for iodine intakes from food >250 µg/d in our study because only 4.3% of the women had such high intakes.

Language development plays a fundamental role in cognition, social development, and learning. Early language deficits may impair long-term social adaptation, cognitive development, and academic achievement and are associated with psychiatric disorders in young adults (42–44). Impairments in cognitive development associated with maternal mild-to-moderate ID have previously been reported in 2 observational studies (4, 5). In a study in the United Kingdom that included 1040 mother-child pairs, Bath et al. (4) found an increased risk of scoring within the lowest quartile on an IQ measure at the age of 8 y (OR: 1.58; 95% CI: 1.09, 2.30) and on reading accuracy (OR: 1.69; 95% CI: 1.15, 2.49) and comprehension (OR: 1.54; 95% CI: 1.06, 2.23) at the age of 9 y in children of mothers with spot urinary iodine below a cutoff of 150 µg creatinine/g during pregnancy (gestational week ≤13; median: 10 wk), indicating mild-to-moderate ID in pregnancy. They also observed a dose-response relation on IQ and reading comprehension when subdividing into 3 categories of exposure (<50, 50–150, >150 µg creatinine/g). Hynes et al. (5) reported lower educational assessment scores (spelling, grammar, and English literacy performance) in Australian children aged 9 y (n = 228) of mothers who had a UIC <150 µg/L during pregnancy (indicating mild-to-moderate ID) than those who had a UIC ≥150 µg/L.

We observed a dose-response relation between maternal iodine intake and externalizing and internalizing behavior problems (Figure 3). The questions on externalizing behavior problems included in our study partly overlapped with screening questions for attention-deficit hyperactivity disorder (ADHD). Mild-to-moderate ID has previously been linked to ADHD in a non-randomized controlled trial in Italy (n = 27 mother-child pairs), in which Vermiglio et al. (45) observed an increased risk of ADHD in children born to mothers from an area with moderate ID (69% fulfilled the diagnostic criteria of ADHD) compared with an area of marginal ID (no cases of ADHD). An increased risk of ADHD has also been reported with generalized resistance to thyroid hormones, indicating the important role thyroid hormone concentrations might play in a possible causal mechanism (46).

Mild-to-moderate ID may affect neurodevelopment by increasing the risk of thyroid disorders, as indicated by Shi et al. (41). Another mechanism could be that ID causes maternal and/or fetal thyroids to be more vulnerable to environmental goitrogens, abundant in certain foods and in cigarettes, causing transient deficits in thyroid hormones during critical periods in neurodevelopment. Román (47) hypothesized that this mechanism is an important cause of autism.

TABLE 3 Adjusted models of associations between iodine from supplements during the first half of pregnancy and child development by different levels of iodine intake from food during pregnancy (Norwegian Mother and Child Cohort Study)[1]

	Percentage	Language delay[2]	Communication delay *z* score[2]	Internalizing behavior problems		Externalizing behavior problems		Not walking at age 17 mo	Fine motor delay *z* score	Gross motor delay *z* score
				+1.5 SD	*z* score	+1.5 SD	*z* score			
Sample size		48,020	48,161	47,978	47,978	47,977	47,977	60,318	47,792	48,087
Cases, *n* (%)		1947 (4.1)		4389 (9.2)		3848 (8.0)		1800 (3.0)		
Iodine from food <160 μg/d										
No supplement	50.9	1	0	1	0	1	0	1	0	0
1–200 μg/d	21.9	1.06 (0.94, 1.19)	0.00 (−0.02, 0.02)	1.14 (1.06, 1.24)	0.04 (0.02, 0.07)	1.07 (0.98, 1.16)	0.02 (−0.00, 0.04)	1.05 (0.93, 1.19)	0.00 (−0.02, 0.03)	0.00 (−0.02, 0.03)
>200 μg/d	1.8	1.02 (0.71, 1.47)	0.04 (−0.02, 0.11)	1.01 (0.80, 1.28)	0.01 (−0.06, 0.07)	1.21 (0.96, 1.54)	0.05 (−0.02, 0.12)	1.15 (0.82, 1.61)	0.00 (−0.07, 0.06)	0.02 (−0.05, 0.09)
P-overall		0.68	0.44	0.004	<0.001	0.11	0.079	0.56	0.91	0.89
Iodine from food ≥160 μg/d										
No supplement	17.5	1	0	1	0	1	0	1	0	0
1–200 μg/d	7.3	1.09 (0.89, 1.32)	0.03 (−0.01, 0.06)	1.05 (0.91, 1.20)	0.02 (−0.02, 0.06)	1.07 (0.92, 1.24)	0.02 (−0.02, 0.06)	1.02 (0.83, 1.26)	−0.03 (−0.07, 0.01)	−0.03 (−0.07, 0.01)
>200 μg/d	0.6	0.92 (0.49, 1.72)	0.09 (−0.05, 0.24)	0.76 (0.48, 1.21)	0.02 (−0.09, 0.12)	1.02 (0.65, 1.60)	0.05 (−0.06, 0.17)	1.04 (0.58, 1.87)	0.00 (−0.12, 0.12)	−0.07 (−0.17, 0.03)
P-overall		0.67	0.19	0.38	0.55	0.70	0.42	0.97	0.42	0.18

[1] Values are adjusted ORs (95% CIs) for associations with dichotomous outcomes and adjusted standardized β coefficients (95% CIs) for continuous outcomes unless otherwise indicated. Results are from multivariable analyses, including interaction terms between iodine from diet and iodine from supplements, and the models were adjusted for maternal age, BMI, parity, educational status, smoking during pregnancy, energy intake, fiber intake, folic acid supplement within the interval from 4 wk before to 8 wk after conception, total EPA/DHA intake, and random effects of sibling clusters. Reported *P* values reflect the potential effect of iodine from supplements on outcomes in participants with a low (<160 μg/d) or high (≥160 μg/d) intake of iodine from foods during pregnancy.

[2] Additionally adjusted for parental bilingualism.

Iodine from supplements and neurodevelopment. Previous studies on iodine supplement use in pregnancy and child neurodevelopmental outcomes in areas with mild-to-moderate ID have shown inconsistent results (3, 39, 48), and to our knowledge there are no randomized controlled trials published to date. In this study we examined the associations in women with iodine intake from food below and above the EAR (160 μg/d) separately because the effect of supplemental iodine might depend on previous iodine status. Our findings of no beneficial effects and some indications of negative effects are supported by findings from the Environment and Childhood cohort in Spain in which an increased risk of low psychomotor (10, 11) and mental scores (11) in children of women who reported intake of iodine from supplements ≥150 μg/d compared with <100 μg/d was observed. On the other hand, Velasco et al. (49) reported a positive impact on psychomotor scores in children of mothers who received 300 μg I from supplements from the first trimester compared with controls in a nonrandomized intervention study. The negative effects of iodine supplement use observed in our study were seen when mothers had iodine intake from food <160 μg/d and initiated iodine supplement use after conception.

There could be several reasons why no beneficial effects of iodine from supplements were observed. Initiating supplement use during pregnancy might be too late and may also provide less iodine than needed to compensate for the effects of a depleted iodine store on thyroid function. A sudden increase in iodine intake, although modest and within the recommendations, might also lead to a "stunning effect," with transient inhibition of maternal or fetal thyroid hormone production (50). In addition, because the iodine supplements reported by the women in MoBa were almost exclusively multisupplements, we cannot eliminate the possibility of other substances in the supplements acting as confounders or effect modifiers.

Iodine intake as a measure of iodine status. To our knowledge, there are no valid biomarkers for assessing iodine status at an individual level (51). UIC is useful as an indicator of ID at the population level but not at the individual level because of large day-to-day variation. In our study, we used estimated iodine intake from an extensive and validated FFQ that most likely reflects long-term iodine intake and thus iodine status. To our knowledge, iodine intake from food has not previously been used as a measure of individual iodine status in studies that have explored associations with health outcomes. In most countries, iodized salt contributes substantially to iodine intake, making FFQs less suited for estimating iodine intake. In Norway this is not the case, and indeed the MoBa FFQ has proven to be a valid tool for assessing iodine intake, as described previously. The use of calculated iodine intake as opposed to UIC allows for distinguishing between iodine from food and from supplements when exploring exposure-outcome associations.

Strengths and limitations. Potential effects of mild-to-moderate ID are most likely small and only detectable in large studies. Strengths of MoBa include the large sample size, prospective design, and extensive collection of data. The iodine situation among pregnant women in Norway, with a high frequency of low intakes and a large variation in exposure, makes MoBa ideal for studying suboptimal iodine intakes. In Norway, the weaning diet of most children includes iodine-fortified baby foods, and children have a higher intake of dairy products than adults relative to their energy intake. Unless dairy products are excluded from the child's diet, Norwegian infants and toddlers most likely get adequate amounts of iodine (52).

Weaknesses include the observational design, which implies that we cannot rule out the possibility of residual confounding. Self-administered questionnaires introduce the risk of measurement errors and misclassifications, but the biases introduced would most likely tend to weaken associations (53). The participation rate of 41% in MoBa introduces the risk of selection bias. However, a previous study of MoBa found that although the prevalence of exposures and outcomes might be biased, exposure-outcome associations did not differ between MoBa and a nationally representative sample (54).

Clinical relevance and implications. ID is easily preventable at a low cost. The results of this study emphasize the urgent need for preventing inadequate iodine intake in women of childbearing age to secure optimal brain development in children. Securing an adequate long-term iodine intake before pregnancy is important because supplementation during pregnancy might not compensate and could even be harmful in mild-to-moderate ID. The estimated attributable risk fractions of having a lower iodine intake than the EAR indicate that mild-to-moderate ID may be an important risk factor for behavior problems and language delay, especially if maternal long-term iodine intake is <100 μg/d.

Future studies with the use of UIC as the exposure variable should exclude iodine supplement users or at least control for iodine supplement use because short-term iodine intake from supplements seems to have a differential impact than long-term intake. Our results show that maternal iodine intake below the EAR during pregnancy is associated with symptoms of impaired child neurodevelopment. Our study does not support recommending iodine supplementation to pregnant women in areas with suboptimal iodine intakes.

Acknowledgments

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Supplemental Methods

Neurodevelopmental outcomes - Items included in the questionnaire at child's age 3 years
Complete questionnaires are available online at https://www.fhi.no/en/studies/moba/for-participants-articles/questionnaires-from-moba/

Behavior problems
Scores were calculated based on selected items from the Child behavior check list (CBCL/ 11/2-5/LDS) (1). Mother reported "Not true", "Somewhat or sometimes true", or "Very true or often true" to the given statements. Selected items are listed below:

Internalizing behavior problems, nine selected items including:

- Three items from the subdomain 'anxious/depressed' ("Clings to adults or too dependent", "Gets too upset when separated from parents", and "Too fearful or anxious")
- Two items from the subdomain 'emotionally reactive' ("Disturbed by any change in routine" and "Sudden changes in moods or feelings")
- Four items from the subdomain 'somatic complaints' ("Constipated, doesn't move bowels", "Doesn't eat well", "Stomach aches or cramps (without medical cause)", and "Vomiting, throwing up (without medical cause)")

Externalizing behavior problems, eleven selected items including:

- Seven items from the subdomain 'aggressive behavior' ("Can't stand waiting, wants everything now", "Defiant", "Demands must be met immediately", "Doesn't seem to feel guilty after misbehaving", "Gets into many fights", "Hits others", and "Punishment doesn't change his/her behavior")
- Four items from the subdomain 'attention problems' ("Can't concentrate, can't pay attention for long", "Can't sit still, restless or hyperactive", "Poorly coordinated or clumsy", and "Quickly shifts from one activity to another")

Language development (question on sentence complexity developed by Dale & Bishop (2)):

About your child's language skills.
(Enter a cross for the option which best describes the way your child talks.)

- Not yet talking
- He/she is talking, but you can't understand him/her
- Talking in one-word utterances, such as "milk" or "down"
- Talking in 2- to 3 word phrases, such as "me got ball" or "give doll"
- Talking in fairly complete sentences, such as "I got doll" or "can I go outside?"
- Talking in long and complicated sentences, such as "when I went to the park, I went on the swings" or "I saw a man standing on the corner"

Communication (six selected items from the Ages and stages questionnaire (ASQ) (3)). Mother reported "Yes", "A few times", or "Not yet" to the given statements. Selected items are listed below:

1. Without showing him/her first, does your child point to the correct picture when you say, "Where is the cat" or "Where is the dog"? Your child must only point at the correct picture
2. When you ask your child to point at his/her eyes, nose, hair, feet, ears, etc., does he/she point correctly at least seven parts of the body? (The child can point at himself/herself, you or a doll.)
3. Does your child use sentences made up of three or four words?
4. Without giving him/her help by pointing or using gestures, ask your child to "Put the shoe on the table" and "Put the book under the chair". Does your child carry out both of these directions correctly?
5. When looking at a picture book, does your child tell you what is happening or what action is taking place in the picture? (For example, "Barking", "Running", "Eating" and "Crying"?) You may ask, "What is the dog (or boy) doing?"
6. Can your child tell you at least two things about an object he/she is familiar with? If you say, for example, "Tell me about your ball", will your child answer by saying something like "It is round, I can throw it, it is big"?

Gross and fine motor development (four selected items from the Ages and stages questionnaire (ASQ) (3)). Mother reported "Yes", "A few times", or "Not yet" to the given statements. Selected items are listed below:

1. Can your child kick a ball by swinging his/her leg forward without holding onto anything for support?
2. Can your child catch a large ball with both hands?
3. When drawing, does your child hold a pencil, crayon or pen between his/her fingers and thumb like an adult does?
4. Can your child undo one or more buttons?

Supplemental Table 1 Associations between maternal iodine intake from food in pregnancy and child development at age 3 years in participants who did not report use of supplemental iodine in pregnancy, crude models[1]

	Communication delay (z-score)	Language delay[2] (incl. severe language delay)	Severe language delay[2]	Internalizing behavior problems +1.5 SD	Internalizing behavior problems z-score	Externalizing behavior problems +1.5 SD	Externalizing behavior problems z-score	Not walking at age 17 months	Gross motor delay z-score	Fine motor delay z-score
Sample size	32,089	32,851	32,593	32,809	32,809	32,809	32,809	41,245	32,905	32,706
Cases *n* (%)		1345 (4.1)	258 (0.79)	2895 (8.8)		2591 (7.9)		1211 (2.9)		
Iodine intake (µg/day)										
25	0.11 (0.05, 0.17)	1.71 (1.25, 2.33)	2.04 (1.01, 4.14)	1.89 (1.55, 2.30)	0.21 (0.15, 0.26)	1.97 (1.61, 2.42)	0.22 (0.16, 0.28)	1.05 (0.77, 1.45)	0.01 (-0.04, 0.07)	0.03 (-0.03, 0.09)
50	0.07 (0.03, 0.11)	1.41 (1.14, 1.73)	1.73 (1.09, 2.74)	1.63 (1.43, 1.86)	0.16 (0.12, 0.20)	1.65 (1.44, 1.90)	0.17 (0.13, 0.21)	1.06 (0.86, 1.31)	0.01 (-0.03, 0.04)	0.04 (-0.00, 0.07)
75	0.03 (0.00, 0.06)	1.16 (1.00, 1.36)	1.47 (1.06, 2.03)	1.41 (1.28, 1.57)	0.12 (0.09, 0.15)	1.39 (1.25, 1.55)	0.12 (0.09, 0.15)	1.06 (0.91, 1.24)	0.00 (-0.03, 0.03)	0.04 (0.01, 0.07)
100	-0.00 (-0.03, 0.03)	1.01 (0.88, 1.17)	1.25 (0.92, 1.72)	1.24 (1.12, 1.36)	0.08 (0.05, 0.10)	1.20 (1.08, 1.33)	0.08 (0.05, 0.11)	1.05 (0.91, 1.22)	-0.00 (-0.03, 0.02)	0.04 (0.01, 0.07)
125	-0.01 (-0.02, 0.01)	0.97 (0.89, 1.06)	1.10 (0.89, 1.34)	1.10 (1.04, 1.17)	0.04 (0.02, 0.05)	1.08 (1.01, 1.15)	0.04 (0.02, 0.06)	1.03 (0.94, 1.13)	-0.00 (-0.02, 0.02)	0.02 (0.01, 0.04)
160 (ref)	0	1	1	1	0	1	0	1	0	0
200	0.01 (0.00, 0.02)	1.06 (1.01, 1.11)	1.05 (0.95, 1.18)	0.98 (0.94, 1.01)	-0.01 (-0.02, 0.00)	0.97 (0.93, 1.00)	-0.03 (-0.04, -0.01)	1.00 (0.95, 1.06)	-0.00 (-0.02, 0.01)	-0.01 (-0.02, 0.00)
225	0.02 (-0.00, 0.04)	1.10 (1.02, 1.20)	1.15 (0.97, 1.35)	0.99 (0.93, 1.05)	-0.00 (-0.02, 0.02)	0.96 (0.90, 1.03)	-0.04 (-0.05, -0.02)	1.02 (0.93, 1.12)	-0.01 (-0.03, 0.01)	-0.01 (-0.03, 0.01)
250	0.03 (-0.00, 0.06)	1.15 (1.02, 1.30)	1.27 (1.00, 1.60)	1.01 (0.93, 1.11)	0.00 (-0.03, 0.03)	0.96 (0.87, 1.06)	-0.04 (-0.07, -0.01)	1.04 (0.90, 1.19)	-0.01 (-0.05, 0.02)	-0.01 (-0.04, 0.02)
300	0.04 (-0.01, 0.10)	1.26 (1.03, 1.54)	1.55 (1.05, 2.28)	1.06 (0.91, 1.25)	0.02 (-0.03, 0.07)	0.96 (0.81, 1.15)	-0.06 (-0.11, -0.01)	1.08 (0.85, 1.37)	-0.02 (-0.08, 0.03)	-0.01 (-0.06, 0.04)
350	0.06 (-0.02, 0.14)	1.37 (1.03, 1.83)	1.90 (1.10, 3.27)	1.12 (0.89, 1.40)	0.04 (-0.04, 0.11)	0.96 (0.75, 1.24)	-0.07 (-0.15, -0.00)	1.12 (0.80, 1.57)	-0.03 (-0.12, 0.05)	-0.01 (-0.08, 0.07)
400	0.07 (-0.03, 0.17)	1.50 (1.03, 2.18)	2.31 (1.14, 4.67)	1.17 (0.87, 1.57)	0.05 (-0.04, 0.15)	0.96 (0.69, 1.33)	-0.09 (-0.19, 0.00)	1.16 (0.75, 1.80)	-0.04 (-0.15, 0.06)	-0.01 (-0.11, 0.09)
P-overall	*P*<0.001	*P*=0.001	*P*=0.015	*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.001	*P*=0.85	*P*=0.84	*P*=0.031
P-non linearity	*P*<0.001	*P*<0.001	*P*=0.006	*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.001			*P*=0.24

[1] Values are odds ratios (95% CIs) for associations with dichotomous outcomes and standardized betas (95% CIs) for continuous outcomes unless otherwise indicated. Results are from multivariable regression analysis adjusting for reported energy intake in the FFQ to adjust for measurement error, and adjusting for random effects of sibling clusters. Iodine intake was modelled by restricted cubic splines with 4 knots (at percentiles 5, 35, 65 and 95 / iodine intakes of 54, 102, 142 and 243 µg/day). Energy intake was modelled linearly for behavior outcomes and piecewise linearly (knots at 8.5 and 11 MJ/d) for all other outcomes.

[2] Language delay was defined when the child was talking in maximum two to three word phrases and also includes severe language delay. Severe language delay was defined when the child was not yet talking, speaking in only one word utterances or in unintelligible speak.

Supplemental Table 2 Associations between maternal iodine intake from food in pregnancy and child development at age 3 years in participants who did not report use of supplemental iodine in pregnancy, adjusted models[1]

	Communication delay[2]	Language delay (incl. severe language delay)[2]	Severe language delay[2]	Internalizing behavior problems	Internalizing behavior problems	Externalizing behavior problems	Externalizing behavior problems	Not walking at age 17 months	Gross motor delay	Fine motor delay
	z-score			+1.5 SD	z-score	+1.5 SD	z-score		z-score	z-score
Sample size	32,089	32,851	32,593	32,809	32,809	32,809	32,809	41,245	32,905	32,706
Cases *n* (%)		1345 (4.1)	258 (0.79)	2895 (8.8)		2591 (7.9)		1211 (2.9)		
Iodine intake (µg/day)										
25	0.06 (0.01, 0.12)	1.45 (1.07, 1.96)	1.82 (0.91, 3.64)	1.66 (1.36, 2.03)	0.16 (0.11, 0.22)	1.71 (1.40, 2.10)	0.17 (0.11, 0.23)	1.05 (0.76, 1.44)	0.00 (-0.06, 0.06)	0.05 (-0.01, 0.11)
50	0.05 (0.01, 0.09)	1.27 (1.04, 1.56)	1.62 (1.03, 2.55)	1.50 (1.31, 1.71)	0.14 (0.10, 0.17)	1.52 (1.32, 1.74)	0.14 (0.10, 0.18)	1.06 (0.86, 1.31)	0.00 (-0.04, 0.04)	0.05 (0.01, 0.09)
75	0.03 (0.00, 0.06)	1.12 (0.96, 1.30)	1.44 (1.05, 1.99)	1.35 (1.22, 1.49)	0.11 (0.08, 0.14)	1.34 (1.21, 1.50)	0.11 (0.08, 0.14)	1.07 (0.92, 1.25)	0.01 (-0.02, 0.04)	0.05 (0.02, 0.08)
100	0.02 (-0.01, 0.04)	1.02 (0.88, 1.18)	1.27 (0.93, 1.75)	1.22 (1.10, 1.34)	0.08 (0.05, 0.10)	1.20 (1.08, 1.33)	0.08 (0.05, 0.11)	1.06 (0.92, 1.23)	0.01 (-0.02, 0.04)	0.04 (0.01, 0.07)
125	0.01 (-0.01, 0.02)	0.99 (0.90, 1.08)	1.11 (0.91, 1.36)	1.10 (1.04, 1.17)	0.04 (0.02, 0.06)	1.10 (1.03, 1.17)	0.05 (0.03, 0.06)	1.04 (0.95, 1.13)	0.01 (-0.01, 0.02)	0.02 (0.01, 0.04)
160 (ref)	0	1	1	1	0	1	0	1	0	0
200	0.00 (-0.01, 0.01)	1.05 (1.00, 1.10)	1.04 (0.94, 1.16)	0.96 (0.93, 1.00)	-0.02 (-0.03, -0.01)	0.94 (0.91, 0.98)	-0.04 (-0.05, -0.03)	1.00 (0.95, 1.06)	-0.01 (-0.02, 0.00)	-0.02 (-0.03, -0.00)
225	0.01 (-0.01, 0.03)	1.09 (1.00, 1.18)	1.13 (0.96, 1.34)	0.96 (0.90, 1.02)	-0.03 (-0.05, -0.01)	0.92 (0.86, 0.99)	-0.06 (-0.08, -0.04)	1.02 (0.92, 1.11)	-0.01 (-0.03, 0.01)	-0.02 (-0.04, -0.00)
250	0.02 (-0.01, 0.05)	1.13 (1.01, 1.28)	1.25 (0.99, 1.58)	0.96 (0.88, 1.06)	-0.03 (-0.06, -0.00)	0.91 (0.82, 1.01)	-0.08 (-0.11, -0.05)	1.03 (0.90, 1.19)	-0.02 (-0.05, 0.01)	-0.03 (-0.06, 0.00)
300	0.03 (-0.02, 0.08)	1.23 (1.00, 1.51)	1.52 (1.04, 2.24)	0.97 (0.83, 1.14)	-0.04 (-0.09, 0.02)	0.88 (0.74, 1.05)	-0.11 (-0.16, -0.06)	1.07 (0.85, 1.36)	-0.03 (-0.08, 0.03)	-0.04 (-0.09, 0.02)
350	0.04 (-0.03, 0.12)	1.34 (1.00, 1.79)	1.85 (1.08, 3.20)	0.98 (0.78, 1.24)	-0.04 (-0.12, 0.03)	0.85 (0.66, 1.10)	-0.15 (-0.22, -0.07)	1.11 (0.79, 1.56)	-0.04 (-0.12, 0.04)	-0.05 (-0.13, 0.03)
400	0.05 (-0.04, 0.15)	1.45 (0.99, 2.12)	2.25 (1.11, 4.55)	0.99 (0.74, 1.34)	-0.05 (-0.14, 0.05)	0.83 (0.59, 1.15)	-0.18 (-0.28, -0.09)	1.15 (0.74, 1.79)	-0.05 (-0.15, 0.06)	-0.06 (-0.16, 0.04)
P-overall	*P*=0.083	*P*=0.024	*P*=0.026	*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.001	*P*=0.81	*P*=0.66	*P*=0.002
P-non linearity		*P*=0.009	*P*=0.010	*P*<0.001	*P*<0.001	*P*=0.008	*P*=0.17			*P*=0.51
Attributable risk fraction (95% CI) Population 1[3]	-	0.051 (-0.048, 0.140)	0.205 (0.004, 0.365)	0.158 (0.101, 0.212)	-	0.158 (0.096, 0.214)	-	0.044 (-0.060, 0.138)	-	-
Attributable risk fraction (95% CI) Population 2[3]	-	0.116 (-0.021, 0.234)	0.310 (0.052, 0.497)	0.241 (0.169, 0.308)	-	0.243 (0.166, 0.314)	-	0.060 (-0.091, 0.190)	-	-

[1] Values are adjusted odds ratios (95% CIs) for associations with dichotomous outcomes and adjusted standardized betas (95% CIs) for continuous outcomes unless otherwise indicated. Results are from multivariable regression analysis. Iodine intake was modelled by restricted cubic splines (4 knots) and the reference level was set to 160µg/day. The models were adjusted for maternal age, parity, education, body mass index, smoking in pregnancy, energy intake, fiber intake, and for random effects of sibling clusters. For language and communication outcomes bilingual parent(-s) and folic acid supplement within the interval from 4 weeks before to 8 weeks after conception were also included in the adjusted models. Energy intake was modelled linearly for behavior outcomes and piecewise linearly (2 knots) for all other outcomes.

[2] Models on language and communication are additionally adjusted for bilingual parent(-s). Language delay was defined when the child was talking in maximum two to three word phrases and also includes severe language delay. Severe language delay was defined when the child was not yet talking, speaking in only one word utterances or in unintelligible speak.

[3] Attributable risk fraction was calculated based on the models and gives the estimated fraction of the adverse outcome in this population attributed to the mothers having a lower iodine intake in pregnancy than the estimated average requirement in pregnancy of 160µg/day. Population 1: All participants with maternal iodine intake from food 0-160µg/day, population 2: participants with iodine intake from food 0-100µg/day.

Supplemental Table 3 Iodine from supplements in first half of pregnancy and child development at 3 years by different levels of iodine intake from food in pregnancy, crude models[1]

Iodine from supplements µg/day	%	Language delay[2]	Communication delay	Internalizing behavior problems		Externalizing behavior problems		Not walking at age 17 months	Fine motor delay	Gross motor delay
			z-score	+1.5SD	z-score	+1.5SD	z-score		z-score	z-score
Sample size		48,020	48,161	47,978	47,978	47,977	47,977	60,318	47,792	48,087
Cases n (%)		1947 (4.1)		4 389 (9.2)		3848 (8.0)		1800 (3.0)		
Iodine from food <160 µg/day										
No supplement	50.9	1	0	1	0	1	0	1	0	0
1-200 µg/day	21.9	0.96 (0.85, 1.08)	-0.03 (-0.05, -0.01)	1.16 (1.07, 1.25)	0.06 (0.04, 0.08)	1.04 (0.94, 1.13)	0.02 (-0.00, 0.04)	1.05 (0.94, 1.19)	0.02 (-0.00, 0.04)	0.02 (-0.01, 0.04)
>200 µg/day	1.8	0.93 (0.65, 1.33)	-0.01 (-0.08, 0.05)	1.06 (0.84, 1.33)	0.04 (-0.02, 0.11)	1.18 (0.93, 1.49)	0.05 (-0.02, 0.12)	1.15 (0.82, 1.60)	0.01 (-0.06, 0.07)	0.01 (-0.06, 0.08)
P-overall[3]		*P*=0.74	*P*=0.032	*P*<0.001	*P*<0.001	*P*=0.28	*P*=0.14	*P*=0.53	*P*=0.24	*P*=0.32
Iodine from food ≥160 µg/day										
No supplement	17.5	1	0	1	0	1	0	1	0	0
1-200 µg/day	7.3	1.01 (0.83, 1.22)	-0.01 (-0.05, 0.03)	1.08 (0.94, 1.23)	0.03 (-0.01, 0.07)	1.05 (0.91, 1.22)	0.02 (-0.02, 0.06)	1.02 (0.83, 1.25)	-0.01 (-0.05, 0.03)	-0.01 (-0.05, 0.03)
>200 µg/day	0.6	0.87 (0.47, 1.60)	0.01 (-0.12, 0.14)	0.78 (0.49, 1.23)	0.03 (-0.08, 0.13)	1.01 (0.65, 1.57)	0.05 (-0.07, 0.17)	1.06 (0.59, 1.91)	0.02 (-0.10, 0.14)	-0.07 (-0.18, 0.03)
P-overall[3]		*P*=0.90	*P*=0.87	*P*=0.29	*P*=0.31	*P*=0.81	*P*=0.39	*P*=0.96	*P*=0.86	*P*=0.35

[1] Values are odds ratios (95% CIs) for associations with dichotomous outcomes and standardized betas (95% CIs) for continuous outcomes unless otherwise indicated. Results are from multivariable analysis including interaction terms between iodine from diet and iodine from supplements, and models are adjusted for reported energy intake in the FFQ to adjust for measurement error and for random effects of sibling clusters.

[2] Language delay was defined when the child was talking in maximum two to three word phrases.

[3] Reported *P*-values reflect the potential effect of iodine from supplements in participants with low (<160µg/day) or high (≥160µg/day) intake of iodine from foods.

Supplemental Table 4 Impact of timing of prenatal maternal iodine supplement use on child development at age 3 years by different levels of maternal iodine intake from food, crude models[1, 2]

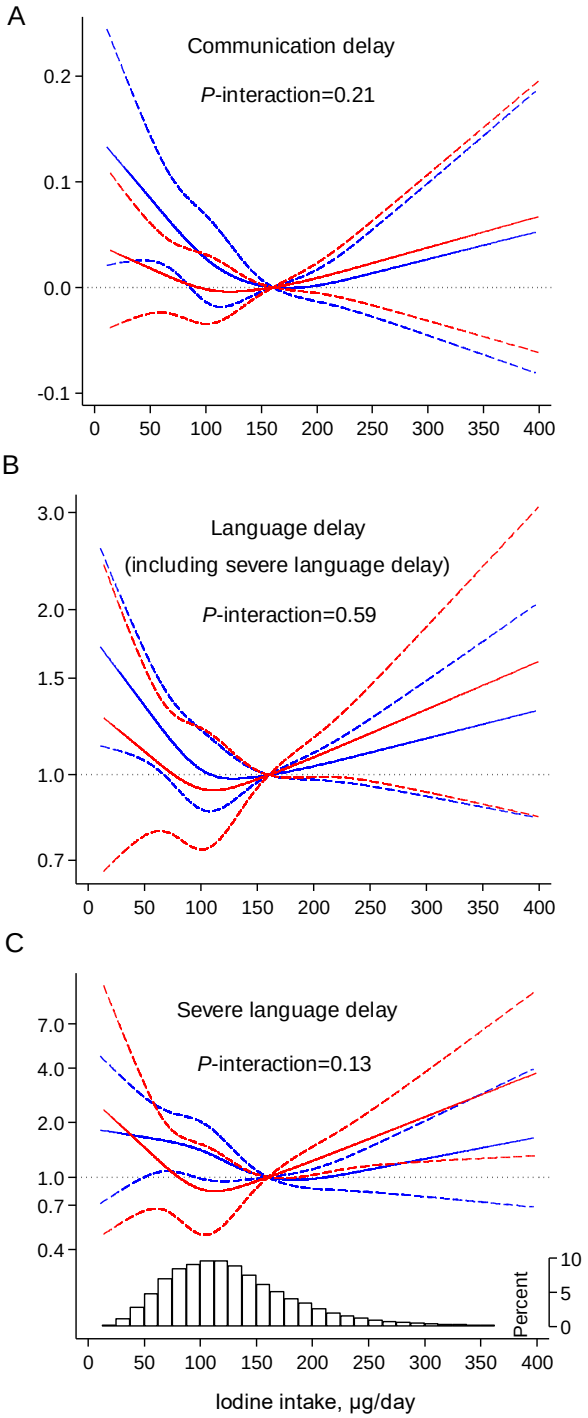
Iodine from supplements µg/day		Language delay[3]	Communication delay	Internalizing behavior problems		Externalizing behavior problems		Not walking at age 17 months	Gross motor delay	Fine motor delay
	%		z-score	+1.5SD	z-score	+1.5SD	z-score		z-score	z-score
Sample size		42,163	42,283	42,118	42,118	42,119	42,119	52,843	42,219	41,958
Cases n (%)		1725 (4.1)		3811 (9.1)		3377 (8.0)		1559 (3.0)		
Iodine from food <160µg/day and first report of iodine supp:										
No supplement (ref)	58.0	1	0	1	0	1	0	1	0	0
Before pregnancy[4]	6.5	0.92 (0.75, 1.14)	-0.06 (-0.09, -0.02)	1.09 (0.95, 1.24)	0.03 (-0.01, 0.07)	0.98 (0.85, 1.13)	0.01 (-0.03, 0.05)	1.01 (0.82, 1.25)	0.00 (-0.04, 0.04)	0.05 (0.01, 0.09)
GW 0-12	4.8	0.96 (0.76, 1.22)	-0.02 (-0.06, 0.03)	1.06 (0.90, 1.23)	0.07 (0.02, 0.11)	1.27 (1.09, 1.48)	0.08 (0.04, 0.13)	1.21 (0.97, 1.52)	0.00 (-0.04, 0.05)	0.03 (-0.02, 0.07)
GW ≥13	5.3	1.00 (0.81, 1.25)	-0.02 (-0.06, 0.02)	1.31 (1.14, 1.51)	0.09 (0.05, 0.14)	1.06 (0.91, 1.24)	0.02 (-0.03, 0.06)	0.88 (0.69, 1.13)	0.00 (-0.04, 0.05)	0.05 (0.01, 0.09)
P-overall[5]		*P*=0.91	*P*=0.012	*P*=0.001	*P*<0.001	*P*=0.021	*P*=0.006	*P*=0.25	*P*=0.99	*P*=0.023
Iodine from food ≥160µg/day and first report of iodine supp:										
No supplement (ref)	19.9	1	0	1	0	1	0	1	0	0
Before pregnancy[4]	2.4	1.05 (0.76, 1.44)	0.01 (-0.06, 0.08)	1.15 (0.92, 1.43)	0.01 (-0.09, 0.08)	0.98 (0.75, 1.26)	0.01 (-0.06, 0.07)	0.86 (0.59, 1.24)	-0.02 (-0.09, 0.04)	0.02 (-0.04, 0.09)
GW 0-12	1.5	1.11 (0.77, 1.61)	0.00 (-0.07, 0.08)	1.04 (0.79, 1.37)	0.07 (-0.01, 0.15)	1.04 (0.77, 1.40)	0.03 (-0.05, 0.11)	0.87 (0.56, 1.35)	0.04 (-0.04, 0.13)	-0.01 (-0.09, 0.06)
GW ≥13	1.7	1.11 (0.78, 1.60)	-0.02 (-0.08, 0.05)	0.96 (0.73, 1.27)	0.01 (-0.06, 0.08)	1.11 (0.84, 1.47)	0.05 (-0.02, 0.13)	1.33 (0.93, 1.91)	-0.01 (-0.09, 0.07)	0.05 (-0.03, 0.13)
P-overall[5]		*P*=0.89	*P*=0.95	*P*=0.66	*P*=0.39	*P*=0.89	*P*=0.53	*P*=0.29	*P*=0.63	*P*=0.55

[1]The analyses were restricted to participants who i) did not report any use of iodine containing supplements in the FFQ or ii) reported use of iodine containing supplements in the food frequency questionnaire (1-200µg/day) and also reported the timing of use in the general questionnaires.
[2] Values are odds ratios (95% CIs) for associations with dichotomous outcomes and standardized betas (95% CIs) for continuous outcomes unless otherwise indicated. Results are from multivariable analysis including interaction terms between iodine from diet and timing of first report of iodine-containing supplement, and the models are adjusted for energy intake to control for measurement error and for random effects of sibling clusters.
[3] Language delay was defined when the child was talking in maximum two to three word phrases.
[4] Reported use of iodine-containing supplements in the time period 0-26 weeks before pregnancy.
[5] Reported *P*-values reflect the potential effect of iodine from supplements in participants with low (<160µg/day) or high (≥160µg/day) intake of iodine from foods.
Abbreviations: GW, gestational week; supp, supplement

Supplemental Table 5 Impact of timing of prenatal maternal iodine supplement use on child development at age 3 years by different levels of maternal iodine intake from food, adjusted models[1, 2]

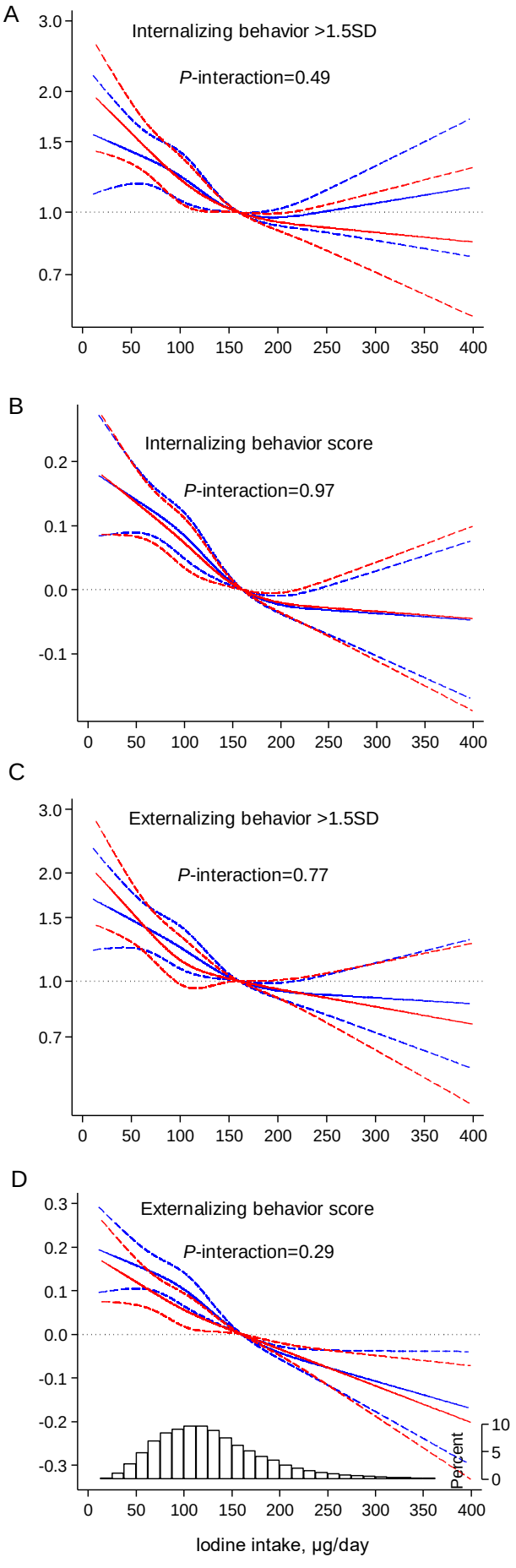
Iodine from supplements µg/day	%	Language delay[3]	Communication delay[3] z-score	Internalizing behavior problems +1.5SD	Internalizing behavior problems z-score	Externalizing behavior problems +1.5SD	Externalizing behavior problems z-score	Not walking at age 17 months	Gross motor delay z-score	Fine motor delay z-score
Sample size		42,163	42,283	42,118	42,118	42,119	42,119	52,843	42,219	41,958
Cases n (%)		1725 (4.1)		3811 (9.1)		3 377 (8.0)		1 559 (3.0)		
Iodine from food <160µg/day and first report of iodine supp:										
No supplement	58.0	1	0	1	0	1	0	1	0	0
Before pregnancy[4]	6.5	1.05 (0.85, 1.30)	-0.02 (-0.05, 0.02)	1.10 (0.96, 1.27)	0.04 (-0.00, 0.08)	1.03 (0.89, 1.19)	0.02 (-0.02, 0.06)	1.01 (0.82, 1.24)	-0.02 (-0.05, 0.02)	0.02 (-0.02, 0.06)
GW 0-12	4.8	1.11 (0.88, 1.41)	0.03 (-0.02, 0.07)	1.01 (0.87, 1.19)	0.04 (-0.00, 0.09)	1.28 (1.09, 1.49)	0.08 (0.03, 0.12)	1.24 (0.99, 1.55)	-0.02 (-0.06, 0.03)	0.00 (-0.05, 0.05)
GW ≥13	5.3	1.09 (0.87, 1.36)	0.00 (-0.04, 0.04)	1.27 (1.10, 1.46)	0.07 (0.02, 0.11)	1.08 (0.92, 1.26)	0.02 (-0.03, 0.06)	0.89 (0.70, 1.14)	-0.03 (-0.07, 0.02)	0.04 (-0.00, 0.09)
P-overall[5]		*P*=0.72	*P*=0.56	*P*=0.007	*P*=0.004	*P*=0.019	*P*=0.012	*P*=0.22	*P*=0.54	*P*=0.23
Iodine from food ≥160µg/day and first report of iodine supp:										
No supplement	19.9	1	0	1	0	1	0	1	0	0
Before pregnancy[4]	2.4	1.21 (0.87, 1.67)	0.06 (0.00, 0.13)	1.16 (0.93, 1.46)	0.03 (-0.03, 0.10)	1.04 (0.77, 1.35)	0.02 (-0.04, 0.09)	0.85 (0.58, 1.24)	-0.05 (-0.12, 0.03)	-0.01 (-0.07, 0.06)
GW 0-12	1.5	1.26 (0.86, 1.83)	0.03 (-0.04, 0.10)	0.99 (0.75, 1.31)	0.05 (-0.02, 0.13)	1.04 (0.77, 1.41)	0.02 (-0.05, 0.10)	0.90 (0.58, 1.40)	-0.01 (-0.09, 0.08)	-0.04 (-0.12, 0.03)
GW ≥13	1.7	1.17 (0.81, 1.69)	0.01 (-0.06, 0.07)	0.92 (0.70, 1.22)	-0.01 (-0.08, 0.06)	1.12 (0.85, 1.49)	0.05 (-0.03, 0.13)	1.35 (0.94, 1.94)	-0.01 (-0.09, 0.07)	0.04 (-0.04, 0.12)
P-overall[5]		*P*=0.41	*P*=0.23	*P*=0.53	*P*=0.42	*P*=0.86	*P*=0.56	*P*=0.27	*P*=0.66	*P*=0.47

[1]The analyses were restricted to participants who i) did not report any use of iodine containing supplements in the FFQ or ii) reported use of iodine containing supplements in the food frequency questionnaire (1-200µg/day) and also reported the timing of use in the general questionnaires.
[2] Values are odds ratios (95% CIs) for associations with dichotomous outcomes and standardized betas (95% CIs) for continuous outcomes unless otherwise indicated. Results are from multivariable analysis including interaction terms between iodine from diet and timing of first report of iodine-containing supplement, and the models are adjusted for maternal age, BMI, parity, education, smoking in pregnancy, energy intake, fiber intake, folic acid supplement use before gestational week 8, total EPA/DHA intake, and for random effects of sibling clusters.
[3] Models are additionally adjusted for bilingual parent(-s). Language delay was defined when the child was talking in maximum two to three word phrases and also includes severe language delay. Severe language delay was defined when the child was not yet talking, speaking in only one word utterances or in unintelligible speak.
[4] Reported use of iodine-containing supplements in the time period 0-26 weeks before pregnancy.
[5] Reported *P*-values reflect the potential effect of iodine from supplements in participants with low (<160µg/day) or high (≥160µg/day) intake of iodine from foods.
Abbreviations: GW, gestational week; supp, supplement



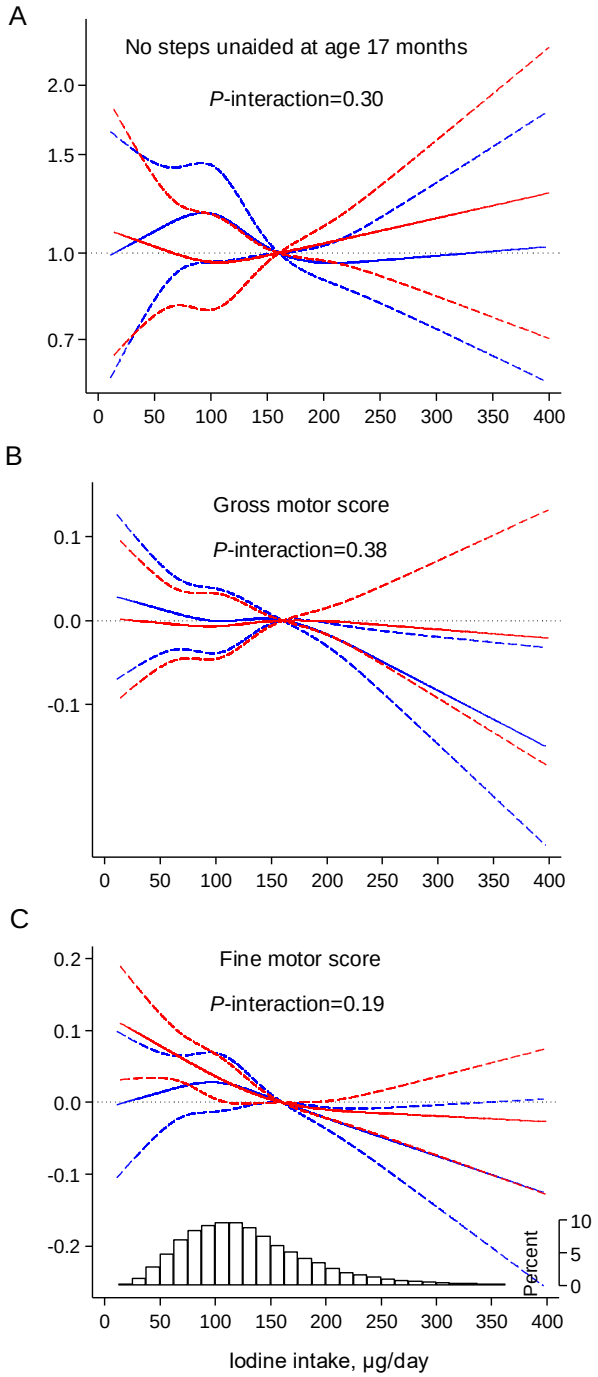
Supplemental Figure 1 Sex specific associations between maternal iodine intake from food in pregnancy (in non-supplement users) and communication- and language outcomes at age 3 years in the Norwegian Mother and Child Cohort Study.

Estimated associations for boys (*n*=16,903) are in blue and girls (*n*=16,144) in red. The models included an interaction term between child sex and iodine intake. Solid lines represent associations modelled by restricted cubic splines (knotpositions at 54, 102, 142 and 243 µg/day), 95% CI are illustrated by dotted lines, and the reference level was set to 160 µg/day. The models were adjusted for maternal age, parity (0, 1, ≥2), education (≤12, 13-16, ≥17 years), body mass index (<18.5, 18.5-24.9, 25-30, >30), smoking in pregnancy (never, occasionally or quit before gestational week 12, daily), folic acid supplement within the interval from 4 weeks before to 8 weeks after conception (yes/no), energy intake (linear splines with knots at 8.5 MJ/d and 11 MJ/d), fiber intake, bilingual parent(-s) (yes/no), and for random effects of sibling clusters.
Language delay (4.1%) (B) was defined when the child was talking in maximum two to three word phrases and also includes severe language delay. Severe language delay (0.8%) (C) was defined when the child was not yet talking, speaking in only one word utterances or in unintelligible speak.



Supplemental Figure 2 Sex specific associations between maternal iodine intake from food in pregnancy (in non-supplement users) and behavior outcomes at age 3 years in the Norwegian Mother and Child Cohort Study.

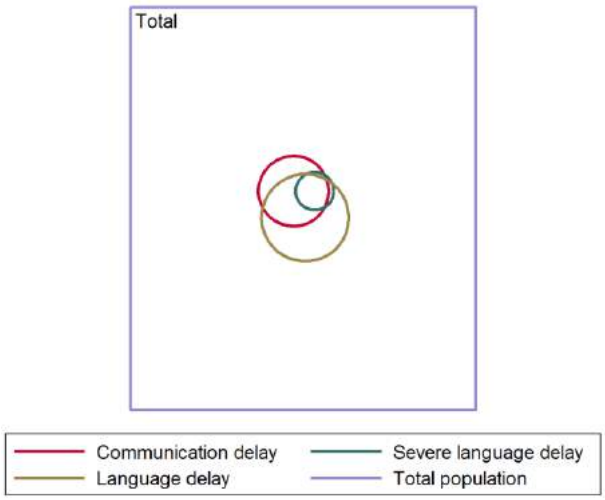
Estimated associations for boys (*n*=16,903) in blue and girls (*n*=16,144) in red. The models included an interaction term between child sex and iodine intake. Solid lines represent restricted cubic splines (knotpositions at 54, 102, 142 and 243 µg/day), 95% CI are illustrated by dotted lines, and the reference level was set to 160 µg/day. The models were adjusted for maternal age, parity (0, 1, ≥2), education (≤12, 13-16, ≥17 years), body mass index (<18.5, 18.5-24.9, 25-30, >30), smoking in pregnancy (never, occasionally or quit before gestational week 12, daily), folic acid supplement within the interval from 4 weeks before to 8 weeks after conception (yes/no), energy intake, fiber intake, and for random effects of sibling clusters.



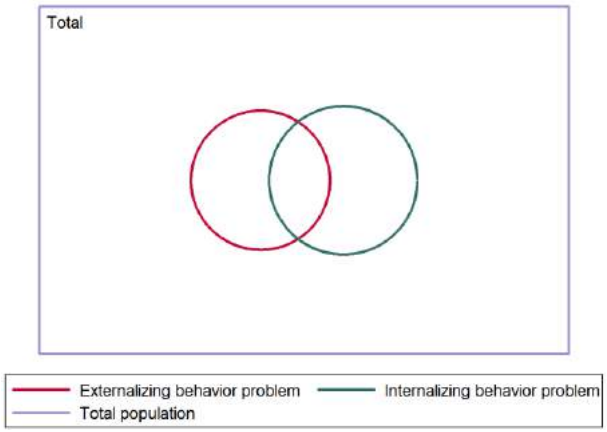
Supplemental Figure 3 Sex specific associations between maternal iodine intake from food in pregnancy (in non-supplement users) and child motor outcomes by 3 years of age in the Norwegian Mother and Child Cohort Study.

Estimated associations for boys (*n*=16,903) are in blue and girls (*n*=16,144) in red. The models included an interaction term between sex and iodine intake. Solid lines represent restricted cubic splines (knotpositions at 54, 102, 142 and 243 µg/day), 95% CI are illustrated by dotted lines and the reference level was set to 160 µg/day. The models were adjusted for maternal age, parity (0, 1, ≥2), education (≤12, 13-16, ≥17 years), body mass index (<18.5, 18.5-24.9, 25-30, >30), smoking in pregnancy (never, occasionally or quit before gestational week 12, daily), folic acid supplement within the interval from 4 weeks before to 8 weeks after conception (yes/no), energy intake (linear splines with knots at 8.5 MJ/d and 11 MJ/d), fiber intake, and for random effects of sibling clusters. Of all children in the sample 2.9% had not started walking yet at age 17 months (A).

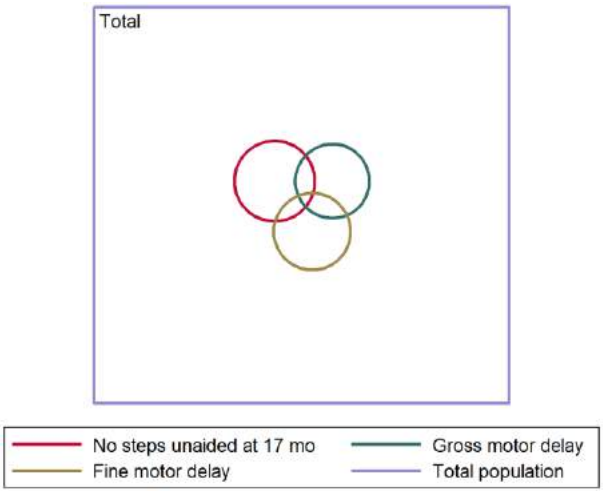
Supplemental Figure 4 Venn diagram illustrating overlap of children characterized with communication delay (+2SD), language delay and severe language delay at age 3 years in the Norwegian Mother and Child Cohort Study.



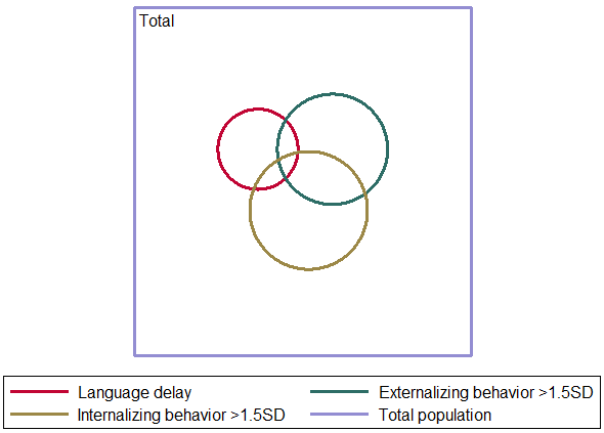
Supplemental Figure 5 Venn diagram illustrating overlap of children characterized with externalizing and internalizing behavior problems (+1.5SD) at age 3 years in the Norwegian Mother and Child Cohort Study.



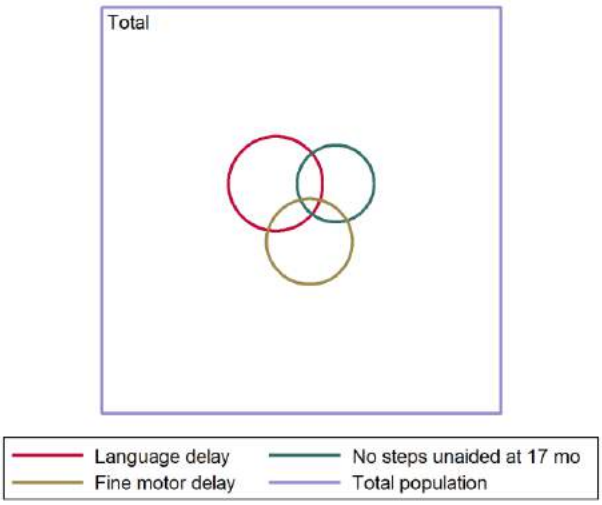
Supplemental Figure 6 Venn diagram illustrating overlap of children with no steps unaided at age 17 months and children characterized with fine or gross motor delay at age 3 years in the Norwegian Mother and Child Cohort Study.



Supplemental Figure 7 Venn diagram illustrating overlap of children with language delay and behavior problems (+1.5SD) at age 3 years in the Norwegian Mother and Child Cohort Study.



Supplemental Figure 8 Venn diagram illustrating overlap of children with language delay, fine motor delay (+2SD) and no steps unaided at age 17 months in the Norwegian Mother and Child Cohort Study.



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PAPER 2

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Article

Maternal Iodine Intake and Offspring Attention-Deficit/Hyperactivity Disorder: Results from a Large Prospective Cohort Study

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Abstract: Current knowledge about the relationship between mild to moderately inadequate maternal iodine intake and/or supplemental iodine on child neurodevelopment is sparse. Using information from 77,164 mother-child pairs in the Norwegian Mother and Child Cohort Study, this study explored associations between maternal iodine intake and child attention-deficit/hyperactivity disorder (ADHD) diagnosis, registered in the Norwegian Patient Registry and maternally-reported child ADHD symptoms at eight years of age. Pregnant women reported food and supplement intakes by questionnaire in gestational week 22. In total, 1725 children (2.2%) were diagnosed with ADHD. In non-users of supplemental iodine (53,360 mothers), we found no association between iodine intake from food and risk of child ADHD diagnosis ($p = 0.89$), while low iodine from food (<200 µg/day) was associated with higher child ADHD symptom scores (adjusted difference in score up to 0.08 standard deviation (SD), $p < 0.001$, $n = 19{,}086$). In the total sample, we found no evidence of beneficial effects of maternal use of iodine-containing supplements ($n = 23{,}804$) on child ADHD diagnosis or symptom score. Initiation of iodine supplement use in gestational weeks 0–12 was associated with an increased risk of child ADHD (both measures). In conclusion, insufficient maternal iodine intake was associated with increased child ADHD symptom scores at eight years of age, but not with ADHD diagnosis. No reduction of risk was associated with maternal iodine supplement use.

Keywords: ADHD; attention-deficit/hyperactivity disorder; iodine; dietary supplements; pregnancy; neurodevelopment; Norwegian mother and child cohort study; MoBa; Norwegian Patient Registry

1. Introduction

Iodine deficiency (ID) is among the most common micronutrient deficiencies worldwide and is recognized by the World Health Organization (WHO) as the number one cause of potentially preventable brain damage [1]. Iodine is essential for the production of thyroid hormones, which in turn are involved in multiple pathways in neurodevelopment [2]. Severe maternal ID is associated with impaired brain development, but less is known about the potential consequences of mild to moderate ID, commonly seen in populations of both low and high income countries [3].

The worldwide prevalence of attention-deficit/hyperactivity disorder (ADHD) in children and adolescents is estimated to be in the range 6.7–7.8%, based on the criteria of the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) [4]. ADHD is in the fifth edition of DSM (DSM-V), defined as a persistent pattern of inattention and/or hyperactivity-impulsivity, that interferes with functioning or development [5]. It is associated with significant morbidity and disability, and impairments persist into adulthood in the majority of cases [6]. The children have an increased risk of school failure, emotional difficulties, poor peer relations, and trouble with the law [6]. ADHD is also often linked with comorbidities, such as oppositional defiant disorder, conduct disorder, autism spectrum disorders, anxiety, depression, and substance use disorders [6]. Causes of ADHD are multifactorial and largely unknown and involve both genetic and environmental factors [7]. The heritability of ADHD is estimated to be 83–92% in children and 56–84% in adults [8]. Several nutritional factors have been investigated as potential causal factors (e.g., zinc, magnesium and polyunsaturated fatty acids), but currently there is no consistent evidence linking diet to ADHD [7]. An association between maternal iodine status and child ADHD has been suggested in several studies [9–11].

This paper is a follow-up of a previous publication from The Norwegian Mother and Child Cohort Study (MoBa), where we found that inadequate iodine intake in pregnancy was associated with maternally reported child language delay, behavior problems (externalizing and internalizing) and fine motor delay, but not with gross motor delay at three years of age, or risk of not walking unaided at 17 months of age [12]. Regarding the maternal use of iodine-containing supplements, we found no evidence of beneficial effects. However, the results indicated a negative impact on child behavior problems when mothers had inadequate iodine intake from food and initiated use of supplemental iodine in the first trimester of pregnancy [12].

The main aim of the current study was to explore the association between iodine intake from food in pregnancy (as a proxy for long-term iodine intake and status) and (i) risk of specialist-diagnosed ADHD in the child and (ii) maternal report of child ADHD symptoms at eight years of age. A second aim was to explore the associations between maternal use of iodine-containing supplements prior to and during pregnancy and the same outcome measures.

2. Materials and Methods

2.1. Subjects and Design

This study is based on data from MoBa, a prospective population-based pregnancy cohort study, conducted by the Norwegian Institute of Public Health [13]. Women pregnant in their first trimester were recruited from all over Norway during the years 1999 to 2008. Participants were recruited to the study by postal invitation before the routine free ultrasound examination at around gestational week 18. The women were asked to provide blood and urine samples at baseline and to answer questionnaires (in Norwegian) at regular intervals during pregnancy and after birth. More than 99% of the participants were of Caucasian origin. Pregnancy and birth records from the Medical Birth Registry of Norway are linked to the MoBa database [14]. The women consented to participation in 41% of the pregnancies. The cohort now includes 114,500 children, 95,200 mothers and 75,200 fathers. The current study is based on version 9 of the quality-assured data files released for research in 2016.

To be included in this study, mothers had to have responded to a general questionnaire at around gestational week (GW) 17, and a food frequency questionnaire (FFQ) at around GW 22. Only singleton pregnancies were included. Mothers reporting the use of thyroid medication at any time during pregnancy were excluded from the study. Only participants with information on all covariates were included in the analysis because of the large sample size and low rates of missing values. FFQs with more than three blank pages or with calculated energy intakes <4.5 MJ or >20 MJ were excluded [15].

A total of 77,164 mother-child pairs were included in this study, and for 27,945 there were data on maternally reported ADHD scores when the child was aged eight years (Figure 1). For the main analysis, restricted to participants who had not reported use of iodine-containing supplements in the FFQ, 53,360 mother-child pairs were included (19,086 for ADHD score).

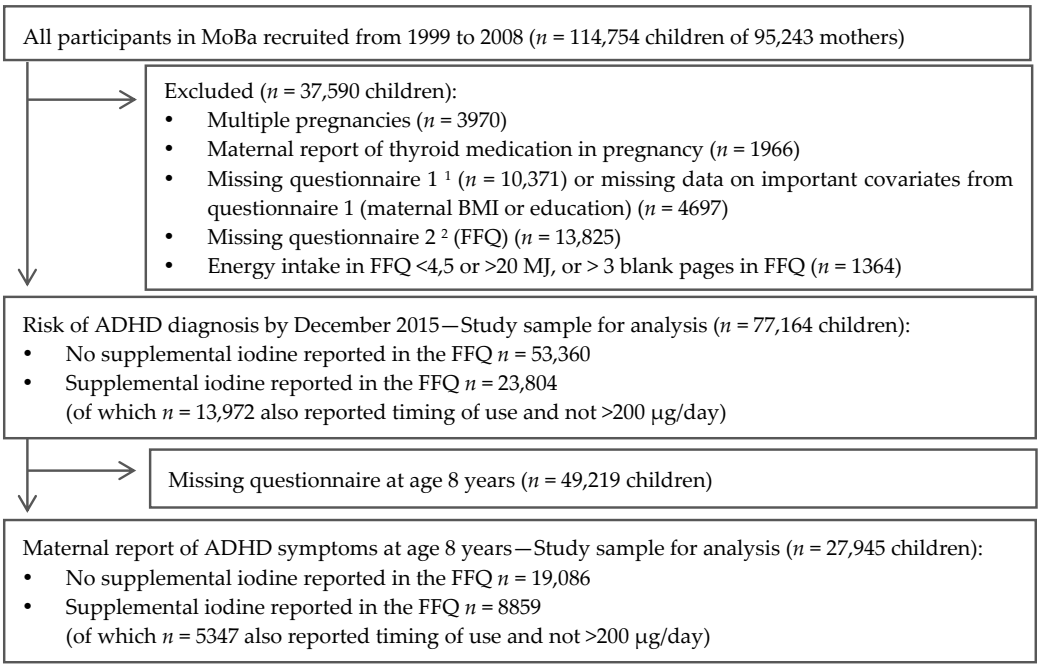

Figure 1. Flow-chart of inclusion. [1] Questionnaire 1 was answered around gestational week 17. [2] The FFQ (questionnaire 2) used in the present study was included in The Norwegian Mother and Child Cohort Study (MoBa) from 2002 and was answered around gestational week 22. FFQ: Food frequency questionnaire, BMI: Body Mass Index, ADHD: attention-deficit/hyperactivity disorder.

2.2. Exposure Variables—Iodine Intake from Food and Supplements

The MoBa FFQ [16] was specifically designed for the MoBa study and was in use from 2002. It was completed by participating women at around GW 22. It is a semi-quantitative questionnaire, designed to capture dietary habits and use of dietary supplements during the first half of pregnancy and included questions about the intake of 255 food items or dishes [15]. Intake of specific foods and nutrients were calculated based on standard Norwegian portion sizes, the Norwegian food composition table, analysis of Norwegian milk and food samples [17,18] and data on the content of more than 1000 food supplements collected from suppliers [19].

As reported previously [12], the MoBa FFQ has been shown to be a valid tool for ranking pregnant women, according to high and low intakes of energy, nutrients and foods [20]. Iodine was validated separately and iodine intake by the FFQ, including supplemental iodine, showed good agreement with the reference methods (triangular validity coefficient for total iodine intake by the FFQ was 0.62 (95% confidence interval (CI): 0.46, 0.77)) [21,22]. When dividing participants into quintiles of their iodine intake estimates, 67% were correctly classified by the FFQ compared to the 4-day weighed food diary (classified into the same or adjacent quintiles), and 63% were correctly classified by the

FFQ compared to 24-h urinary iodine. Less than 5% were grossly misclassified [20]. In non-users of iodine supplements, estimated median iodine intake from food was 122 μg/day, calculated from the FFQ, 120 μg/day from the 4-day food diary, and 122 μg/day, based on 24-h urinary iodine excretion (assuming that 90% is excreted in the urine) [21,22].

An analysis of urinary iodine in spot samples from GW 18 has also been performed in a MoBa subsample comprising women with singleton deliveries (Abel et al. [23]). Median spot urinary iodine concentration (UIC) in non-users of iodine supplements and thyroid medication (n = 1950) was 61 μg/L (interquartile range (IQR): 32–104 μg/L). In iodine supplement users (n = 988), the median UIC was 86 μg/L (IQR: 43–140 μg/L). Total iodine intake, calculated by the FFQ, correlated with spot UIC (μg/g creatinine) (Spearman's correlation: $r = 0.36$, $p < 0.001$).

Iodine intake from supplements was categorized into three groups (0, 1–200 and >200 μg/day), and the timing of initiation of iodine containing supplements up to GW 22 was divided in four categories (never, week 0–26 before pregnancy, GW 0–12 and GW $\geq$ 13).

2.3. ADHD Diagnosis

We obtained information about children's ADHD diagnoses from the Norwegian Patient Registry (NPR) [24]. From 2008, all government-owned and government-financed hospitals and outpatient clinics have mandatorily reported individual level diagnoses, defined in the tenth revision of the International Classification of Disease (ICD-10) [25], to the NPR, in order to receive financial reimbursement. Using individual personal identification numbers, diagnostic information from NPR was linked to MoBa. Thus, all MoBa children registered with an ICD-10-diagnosis of hyperkinetic disorder (HKD, coded as F90.0, F90.1, F90.8, or F90.9) between 2008 and 2015 were identified and regarded as having ADHD.

In an international meta-study, prevalence estimates were 4.1% lower using the ICD-10 than the DSM-IV criteria [26]. HKD requires the combination of persisting inattentive and hyperactive symptoms before the age of six and impairment in two or more settings, and as a result HKD is a severe subtype nested within ADHD, as defined by the DSM [27]. In comparison to ADHD, as defined by the DSM, HKD is characterized by a higher proportion of individuals with impaired language and motor development [28].

2.4. ADHD Symptom Score

Child ADHD symptoms were assessed in the eight-year-old questionnaire from MoBa on a four-point Likert scale (never/rarely, sometimes, often, or very often) covering inattention problems (nine items) and hyperactivity/impulsivity (nine items) from the ADHD Rating Scale [29]. Mean scores for inattention symptoms, hyperactivity symptoms, and total ADHD symptoms were calculated and standardized. The ADHD subscales of inattention and hyperactivity were correlated (Spearman's correlation coefficient: $r = 0.53$; $p < 0.001$). There was high agreement between maternally reported ADHD scores at eight years of age and registered ADHD diagnosis, and the median score was +2.4 SD (IQR: 1.3, 3.6) in children with ADHD diagnoses.

2.5. Covariates

A predefined set of covariates were included in the analysis, based on previous knowledge and a theoretic causal diagram (Supplemental Figure S1). Variables on birth outcomes and data from after birth were not included, as they could represent potential mediators on the causal pathway. Also, maternal mental health was not included since it could be an indicator of thyroid dysfunction [30]. For models with continuous outcome measures, child sex and birth season were included, to improve the precision of effect estimates, as these are important determinants of ADHD. Included covariates were obtained from different sources: Maternal age, child sex, and birth season (January–April, May–August, September–December) were obtained from the Medical Birth Registry of Norway. Maternally reported pre-pregnancy body weight and height, for the calculation of body mass index

(BMI), maternal education (≤12, 13–16, ≥17 years), parity (previous pregnancies ≥22 weeks: 0, 1, ≥2), and use of folic acid supplements within the interval from 4 weeks beforehand, to 8 weeks after conception (yes/no) were obtained from the MoBa questionnaire 1 at GW 17. Energy intake, fiber intake (as a marker of a healthy dietary pattern), and total intake of the long chain polyunsaturated *n*-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from food and dietary supplements, were calculated, based on the FFQ. Information on smoking in pregnancy was obtained from questionnaire 1 and, if available, questionnaires 3 (GW 30) and 4 (child's age: six months) for three categories: no reported smoking in pregnancy, reported occasional smoking or stopped smoking before GW 12, and daily smoking at any time in pregnancy and had not stopped smoking before GW 12.

Other potential covariates were explored, but not included in the final analysis, since they had no/only negligible effects on the estimates of interest—maternal intake of alcohol (g/day), year of birth, marital status, paternal education, parents' incomes, bilingual parent(s) (mother tongue other than Norwegian: yes/no), and maternal chronic illness (asthma, diabetes, inflammatory bowel disease, rheumatic disease, epilepsy, multiple sclerosis or cancer: yes/no).

2.6. Ethics

The MoBa was conducted according to the guidelines laid down in the Declaration of Helsinki and written informed consent was obtained from all participants. MoBa has obtained a license from the Norwegian Data Inspectorate. The current study was approved by The Regional Committee for Medical Research Ethics South East Norway 2013/594.

2.7. Statistics

The association between iodine intake and risk of ADHD diagnoses was explored with Cox proportional hazards regression. Associations to maternally reported ADHD symptoms at eight years of age were modelled by generalized linear models, with gamma family and log link functions. All models were adjusted for random effects of sibling clusters, since some mothers participated with more than one pregnancy. Results are reported as hazard ratios (HR) for ADHD diagnoses and standardized betas for ADHD symptom scores and include robust 95% confidence intervals (CI). A p-value < 0.05 was considered statistically significant.

To isolate the effect of long term iodine intake, we performed analyses on associations between iodine intake from food and ADHD outcomes, restricted to participants who had not reported the use of supplemental iodine in the FFQ. We examined a potential nonlinear dose-response relationship between iodine intake from food and ADHD diagnosis, by modelling iodine intake using restricted cubic splines with four knots (at percentiles 5, 35, 65 and 95, corresponding to iodine intakes of 54, 102, 143 and 245 μg/day).

All regression models (including crude models) were adjusted for energy intake (as two piecewise linear splines, knot position at 10.5 MJ) to control for measurement error in calculated iodine intake. Adjusted models also included the following baseline maternal and family characteristics based on a causal diagram: maternal age, education, parity, pre-pregnancy BMI (including BMI squared), fiber intake, and smoking in pregnancy. Child sex and birth season were also included in models with continuous outcome variables, since they are important predictors of ADHD. Possible interaction effects were explored for maternal BMI, age, smoking, child sex and parity. The reference level of iodine intake was set at 160 μg/day, the estimated average requirement (EAR) for iodine during pregnancy by the Institute of Medicine [31]. *P*-values are reported for overall associations between exposure and outcomes, by testing the coefficients of all spline transformations equal to zero. The tests for non-linearity were performed by testing the coefficients of the second and third spline transformations equal to zero. Potential interactions were explored by testing all interaction coefficients equal to zero. Graphs and tabular results were calculated based on the spline models.

We also explored associations between iodine intake from food and ADHD outcome with iodine intake categorized (six categories), and the results were in agreement with results from the flexible spline models (results not included).

The impact of dosage of iodine from supplements was explored by including interaction terms between iodine from supplements (divided in three categories: 0, 1–200 and >200 μg/day) and iodine from food (in two categories: less than the EAR (<160) and above (≥160 μg/day)). The models were adjusted with the same covariates as described above, but in addition, maternal folic acid supplement within the interval from 4 weeks beforehand to 8 weeks after conception and total EPA/DHA intake were included in the adjusted models.

The impact of the timing of introduction of iodine-containing supplements (reported use 0–26 weeks before conception, first reported use GW 0–12, or GW ≥ 13) was explored in participants who had reported an intake of 1–200 μg/day of iodine from supplements in the FFQ, and who had also reported timing of use in the general questionnaires. Timing was explored in the same way as dosage, including an interaction term with iodine from food (above/below the EAR) and adjusting for the same covariates.

Additionally, we performed matched control analyses to further assess the potential causal effects of iodine supplement use (both for dosage and timing). Since supplemental iodine was not generally recommended for pregnant women in Norway at the time, we restricted the control group to participants who had reported the use of supplemental vitamins and minerals other than the recommended (which included folic acid, vitamin D, and iron (only in iron deficient individuals)). This was possible since some multi-supplements contained iodine whereas others did not. More comparable controls enabled us to control for the health-seeking behavior of taking an additional supplement as a precaution, and, to some extent, to control for confounding by other nutrients in the multi-supplements.

Statistical analyses were performed using STATA software (version 14.0; Stata Corp., College Station, TX, USA). Including the package xblc for calculating tabular estimates based on the flexible spline models [32].

3. Results

3.1. Background Characteristics

The calculated iodine intake from food (not supplements) in the first half of pregnancy ranged from 9 to 792 μg/day (median: 121 μg/day; IQR: 89–162 μg/day) and 74% had an estimated intake from food lower than the EAR in pregnancy (160 μg/day). Supplemental iodine was reported by 31% of the pregnant women (range of the average intake during GW 0–22: 1–1264 μg/day, median: 107 μg/day; IQR: 58–150 μg/day). ADHD diagnosis was registered in 1725 children (2.2%) by December 2015, and the median age at diagnosis was 8.2 years (IQR: 7.0, 9.5 years). The median age of all children in our MoBa sample (n = 77,164) was 9.9 years in December 2015 (range: 6.4–13.8 years).

The maternal and child characteristics by iodine intake from food and supplements are shown in Tables 1 and 2. There were only minor differences in background characteristics between exposure groups. Mothers with the estimated highest iodine intake from food (>250 μg/day) included more mothers under the age of 25 years (17% vs. 10% of mothers with iodine intake ≤250 μg/day), more mothers with ≤12 years education (42% vs. 30%), and a higher prevalence of any smoking in pregnancy (26% vs. 21%). Maternal iodine intake from food was mostly determined by the intake of milk and yoghurt (Spearman's correlation coefficient: r = 0.85; p < 0.001), but iodine intake from food was also related to the calculated total energy intake (r = 0.56, p < 0.001) and to other nutrients and foods (Table 2). Iodine supplement use was more commonly reported in mothers with no previous pregnancies (35% vs. 27% in mothers with parity ≥1), and less frequently in the mothers with ≤12 years education (27% vs. 32% in mothers with >12 years education). The use of folic acid supplements and/or n-3 fatty acid supplements were more prevalent in mothers who used supplemental iodine

(95% vs. 82% in non-users). Iodine intake from food did not differ between iodine supplement users and non-users (difference in means 0.3 μg/day, $p = 0.56$).

Table 1. Maternal and child characteristics by maternal iodine intake from food and from supplements in first half of pregnancy [1].

	Iodine Intake from Food (μg/Day)				Supplemental Iodine (μg/Day)			Total
	<100	100–159.9	160–250	>250	0	1–200	>200	
Mother-child pairs, *n* (%)	25,637 (33.2)	31,688 (41.1)	16,322 (21.1)	3517 (4.6)	53,360 (69.2)	21,940 (28.4)	1864 (2.4)	77,164 (100)
Maternal age at delivery, years	30.1 (4.5)	30.4 (4.4)	30.1 (4.6)	29.4 (4.9)	30.1 (4.6)	30.3 (4.4)	30.0 (4.8)	30.2 (4.5)
Pre-pregnancy BMI, kg/m^2	24.2 (4.4)	23.9 (4.2)	24.0 (4.2)	24.4 (4.6)	24.1 (4.3)	23.9 (4.2)	23.9 (4.3)	24.0 (4.3)
Parity, %								
0	49.9	46.2	45.3	48.4	44.7	52.7	58.2	47.3
1	34.9	36.3	35.8	32.4	36.7	33.4	31.0	35.6
2 or more	15.2	17.4	18.9	19.2	18.6	13.9	10.8	17.1
Maternal education								
≤12 years	31.1	27.9	32.1	41.7	32.2	26.4	30.3	30.5
13–16 years	42.4	43.9	43.3	39.0	42.3	44.9	42.9	43.1
>16 years	26.5	28.2	24.6	19.3	25.5	28.7	26.8	26.5
Married/cohabitant	96.7	97.0	96.6	94.9	96.7	96.8	95.9	96.7
Smoking in pregnancy								
No	77.8	79.8	78.5	74.0	78.1	79.8	78.2	78.6
Occasionally	16.2	14.9	15.1	16.5	15.5	15.2	16.1	15.5
Daily	6.0	5.3	6.4	9.5	6.4	5.0	5.7	5.9
Chronic illness	11.6	9.5	9.3	10.5	9.8	11.0	12.0	10.2
Parents' income								
Low	25.1	25.1	29.0	33.4	27.1	24.7	23.5	26.3
Medium	40.7	41.2	42.0	42.6	41.4	40.9	41.3	41.3
High	31.8	31.2	26.0	19.8	28.7	32.1	32.5	29.8
Missing	2.4	2.5	3.0	4.3	2.8	2.4	2.7	2.6
Child sex								
Boys	51.2	51.2	51.0	51.6	51.5	50.5	51.2	51.2
Girls	48.8	48.8	49.0	48.4	48.5	49.5	48.8	48.8
Bilingual parent(s)	10.7	10.3	8.9	9.3	9.8	10.6	12.8	10.1
ADHD diagnosis by December 2015	2.1	2.1	2.6	3.4	2.2	2.3	2.1	2.2

[1] Values are presented as mean ± standard deviation (SD) or percentages unless otherwise indicated. BMI: body mass index; ADHD: attention-deficit/hyperactivity disorder.

3.2. Iodine Intake from Food and Risk of ADHD

Associations between maternal iodine intake from food and child ADHD are illustrated in Figures 2 and 3. Iodine from food was significantly associated with maternally reported child ADHD symptoms at eight years of age (adjusted p overall = 0.001) (Figure 3), but not with risk of child ADHD diagnosis (Figure 2). The negative effect associated with a low iodine intake (<200 μg/day) on ADHD symptoms was primarily seen in the inattention subscale (p overall < 0.001), and it did not reach statistical significance for the hyperactivity subscale (p overall = 0.19). Tabular results from unadjusted and adjusted analyses are provided in Supplemental Tables S1 and S2.

No significant interaction effects were detected for iodine with the covariates, BMI, education, parity, smoking, or child sex. The associations between maternal iodine intake from food and ADHD outcomes by child sex are presented in Supplemental Figure S2.

Table 2. Maternal dietary characteristics by maternal iodine intake from food and from supplements (in micrograms per day) during the first half of pregnancy [1].

	Iodine Intake from Food				Supplemental Iodine			Total
	<100	100–159.9	160–250	>250	0	1–200	>200	
Energy intake, MJ/day	8.2 (2.0)	9.7 (2.1)	11.3 (2.4)	13.5 (2.8)	9.7 (2.6)	9.7 (2.6)	9.9 (2.6)	9.7 (2.6)
Iodine from food, µg/day	74 (18)	127 (17)	193 (24)	304 (55)	132 (61)	131 (61)	133 (64)	132 (61)
Food intake, g/day								
Milk/yoghurt	162 (123)	411 (168)	756 (246)	1435 (443)	447 (360)	448 (362)	463 (386)	448 (362)
Fish, lean	16 (11)	22 (13)	25 (15)	28 (18)	21 (14)	20 (13)	21 (15)	21 (14)
Fish, fatty	9 (9)	12 (13)	16 (18)	19 (23)	12 (14)	12 (14)	12 (12)	12 (14)
Eggs	9 (9)	12 (12)	13 (14)	15 (17)	11 (12)	12 (13)	12 (13)	11 (12)
Fruits and vegetables	388 (217)	453 (246)	502 (280)	559 (344)	443 (253)	451 (254)	489 (308)	447 (255)
Nutrient intake, g/day								
Protein	71 (14)	86 (14)	104 (16)	132 (21)	87 (21)	87 (21)	89 (22)	87 (21)
Sugar	55 (37)	60 (36)	70 (40)	84 (50)	62 (39)	62 (37)	62 (37)	62 (38)
Fiber	27 (9)	31 (10)	35 (11)	39 (13)	31 (10)	31 (11)	33 (12)	31 (10)
Alcohol	0.1 (0.6)	0.1 (0.7)	0.1 (0.5)	0.1 (0.7)	0.1 (0.6)	0.1 (0.7)	0.1 (0.3)	0.1 (0.6)
Iodine from source, µg/day								
Milk including yoghurt	20 (16)	56 (23)	107 (35)	206 (64)	62 (52)	61 (52)	63 (55)	62 (52)
Fish	16 (11)	24 (14)	30 (19)	35 (27)	23 (16)	23 (16)	22 (16)	23 (16)
Eggs	4 (4)	5 (6)	6 (6)	7 (8)	5 (5)	5 (6)	5 (6)	5 (6)
Supplements	35 (72)	35 (72)	35 (71)	37 (77)	-	95 (51)	336 (135)	35 (72)
Iodine supplement								
0 µg/day	69.0	69.1	69.4	69.3	100	-	-	69.2
1–99 µg/day	15.8	15.1	14.8	14.8	-	53.7	-	15.3
100–199 µg/day	12.6	13.4	13.4	13.1	-	46.3	-	13.2
≥200 µg/day	2.5	2.3	2.4	2.8	-	-	100	2.4
n-3 FA supplement [2]	64.9	69.7	69.7	66.4	63.4	77.4	86.0	68.0
Folic acid supplement [3]	73.9	73.9	71.0	66.3	68.6	82.6	84.5	72.9
Any supplement (in FFQ)	83.8	86.7	86.3	83.7	79.1	100	100	85.5

[1] Values are presented as mean ± standard deviation (SD) or percentages unless otherwise indicated; [2] Long chain *n*-3 polyunsaturated fatty acids (FA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA); [3] Any reported use of folic acid from 4 weeks before to 8 weeks after conception reported in questionnaire 1 (not in FFQ). FFQ: Food frequency questionnaire.

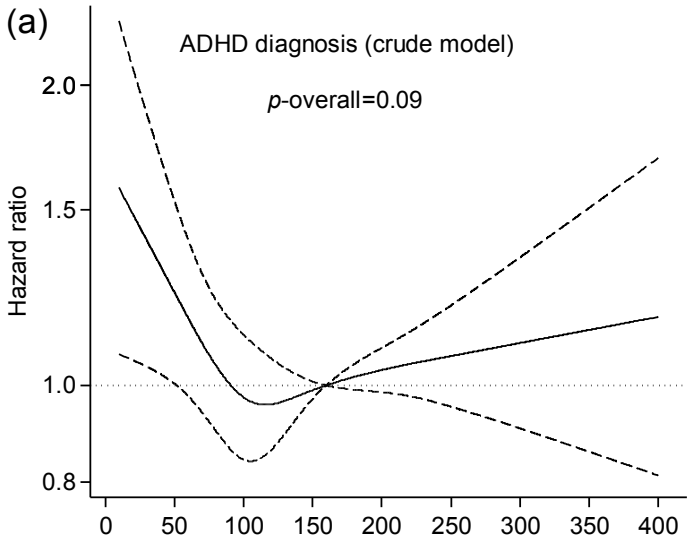

Figure 2. *Cont.*

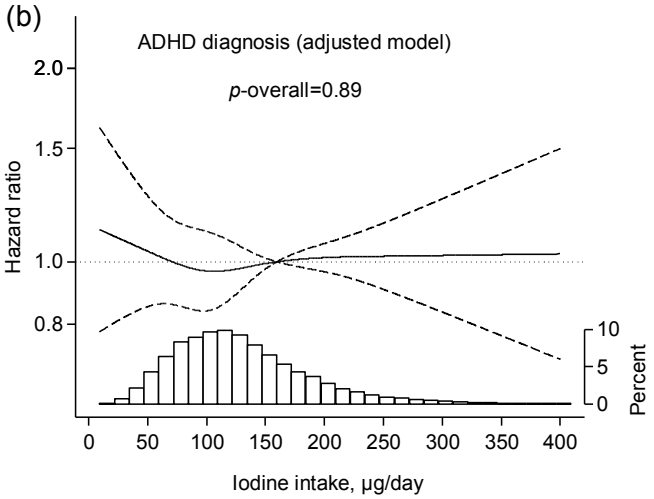

Figure 2. Association between maternal iodine intake from food and proportional risk of child ADHD diagnosis. Results are from multivariable regression analysis and are restricted to non-users of iodine supplements during first half of pregnancy (n = 53,360 mother–child pairs). Iodine intake was modelled by restricted cubic splines (four knots), and the reference level was set to 160 µg/day. Dashed lines represent 95% confidence limits. The histogram (**b**) illustrates the distribution of iodine intake. Both models (**a**,**b**) were adjusted for random effects of sibling clusters and for energy intake to control for measurement error. The adjusted model (**b**) was additionally adjusted for maternal age, BMI, parity, education, smoking in pregnancy, and fiber intake. The vertical axis on hazard ratios are on the log scale. ADHD: attention-deficit/hyperactivity disorder.

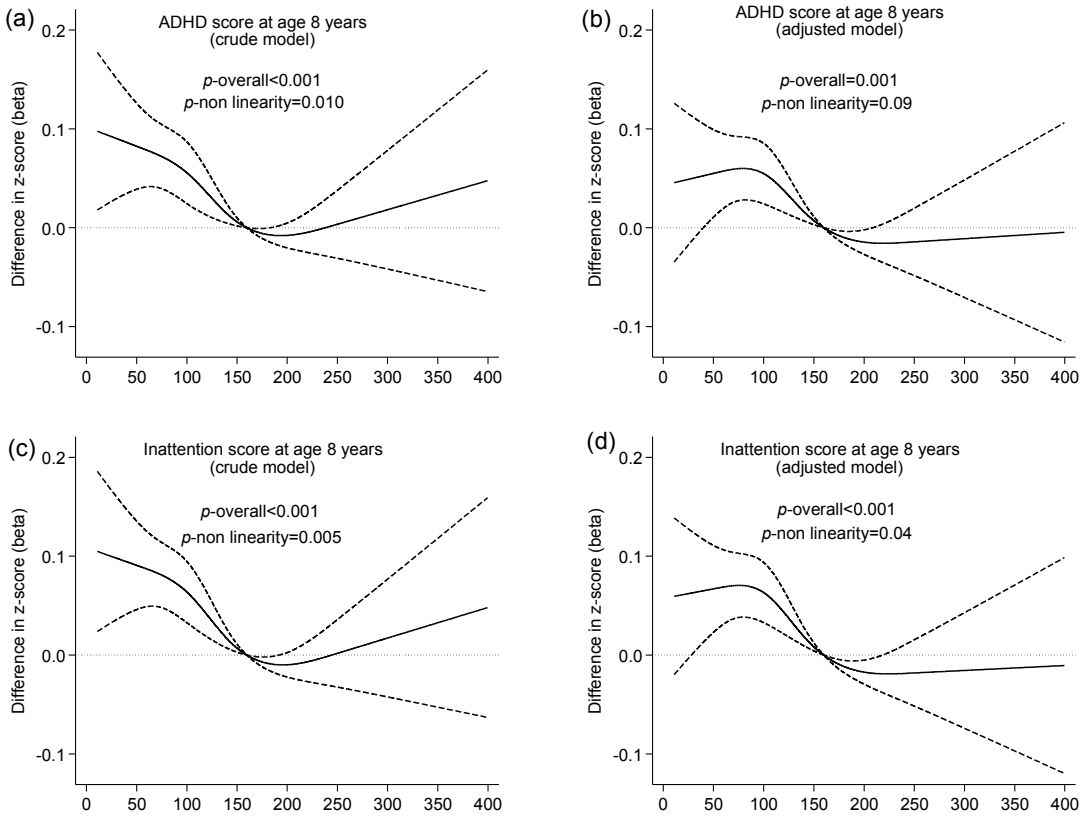

Figure 3. *Cont.*

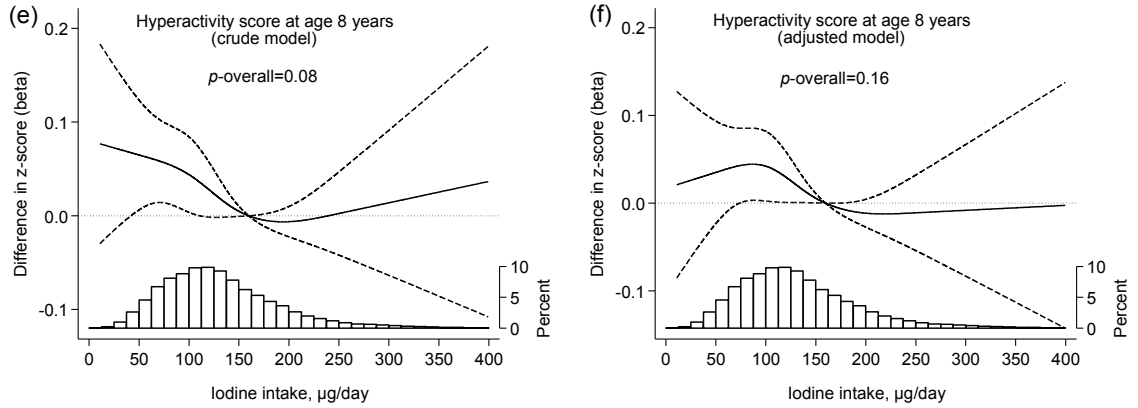

Figure 3. Association between maternal iodine intake from food and standardized score on maternally reported child ADHD symptoms at age eight years. Results are from multivariable regression analysis and restricted to non-users of iodine supplements during the first half of pregnancy (n = 19,086 mother-child pairs). Iodine intake was modelled by restricted cubic splines (four knots), and the reference level was set to 160 µg/day. Dashed lines represent 95% confidence limits. The histograms (**e**,**f**) illustrate the distribution of iodine intake. Crude models (**a**,**c**,**e**) were adjusted for maternal energy intake and for random effects of sibling clusters. Adjusted models (**b**,**d**,**f**) were additionally adjusted for maternal age, parity, education, body mass index, smoking in pregnancy, fiber intake, child sex, and birth season.

3.3. Iodine Intake from Supplements and Risk of ADHD

Supplemental iodine originated almost exclusively from multi-nutrient supplements, and only nine mothers reported use of supplements only containing iodine.

Among iodine supplement users who reported taking up to 200 µg supplemental iodine per day in the FFQ, 64% also gave information on timing of use in the general questionnaires. Of these mothers, 39% reported their first use at 0–26 weeks before pregnancy, 29% in GW 0–12 and 32% in GW 13 or later. There was no data on the dosage or frequency of use before pregnancy, only information on any use (yes or no) in the given time period.

The potential impact of iodine from supplements was explored in two groups by maternal iodine intake from food (less than or above the EAR of iodine from food) (Tables 3 and 4).

The maternal use of supplemental iodine was associated with an increased risk of child ADHD diagnosis (Table 3) and a higher mean ADHD symptom score (Table 4). The effect estimates were somewhat attenuated when restricting the reference group to participants who had reported taking supplements containing one or more vitamins or minerals other than the recommended supplements (folic acid, vitamin D, and iron) (matched controls). In participants with low iodine intakes from food, iodine supplement use initiated in GW 0–12 was associated with a ~29% increased risk of ADHD diagnosis (95% CI: 0–67%, p = 0.053) and a 0.06 SD higher average score on ADHD symptoms at eight years of age (95% CI: 0.01–0.11, p = 0.037) (Table 4). In participants with high iodine intakes from food, the results were not consistent. Initiating iodine supplement use in the first trimester was associated with an increased risk of ADHD diagnosis, whereas use before pregnancy was associated with increased child ADHD symptom scores.

Table 3. Use of iodine-containing supplements in pregnancy and risk of child ADHD diagnosis (n = 77,164) [1].

	ADHD Diagnosis			
	n	**Crude Model**	**Adjusted Model [2]**	**Adjusted Model [2] Matched Controls**
Iodine from food <160 µg/day				
Iodine from supplement:				
No (reference)	39,597 (11,057 [3])	1	1	1
1–200 µg/day	16,355	1.03 (0.91, 1.17)	1.13 (0.99, 1.28)	1.01 (0.86, 1.20)
>200 µg/day	1373	1.05 (0.71, 1.55)	1.07 (0.72, 1.59)	0.95 (0.63, 1.43)
First report of iodine [4]				
Before pregnancy [5]	4018	1.02 (0.81, 1.28)	1.24 (0.99, 1.56)	1.11 (0.86, 1.43)
Gestational week 0–12	2970	**1.34 (1.07, 1.68)**	**1.47 (1.17, 1.85)**	1.29 (1.00, 1.67)
Gestational week ≥13	3402	1.04 (0.82, 1.32)	1.11 (0.87, 1.41)	0.98 (0.75, 1.27)
Iodine from food ≥160 µg/day				
Iodine from supplement:				
No (reference)	13,763 (4152 [3])	1	1	1
1–200 µg/day	5585	1.11 (0.92, 1.34)	1.18 (0.98, 1.43)	1.08 (0.84, 1.37)
>200 µg/day	491	1.15 (0.66, 2.00)	1.16 (0.66, 2.01)	1.03 (0.58, 1.83)
First report of iodine [4]				
Before pregnancy [5]	1460	0.97 (0.69, 1.37)	1.21 (0.85, 1.71)	1.09 (0.74, 1.59)
Gestational week 0–12	1020	**1.40 (1.01, 1.95)**	**1.50 (1.07, 2.10)**	1.35 (0.93, 1.96)
Gestational week ≥13	1102	0.99 (0.68, 1.44)	1.04 (0.71, 1.52)	0.93 (0.61, 1.40)

[1] Values are hazard ratios (95% CIs) unless otherwise indicated. Significant associations ($p < 0.05$) are highlighted. All models (including crude models) were adjusted for random effects of sibling clusters and for energy intake, to control for measurement error. Models estimating the impact of supplemental iodine (dosage and timing) included interaction terms between iodine from diet and iodine from supplements; [2] The adjusted models were additionally adjusted for maternal age, BMI, parity, education, smoking in pregnancy, fiber intake, folic acid supplement within the interval from 4 weeks beforehand to 8 weeks after conception, and total EPA and DHA intake; [3] Matched controls: controls restricted to mothers who reported the intake of supplemental vitamins and/or minerals other than the recommended; [4] Restricted to participants who reported taking up to 200 µg/day of supplemental iodine in the food frequency questionnaire and who also gave information on timing of supplement use in the general questionnaires; [5] 0–26 weeks before conception.

Table 4. Use of iodine-containing supplements in pregnancy and maternally reported ADHD symptoms at eight years of age (n = 27,945) [1].

	N	ADHD Score			Inattention Score		Hyperactivity Score	
		Crude Model	**Adjusted Model**	**Adjusted Model Matched Controls [2]**	**Crude Model**	**Adjusted Model**	**Crude Model**	**Adjusted Model**
Iodine from food <160 µg/day								
Iodine from supplement:								
No (reference)	14,089 (4133)	0	0	0	0	0	0	0
1–200 µg/day	6115	**0.06 (0.04, 0.09)**	**0.05 (0.03, 0.08)**	0.02 (−0.01, 0.05)	**0.06 (0.03, 0.08)**	**0.05 (0.02, 0.07)**	**0.06 (0.03, 0.10)**	**0.06 (0.03, 0.10)**
>200 µg/day	457	0.07 (−0.00, 0.15)	0.06 (−0.02, 0.13)	0.02 (−0.06, 0.10)	0.07 (−0.00, 0.15)	0.06 (−0.02, 0.13)	0.07 (−0.03, 0.17)	0.06 (−0.03, 0.16)
First report of iodine supplement [3]:								
Before pregnancy [4]	1650	**0.04 (0.00, 0.09)**	**0.04 (0.00, 0.08)**	0.01 (−0.04, 0.05)	**0.06 (0.02, 0.10)**	**0.05 (0.01, 0.09)**	0.02 (−0.03, 0.08)	0.03 (−0.02, 0.08)
Gestational week 0–12	1203	**0.12 (0.07, 0.17)**	**0.10 (0.05, 0.15)**	**0.06 (0.01, 0.11)**	**0.11 (0.06, 0.15)**	**0.07 (0.03, 0.12)**	**0.14 (0.08, 0.20)**	**0.13 (0.07, 0.19)**
Gestational week ≥13	1264	**0.06 (0.01, 0.11)**	**0.05 (0.00, 0.10)**	0.01 (−0.04, 0.07)	**0.05 (0.01, 0.10)**	0.04 (−0.01, 0.09)	**0.07 (0.01, 0.14)**	**0.07 (0.01, 0.13)**
Iodine from food ≥160 µg/day								
Iodine from supplement:								
No (reference)	4997 (1593)	0	0	0	0	0	0	0
1–200 µg/day	2133	0.02 (−0.02, 0.06)	0.02 (−0.02, 0.07)	0.02 (−0.04, 0.07)	0.02 (−0.02, 0.07)	0.02 (−0.02, 0.06)	0.02 (−0.04, 0.07)	0.02 (−0.03, 0.08)
>200 µg/day	154	0.00 (−0.13, 0.14)	0.01 (−0.11, 0.13)	0.01 (−0.11, 0.13)	0.02 (−0.11, 0.15)	0.05 (−0.07, 0.17)	−0.02 (−0.20, 0.17)	−0.04 (−0.21, 0.13)
First report of iodine supplement [3]:								
Before pregnancy [4]	611	0.06 (−0.02, 0.13)	**0.08 (0.00, 0.15)**	0.07 (−0.02, 0.15)	0.05 (−0.02, 0.13)	0.06 (−0.01, 0.14)	0.06 (−0.03, 0.16)	0.09 (−0.00, 0.19)
Gestational week 0–12	397	0.03 (−0.05, 0.12)	0.02 (−0.07, 0.10)	0.00 (−0.09, 0.10)	0.04 (−0.04, 0.13)	0.02 (−0.06, 0.10)	0.02 (−0.09, 0.13)	0.02 (−0.09, 0.13)
Gestational week ≥13	439	0.02 (−0.07, 0.10)	0.01 (−0.07, 0.09)	0.00 (−0.09, 0.09)	0.01 (−0.07, 0.09)	0.00 (−0.08, 0.08)	0.03 (−0.08, 0.14)	0.02 (−0.09, 0.13)

[1] Values are standardized beta coefficients (95% CIs) unless otherwise indicated. Significant associations ($p < 0.05$) are highlighted. Models included interaction terms between iodine from diet and iodine from supplements. All models (including crude models) were adjusted for random effects of sibling clusters and for energy intake, to control for measurement error. The adjusted models were additionally adjusted for maternal age, BMI, parity, education, smoking in pregnancy, fiber intake, child sex, and birth season, folic acid supplement within the interval from 4 weeks beforehand to 8 weeks after conception, and total EPA and DHA intake; [2] Matched controls: controls restricted to mothers who reported the intake of supplemental vitamins and/or minerals other than the recommended; [3] Restricted to participants who reported taking up to 200 µg/day of supplemental iodine in the food frequency questionnaire and who also gave information on the timing of supplement use in the general questionnaires; [4] 0–26 weeks before conception.

4. Discussion

The main findings in this study were that maternal iodine intake of less than ~200 µg/day was associated with an increased risk of maternally reported child ADHD symptoms at eight years of age, but not significantly with risk of child ADHD diagnosis. Also, we found no evidence of any beneficial effect of supplemental iodine in pregnancy. On the contrary, initiating iodine supplement use within the first trimester in mothers with inadequate iodine intake from food (<EAR) was associated with both an increased risk of ADHD diagnosis and higher ADHD symptom score at eight years of age. A negative effect of iodine supplement use was also indicated for mothers with adequate iodine in their diet.

4.1. Iodine from Food and ADHD

Short-term use of supplemental iodine might have a different impact on thyroid function than long-term iodine intake [12,33]. We therefore only included non-users of iodine supplements when exploring the effects of iodine intake from food.

We have previously reported that low maternal iodine intake from food in pregnancy (below ~200 µg/day) was related to increased scores on maternally reported child behavior problems at three years of age in a dose-response manner ($p < 0.001$) [12]. The current study shows that this result prevails for maternally reported child ADHD symptoms at eight years of age. In comparison, the recommended iodine intake in pregnancy by the WHO is 250 µg/day [34]. The association curve for maternal iodine intake and child ADHD diagnosis displayed a similar shape as the symptom scores, but did not reach significance. This might indicate that the change in risk of ADHD diagnosis was too low to be detected in our sample, but given the wide confidence intervals, we cannot exclude an effect. Another possible explanation might be that there is a tendency that children with both ADHD and more pervasive neurodevelopmental disorders are registered under their primary diagnosis only (e.g., autism) in the NPR. The rate of registered comorbidities to ADHD in the NPR is much lower than would be expected [35]. This could result in a selection bias for the outcome, potentially also related to our exposure of interest, and thus influence the association that we study. In administrative registries like the NPR, we find only those individuals that actually are assessed and diagnosed by a specialist. The "true" prevalence of ADHD in Norway is not known, as not all suffering from ADHD seek specialist evaluation. Also, as the prevalence of formally diagnosed ADHD shows large regional differences, it is assumed that other factors, apart from symptom levels and impairment, influence the diagnostic process [36]. Thus, we might hypothesize that the variation in ADHD symptom scores is more closely related to the impact of iodine levels than the formal diagnosis.

Iodine status is closely linked to risks of thyroid dysfunction and thyroid autoimmunity [37,38]. In addition, ID might make the maternal thyroid more vulnerable to environmental goitrogens, i.e., substances inhibiting the uptake of iodine in the thyroid or the production of the thyroid hormones, present both in the diet and in cigarette smoke [39]. Several previous studies have explored maternal iodine nutrition and/or thyroid hormone status in pregnancy and the risk of child externalizing behavior or ADHD symptoms. In 1993, Hauser et al. [40] documented a link between thyroid hormones and risk of ADHD. They reported a strong association between the genetic disease, generalized resistance to thyroid hormones—characterized by reduced responsiveness to the actions of thyroid hormones—and risk of ADHD. In 2004, Vermiglio et al. [9] performed a non-randomized prospective study, comparing participants from an ID area, to participants from an iodine sufficient area (mean UIE in schoolchildren: 48 µg/day and 95 µg/day). Eight of the eleven mothers from the iodine deficient area became hypothyroxinemic early in pregnancy, and seven of their children were later diagnosed with ADHD. None of the 16 children from the iodine sufficient area were diagnosed with ADHD. Similarly, a Russian study ($n = 2397$ children) reported a higher prevalence of attention deficit syndrome (without hyperactivity) in an area with ID compared to an area without ID [11] (only the abstract available in English).

In the Generation R study there have been several publications reporting associations between maternal thyroid or iodine measures and child behavior. In a sub-study (n = 692 mother-child pairs), van Mil et al. [10] found that mothers with low urinary iodine early in pregnancy (below the 10th percentile; UIC < 136 μg/g creatinine) gave birth to children with a higher risk of impaired executive functioning at four years of age (mainly inhibition and working memory, which are both related to ADHD symptomatology). Ghassabian et al. [41] reported that elevated maternal thyroid-stimulating hormone concentration (within the normal range), but not hypothyroxinaemia, was associated with higher scores on externalizing problems up to three years of age. Later, Modesto et al. [42] reported that maternal hypothoroxinemia around GW ~14 was associated with increased child ADHD symptoms at eight years of age. Ghassabian et al. [43] reported that children of mothers testing positive for thyroid peroxidase antibodies (identified in 4.7% of 3139 mothers measured in GW ~14) had an increased risk of scoring high on parent-reported ADHD-symptoms at three years of age (odds ratio = 1.77, 95% CI: 1.15, 2.72).

To our knowledge, the largest study to date is a Danish register-based cohort study including n = 857,014 singleton births between 1991 and 2004 [44]. Children born to mothers who were diagnosed with thyroid disorders in pregnancy or later (3.5%) had a higher risk of ADHD diagnosis (HR 1.18, 95% CI 1.03, 1.36 for hyperthyroidism, and HR 1.10, 95% CI: 0.98, 1.25 for hypothyroidism).

Taken together, an increased risk of symptoms of ADHD in the child when the maternal diet provides inadequate iodine seems plausible, and the results in our study add supporting evidence for a link.

4.2. Iodine from Supplements and ADHD

The results in this study support our previous findings, where we reported that mothers with insufficient iodine intake from food, who started using iodine-containing supplements in pregnancy, gave birth to children with increased behavior problems at three years of age [12]. Effect estimates in the present study were however, attenuated when restricting the control group to mothers who used supplements without iodine (other than the recommended vitamin D, folic acid and iron). This most likely indicates a confounding effect of maternal "health seeking behavior". Alternatively, it might indicate confounding or effect modification by other substances in multi-supplements (i.e., other nutrients in the supplements exerting a negative effect on the child's brain development). However, the associations with ADHD outcomes were robust and remained borderline significant for participants with a low iodine intake from food who initiated iodine supplement use in the first trimester.

A Cochrane review, published in 2017, entitled "Iodine supplementation for women during the preconception, pregnancy and postpartum period" summarized findings from relevant randomized controlled trials (RCT) on iodine supplementation [45]. The authors concluded that there is not enough data to reach any meaningful conclusions on the potential benefits and harms of supplementing women in areas with mild to moderate ID, and that more studies are needed. They reported nonsignificant increased risks of both hypo- and hyperthyroidism in pregnancy in supplemented mothers, but the confidence intervals were wide, due to the small number of participants included. It is however, not unlikely that an abrupt increase in iodine intake, particularly in women with low iodine intakes from food, could cause a "stunning effect" of the thyroid and a temporary imbalance in thyroid hormones [33]. The developing brain might be most vulnerable to such imbalances during the first trimester, since the fetus is entirely dependent on the maternal supply of thyroid hormones in early pregnancy.

Just recently, the world's first RCT study, exploring the effect of iodine supplementation in mildly iodine deficient pregnant women (median UIC: 131 μg/L) on child neurodevelopment was published [46]. Gowachirapant et al. did not find any effects on a range of outcomes, including intelligence quotient (IQ), executive functioning, and behavior problems. However, it is important to notice that a substantial part of the study participants had a UIC higher than 150 μg/L; supplement

use was not initiated until gestational week 10.7 (SD: 2.7), and the study was underpowered to detect differences of less than five IQ points between the groups. The results showed that the children of the supplemented women scored more poorly on the tests, but the differences were small and not statistically significant.

4.3. Strengths and Limitations

Strengths of this study include the large sample size, prospective design, extensive collection of data, and the possibility of linking the cohort to national registries like the NPR securing minimal loss to follow up. Maternally reported child ADHD symptoms provided an alternative, continuous outcome measure, strengthening the potential of identifying risks of ADHD.

The considerable variation in iodine intake between mothers and the high prevalence of low intake make MoBa ideal for exploring maternal iodine intake as an exposure. In Norway, tap water and iodized salt contribute only negligible amounts of iodine and there are only few food sources of iodine. A dietary survey method is thus suitable for estimating iodine intake at an individual level, as was previously documented in a validation study in MoBa (referenced in the Methods section [20,22]). However, there will always be uncertainty related to the dietary estimates, due to potential recall bias when reporting average food and supplement intakes in the first half of pregnancy, and to variation in iodine concentration in food items.

There were no substantial differences in maternal age, BMI, parity, or socioeconomic status indicators, either by maternal iodine intake or by iodine supplement use. This might be explained by a very low awareness of iodine among pregnant women in Norway [47], and no existing guidelines for iodine supplement use. Also, milk consumption is not closely related to having a healthy diet. This reduces the risk of confounding by lifestyle or dietary factors.

Some multi-supplements reported by the MoBa mothers contained iodine, whereas others did not. This allowed us to apply a quasi-experimental design with matched controls, which strengthened the evidence for a causal link.

Weaknesses included the observational design, meaning that we cannot rule out the possibility of residual confounding. For example, heritable psychological traits are associated with medication use during pregnancy (e.g., herbal preparations) [48]. Passive genetic transmission of ADHD-like traits could therefore confound the parent-offspring associations. Furthermore, the associations identified in our study might be affected by selection bias and by misclassification of both exposure and outcome variables. The low participation rate (41%) is a concern and participants in MoBa are not representative of the general pregnant population [13]. The possible impact of self-selection in MoBa has been evaluated and the results showed that the non-representativeness does influence prevalence estimates, but does not necessarily affect exposure-outcome associations [49,50].

4.4. Clinical Relevance and Implications

This study adds supporting evidence that insufficient maternal iodine intake might be a risk factor for offspring ADHD. Our analyses also indicate an increase in the risk of ADHD in children of mothers using iodine-containing supplements, and this is alarming given the high rate of supplement use in many countries. The current recommendation from the WHO is to promote iodine supplements in areas of insufficient iodine intake for women of childbearing age and in pregnancy and lactation [34]. The results observed in this study emphasize the urgent need for large and well-designed RCTs on the impact of initiating iodine supplementation in the first trimester, and ideally well before GW 12. Our study indicates that iodine-containing supplements should not be encouraged for pregnant women in the first trimester, especially in areas with mild to moderately insufficient iodine intakes. Our results also emphasize the need for ensuring sufficient iodine intake at the population level, in order to ensure sufficient iodine status in women of childbearing age before they enter pregnancy.

5. Conclusions

This study showed that low maternal iodine intake during pregnancy was associated with increased ADHD symptom score at eight years of age, but not with the risk of child ADHD diagnosis. There was no indication of any beneficial effects of maternal use of supplemental iodine on child ADHD, and initiating iodine supplement use in the first trimester was associated with an increased risk.

Supplementary Materials: The following are available online at www.mdpi.com/2072-6643/9/11/1239/s1, Figure S1: Conceptual model (simplified directed acyclic diagram (DAG)), Figure S2: Sex specific associations between maternal iodine intake from food (in non-supplement users) and child ADHD, Table S1: Associations between maternal iodine intake from food in participants who did not report use of supplemental iodine in the FFQ and risk of child ADHD diagnosis, Table S2: Association between maternal iodine intake from food in participants who did not report use of supplemental iodine in the FFQ) and score on maternally reported ADHD-symptoms at age 8 years.

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Conflicts of Interest: The first author of this paper is employed by a Norwegian dairy company (TINE SA), and she participates in this project as an industrial PhD-student financed partly by the dairy company and partly by The Research Council of Norway. This project is designed, owned and administered by The Norwegian Institute of Public Health and analysis of the data follow from protocol. All results of analysis in the project are to be published regardless of the results. The dairy company supports the study to raise awareness on the importance of iodine and to gain more knowledge about the potential health effects of milk in the Norwegian diet. Apart from the PhD-student, no one from the dairy company has been involved in the study, and in itself, the company had no direct influence on the analysis and interpretation of the results. The other authors had no conflicts of interest.

Abbreviations

ADHD	Attention-deficit/hyperactivity disorder
DSM	The diagnostic and statistical manual of mental disorders
EAR	Estimated average requirement
FFQ	Food frequency questionnaire
GW	Gestational week
HKD	Hyperkinetic disorder
ICD	International Classification of Disease
ID	Iodine deficiency
IQ	Intelligence quotient
MoBa	The Norwegian Mother and Child Cohort Study
NPR	Norwegian Patient Registry
UIC	Urinary iodine concentration

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ONLINE SUPPORTING MATERIAL

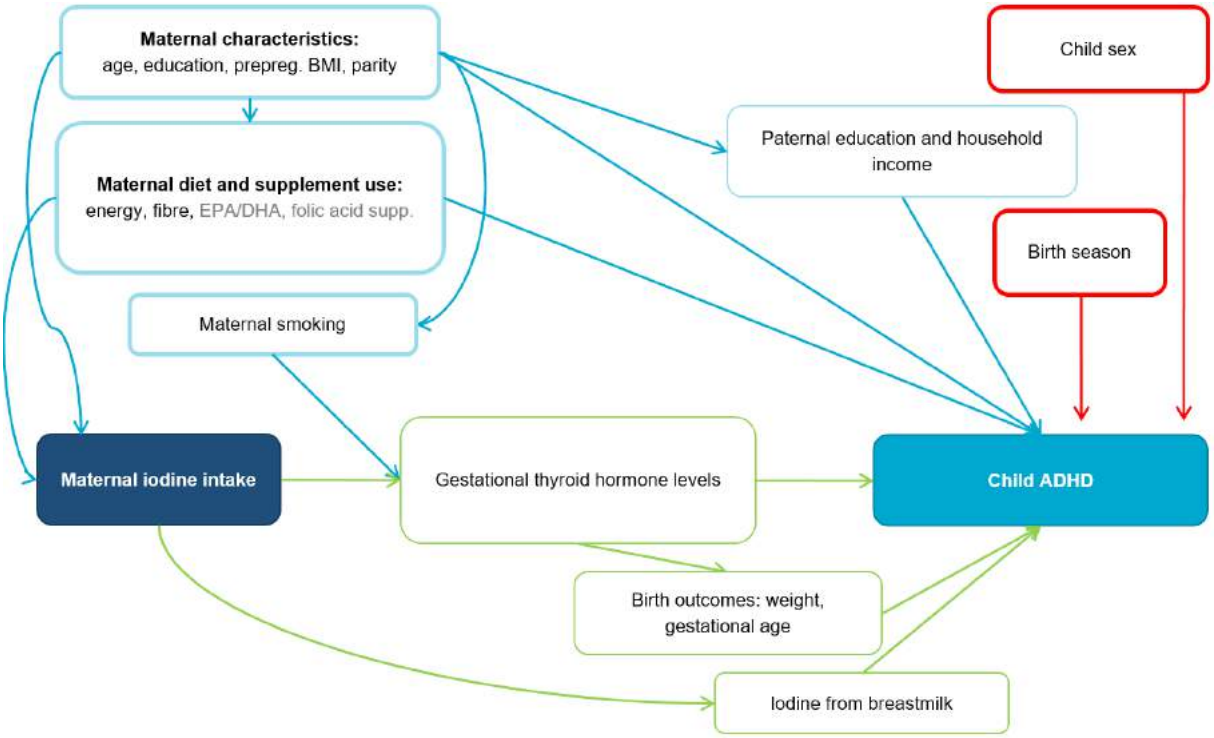

Figure S1 Conceptual model (simplified directed acyclic diagram (DAG))

The association between maternal iodine intake and child ADHD. Potential causal pathways are illustrated in green and confounding pathways in blue. Important determinants of the outcome are in red. Intake of the n-3 fatty acids EPA and DHA and reported use of folic acid supplements were only included as confounders when iodine from supplements was the exposure.

Table S1 Associations between maternal iodine intake from food in participants who did not report use of supplemental iodine in the FFQ and risk of child ADHD diagnosis (*n*=53,360)[1]

	ADHD Crude model	ADHD Adjusted model[1]
Iodine from food, µg/d:		
25	1.44 (1.05, 1.97)	1.09 (0.81, 1.46)
50	1.24 (1.00, 1.53)	1.04 (0.85, 1.27)
75	1.07 (0.92, 1.25)	0.99 (0.85, 1.15)
100	0.97 (0.84, 1.12)	0.97 (0.84, 1.12)
125	0.96 (0.88, 1.05)	0.97 (0.89, 1.07)
160 (ref)	1	1
200	1.04 (0.98, 1.09)	1.01 (0.97, 1.07)
225	1.05 (0.97, 1.14)	1.02 (0.94, 1.10)
250	1.07 (0.95, 1.20)	1.02 (0.91, 1.15)
300	1.10 (0.90, 1.34)	1.02 (0.83, 1.25)
350	1.14 (0.86, 1.51)	1.03 (0.77, 1.37)
400	1.17 (0.81, 1.69)	1.03 (0.71, 1.50)
p-overall	*p*=0.09	*p*=0.89

[1] Values are hazard ratios (95% CIs) unless otherwise indicated. Both models (including crude model) were adjusted for random effects of sibling clusters and for energy intake to control for measurement error. Adjusted model was additionally adjusted for maternal age, BMI, parity, education, smoking in pregnancy, and fiber intake.

Abbreviation: FFQ, Food frequency questionnaire

Table S2 Association between maternal iodine intake from food in participants who did not report use of supplemental iodine in the FFQ) and score on maternally reported ADHD-symptoms at age 8 y (*n*=19 086)[1]

	ADHD score Crude model	ADHD score Adjusted model	Inattention score Crude model	Inattention score Adjusted model	Hyperactivity score Crude model	Hyperactivity score Adjusted model
Iodine from food, µg/d:						
25	0.09 (0.03, 0.16)	0.05 (-0.02, 0.12)	0.10 (0.03, 0.17)	0.06 (-0.00, 0.13)	0.07 (-0.02, 0.16)	0.03 (-0.06, 0.11)
50	0.08 (0.04, 0.13)	0.06 (0.01, 0.10)	0.09 (0.05, 0.14)	0.07 (0.02, 0.11)	0.06 (0.01, 0.12)	0.04 (-0.02, 0.09)
75	0.07 (0.04, 0.10)	0.06 (0.03, 0.09)	0.08 (0.05, 0.11)	0.07 (0.04, 0.10)	0.06 (0.01, 0.10)	0.04 (0.00, 0.09)
100	0.06 (0.02, 0.09)	0.05 (0.02, 0.09)	0.06 (0.03, 0.10)	0.06 (0.03, 0.09)	0.04 (0.00, 0.08)	0.04 (0.00, 0.08)
125	0.03 (0.01, 0.05)	0.03 (0.01, 0.05)	0.03 (0.02, 0.05)	0.04 (0.02, 0.06)	0.02 (-0.00, 0.05)	0.03 (0.00, 0.05)
160 (ref)	0	0	0	0	0	0
200	-0.01 (-0.02, 0.00)	-0.01 (-0.03, -0.00)	-0.01 (-0.02, 0.00)	-0.02 (-0.03, -0.00)	-0.01 (-0.02, 0.01)	-0.01 (-0.03, 0.00)
225	-0.00 (-0.03, 0.02)	-0.02 (-0.04, 0.01)	-0.01 (-0.03, 0.02)	-0.02 (-0.04, 0.00)	-0.00 (-0.03, 0.03)	-0.01 (-0.04, 0.02)
250	0.00 (-0.03, 0.04)	-0.01 (-0.05, 0.02)	0.00 (-0.03, 0.04)	-0.02 (-0.05, 0.02)	0.00 (-0.04, 0.05)	-0.01 (-0.05, 0.03)
300	0.02 (-0.04, 0.08)	-0.01 (-0.07, 0.05)	0.02 (-0.04, 0.08)	-0.02 (-0.07, 0.04)	0.01 (-0.06, 0.09)	-0.01 (-0.08, 0.07)
350	0.03 (-0.05, 0.12)	-0.01 (-0.09, 0.08)	0.03 (-0.05, 0.12)	-0.01 (-0.10, 0.07)	0.03 (-0.09, 0.14)	-0.01 (-0.11, 0.10)
400	0.05 (-0.06, 0.16)	-0.00 (-0.12, 0.11)	0.05 (-0.06, 0.16)	-0.01 (-0.12, 0.10)	0.04 (-0.11, 0.18)	-0.00 (-0.14, 0.14)
p-overall	*p*<0.001	*p* =0.001	*p* <0.001	*p* <0.001	*p* =0.08	*p* =0.16
p-non linearity	*p*=0.010	*p* =0.09	*p* =0.005	*p* =0.043		

[1] Values are standardized beta coefficients (95% CIs) unless otherwise indicated. Both models (including crude model) were adjusted for random effects of sibling clusters and for energy intake to control for measurement error. Adjusted model was additionally adjusted for maternal age, BMI, parity, education, smoking in pregnancy, fiber intake, child sex, and birth season.

Abbreviation: FFQ, Food frequency questionnaire

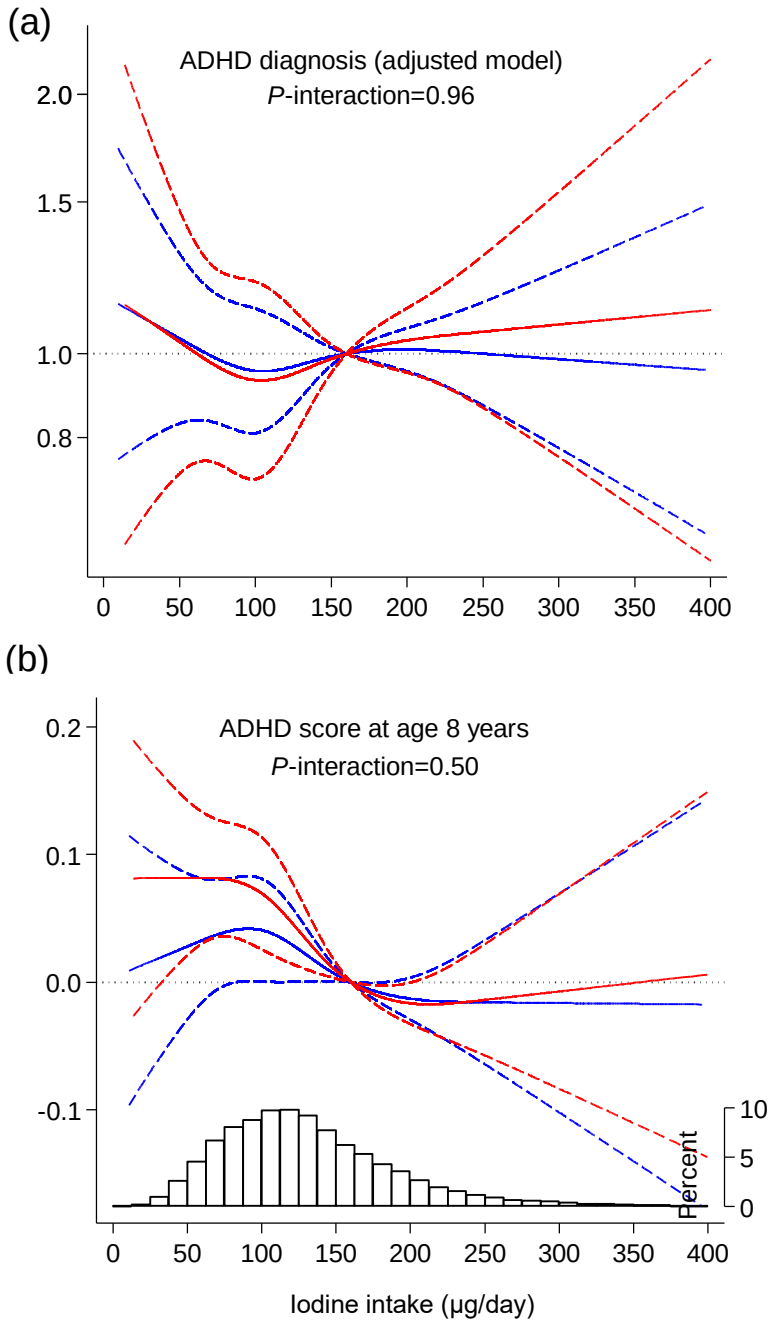

Figure S2 Sex specific associations between maternal iodine intake from food (in non-supplement users) and child ADHD

Estimated associations for boys in blue and girls in red. The models included an interaction term between gender and iodine intake. Solid lines represent restricted cubic splines (knotpositions at 54, 102, 143 and 245 µg/d), 95% CI are illustrated by dotted lines, and the reference level was set to 160 µg/d (the estimated average requirement of iodine in pregnancy by the Institute of Medicine). The histogram in (b) illustrates the distribution of maternal iodine intake. The models (a) and (b) were adjusted for maternal age, body mass index, parity (0, 1, ≥2), education (≤12, 13-16, ≥17 years), smoking in pregnancy (never, occasionally or quit before gestational week 12, daily), energy intake, fiber intake, and for random effects of sibling clusters. Model (b) was additionally adjusted for birth season.

PAPER 3

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Iodine Intake is Associated with Thyroid Function in Mild to Moderately Iodine Deficient Pregnant Women

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Background: Studies indicate that mild to moderate iodine deficiency in pregnancy may have a long-term negative impact on child neurodevelopment. These effects are likely mediated via changes in maternal thyroid function, since iodine is essential for the production of thyroid hormones. However, the impact of iodine availability on thyroid function during pregnancy and on thyroid function reference ranges are understudied. The aim of this study was to investigate the association between iodine intake and thyroid function during pregnancy.
Design: In a population-based pregnancy cohort including 2910 pregnant women participating in The Norwegian Mother and Child Cohort Study, we explored cross sectional associations of maternal iodine intake measured (1) by a food frequency questionnaire and (2) as iodine concentration in a spot urine sample, with plasma thyroid hormones and antibodies.
Results: Biological samples were collected in mean gestational week 18.5 (standard deviation 1.3) and diet was assessed in gestational week 22. Median iodine intake from food was 121 μg/day (interquartile range 90, 160), and 40% reported use of iodine-containing supplements in pregnancy. Median urinary iodine concentration (UIC) was 59 μg/L among those who did not use supplements and 98 μg/L in the women reporting current use at the time of sampling, indicating mild to moderate iodine deficiency in both groups. Iodine intake as measured by the food frequency questionnaire was not associated with the outcome measures, while UIC was inversely associated with FT3 ($p=0.002$) and FT4 ($p<0.001$). Introduction of an iodine-containing supplement after gestational week 12 was associated with indications of lower thyroid hormone production (lower FT4, $p=0.027$, and nonsignificantly lower FT3, $p=0.17$). The 2.5th and 97.5th percentiles of TSH, FT4, and FT3 were not significantly different by groups defined by calculated iodine intake or by UIC.
Conclusion: The results indicate that mild to moderate iodine deficiency affect thyroid function in pregnancy. However, the differences were small, suggesting that normal reference ranges can be determined based on data also from mildly iodine deficient populations, but this needs to be further studied. Introducing an iodine-containing supplement might temporarily inhibit thyroid hormone production and/or release.

Keywords: iodine, pregnancy, thyroid function, dietary supplements, The Norwegian Mother and Child Cohort Study, MoBa

Introduction

Results from observational studies, including the Norwegian Mother and Child Cohort Study (MoBa), have indicated that even mild to moderate iodine deficiency (ID) in pregnancy might negatively affect child neurodevelopment (1–4). Iodine is an essential micronutrient, as it is an integral part of the thyroid hormones thyroxine (T4) and triiodothyronine (T3). Severe ID results in depleted iodine stores and a failure to sustain normal thyroid hormone levels

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(5). The fetus is entirely dependent on an adequate and stable supply of these hormones, especially during the first half of pregnancy, until the fetal thyroid gland is developed. Thyroid hormones are essential in fetal growth, and particularly for cells in the central nervous system and for the structural and functional development of the brain (6). In areas with chronic moderate to severe ID, children score an estimated 8–10 points lower on IQ tests (7). Other dietary factors may contribute to aggravate symptoms of ID. Examples are deficiencies of other nutrients that are important for thyroid function (e.g., selenium and iron) (8), and intake of cruciferous vegetables that are known to inhibit iodine uptake and utilization by the thyroid due to high content of thiocyanate (9). Excessive iodine intake during pregnancy may also result in thyroid hypofunction (10). The association of iodine intake with thyroid function is U-shaped, and studies indicate that the range of optimal intake is narrow (11).

The recommended daily intake of iodine for pregnant women varies somewhat. The World Health Organization (WHO) recommends 250 μg/day (or a population median urinary iodine concentration (UIC) ≥150μg/L), which is almost 70% higher than that of nonpregnant women, to compensate both for an increased turnover of iodine in pregnancy and depleted iodine stores in many populations (12). The European Food Safety Authority has a lower recommendation of 200 μg/day but states that it is for populations with adequate iodine status prior to conception (13). The Nordic recommendation is even lower, at 175 μg/day (14), while in the United States, the recommended daily intake is 220 μg/day and the estimated average requirement is 160 μg/day (15,16). These recommendations are based only on limited data because the association of iodine intake with thyroid function during pregnancy has remained understudied. However, all the above-mentioned recommendations unanimously recommend 150 μg/day for nonpregnant women (12–15).

During pregnancy, thyroid hormone production increases in order to meet the needs of the mother and fetus. Thus, thyroid hormone concentrations in pregnant women differ from nonpregnant women, and also change considerably throughout the gestation period (15). Other important determinants of measured hormone levels are body mass index (BMI), ethnicity, smoking habits, thyroid autoimmunity, and method of analysis, especially for free thyroid hormones (17). The 2017 guidelines of the American Thyroid Association (ATA) recommend using population- and assay-specific reference ranges for the thyroid hormones in defining diagnostic criteria for thyroid disorders and to avoid basing reference values on ID populations (15). However, there is a lack of knowledge regarding the potential impact of mild to moderate ID on thyroid hormone reference ranges (i.e., the 2.5–97.5 % range) (17).

According to the World Health Organization (18), iodine supplements should be recommended for pregnant women in areas of inadequate iodine intake. Although the majority of US pregnant women have a sufficient iodine intake, the ATA recommends that all pregnant and lactating women in the United States use iodine supplements (19). Yet, there is uncertainty regarding the benefit and safety of recommending iodine supplements for pregnant women in areas with mild to moderate ID (20). In severe ID, iodine supplementation reduces the risk of thyroid hypofunction, but in mild to moderate ID, results from studies are not consistent (5). Some studies indicate that iodine supplementation may result in a temporary ''stunning effect'' of the thyroid gland with a transient lower thyroid hormone production (21,22). Other studies find that supplements may be beneficial or have no effect on thyroid function or child outcomes (20,23–25). In MoBa, maternal iodine supplement use initiated in pregnancy was related to increased risk of child behavior problems and ADHD (3,4), but not to language or motor development (3).

In a population of pregnant women from a mild to moderately ID population we aimed to

1. Study the associations of iodine intake measured by (i) a food frequency questionnaire (FFQ) and (ii) by urinary iodine concentration (UIC), with biomarkers of thyroid function;
2. Define thyroid function reference ranges for thyrotropin (TSH), FT4, and FT3 and investigate whether these differ according to iodine intake; and
3. Investigate the impact of current iodine supplement use, and also of timing of initiation, on thyroid function and autoimmunity.

Subjects and Methods

Subjects and design

This study is embedded within MoBa eTox, a substudy of the prospective population-based pregnancy cohort MoBa conducted by the Norwegian Institute of Public Health (26). Pregnant women in their first trimester were recruited from all over Norway during the years 1999 to 2008 and asked to answer questionnaires (in Norwegian) at regular intervals during pregnancy and after delivery. More than 99% of participants are of Caucasian origin. Pregnancy and birth records from the Medical Birth Registry of Norway are linked to the MoBa database (27). The women consented to participation in 41% of the pregnancies. The cohort now includes 114,500 children, 95,200 mothers, and 75,200 fathers. The current study is based on version 10 of the quality-assured data files released for research in 2017.

The participants in MoBa eTox ($n = 2999$) is a selected sample of MoBa consisting of participants who had responded to all questionnaires in MoBa up to child's age 3 years and had delivered all biological samples in mid pregnancy and at birth. Only singleton pregnancies were included. Children with autism, suspected autism, or symptoms of severe language delay were reserved for another substudy and those mother-child pairs were thus not included in MoBa eTox.

For our study ($n = 2910$), we additionally excluded women reporting use of thyroid medication in pregnancy (gestational week 0–24), and women with calculated energy intakes <4.5 MJ or >20 MJ or more than 3 blank pages in the FFQ (28) (see flowchart of inclusion in Fig. 1).

Exposure variables – iodine intake and UIC

The MoBa FFQ was specifically designed for the MoBa study and in use from 2002 (29). It was completed by participating women around gestational week (GW) 22, and is a semi-quantitative questionnaire designed to capture dietary habits and use of dietary supplements during the first half of pregnancy. It included questions about intake of 255 food

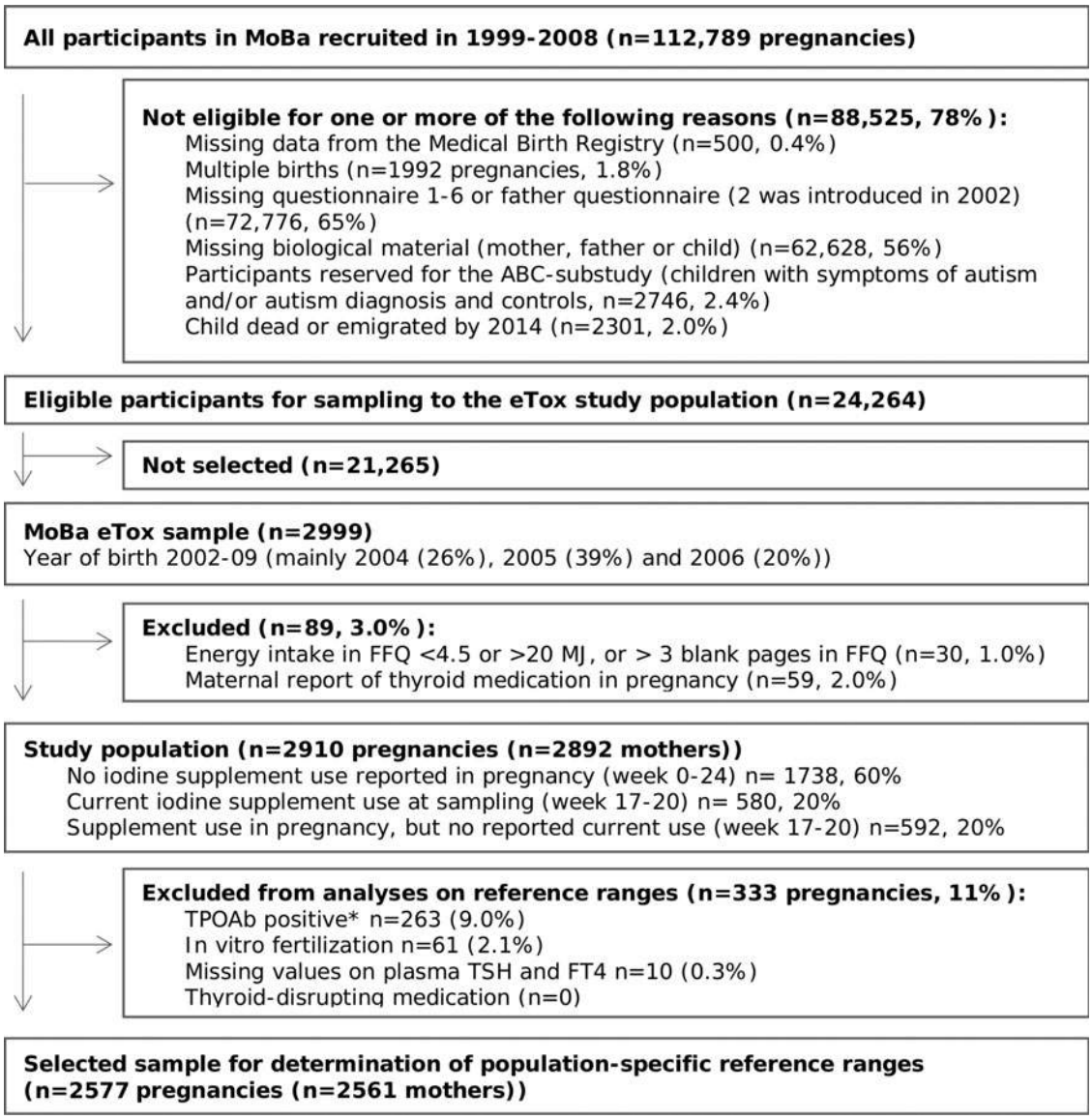

FIG. 1. Flow chart of inclusion. *TPOAb positive according to manufacturer-defined cutoff for nonpregnant women (TPOAb >4.11 IU/mL). FFQ, food frequency questionnaire; FT4, free thyroxine; MoBa, Norwegian Mother and Child Cohort Study; TPOAb, thyroid peroxidase antibodies; TSH, thyrotropin.

items or dishes (28). Intake of specific foods and nutrients were calculated based on standard Norwegian portion sizes, the Norwegian food composition table, analysis of Norwegian food samples (30,31), and data on the content of more than 1000 dietary supplements collected from suppliers (32).

As reported previously (3),the MoBa FFQ was shown to be a valid tool for ranking pregnant women according to high and low intakes of energy, nutrients, and foods (33). Milk and seafood are the main dietary sources of iodine in Norway; thus, iodine intake is highly variable depending on individual food choices (34). Iodine was validated separately and iodine intake by the FFQ, including supplemental iodine, showed good agreement with the reference methods (24 h urinary iodine excretion and 4 days weighed food diary; triangular validity coefficient for total iodine intake by the FFQ was 0.62 [95% confidence interval 0.46, 0.77]) (35,36).

Timing of iodine intake from supplements was reported in the general questionnaires one (GW 17), and three (GW 30). Supplement use was coded in three categories (no use in pregnancy, current use in GW 17-20, and use reported in pregnancy but not in GW 17-20). Timing of first report of iodine-containing supplements was coded in four categories (never, week 0–26 before pregnancy, GW 0-12, and GW 13-20).

Blood and urine samples were collected at the routine ultrasound examination offered in GW 18 (37). UIC was explored both as a crude measure (μg/L) and adjusted for hydration status by the ''residual method'' (38) (i.e., residuals after regressing log UIC on log urinary creatinine plus predicted UIC at the median creatinine concentration, obtaining UIC adjusted for creatinine [UIC$\sim$Cr]). The method is frequently used in nutritional epidemiology to control for energy-intake in dietary surveys and has also been reported

for UIC adjustment (39). UIC ~ Cr represents an estimate of UIC at a median hydration state.

Outcome variables: Thyroid function biomarkers

TSH, free T4 (FT4), free T3 (FT3), thyroid peroxidase antibodies (TPOAb), and thyroglobulin antibodies (TgAb) were measured in plasma samples from mid pregnancy (mean gestational week 18.5, standard deviation 1.3). For TPOAb and TgAb, the 92nd percentile was chosen as a cutoff for defining antibody positivity based on previous literature (40) and on exploring associations between measured antibody concentrations and TSH and FT4 concentrations in our study sample (Supplementary Figs. S1 and S2; Supplementary Data are available online at www.liebertpub.com/thy). For TSH, FT4, and FT3, the cutoffs for defining low and high values were set to the 2.5th and 97.5th percentiles in a subsample of TPOAb negative women after exclusion of women that underwent *in vitro* fertilization (IVF).

Analytical procedures

Samples of urine and blood were collected at the 50 participating hospitals, and samples were shipped by ordinary mail (unrefrigerated shipment) in a vacutainer for long-term freezing at a central biorepository (37). Storage temperature was −80°C (−20°C for whole blood).

UIC was determined at the National Institute for Health and Welfare (THL) in Helsinki (Finland) by inductively coupled plasma–mass spectrometry (ICP-MS) using an Agilent 7800 ICP-MS system (Agilent Technologies Inc., Santa Clara, CA). In brief, 100 μL of urine sample was extracted using ammonium hydroxide solution. Tellurium was used as an internal standard. On the ICP-MS, m/z = 127 was scanned for iodine determination. The limit of quantification of the method was 2 μg/L and the linearity was $t(r = 0.9999$ up to 1500 μg/L). The National Institute of Standards and Technology standard reference materials SRM2670a (with certified mass concentration value) and SRM3668 Level 1 and Level 2 were used to validate the method. The coefficient of variation (CV) of control samples was 3.0–4.8%. The laboratory at THL participated in the Ensuring the Quality of Urinary Iodine Procedures Program organized by the Centers for Disease Control and Prevention three times per year.

Urinary creatinine was analyzed by an enzymatic method using Multigent Creatinine (Enzymatic) Assay (SENTINEL CH. Sp.A.) using the ARCHITECT® c System (Abbott Laboratories, Abbott Park, IL) at THL. CV of control samples was 1.4–1.7%. The laboratory participated in an external quality assessment scheme for urinary creatinine organized by Labquality (Finland).

Plasma TSH, FT3, FT4, TPOAb, and TgAb concentrations were analyzed at THL using chemiluminescent microparticle immunoassays. Reagents and equipment (Architect ci8200 analyzer) were from Abbott Laboratories. CV of control samples (Bio-Rad Liquichek Immunoassay Plus lot 40860 high and low level, and in-house control, $n = 181$) were as follows: TSH 1.8–2.9%, FT3 2.5–4.7%, FT4 2.7–8.5%, TPOAb 3.7–5.2%, TgAb 4.1–4.4%. The laboratory participated in external quality assessment schemes for thyroid hormones and antibodies organized by Labquality (Finland).

Plasma ferritin was analyzed at THL using a chemiluminescent microparticle immunoassay. The coefficient of variation of control samples (Bio-Rad Liquichek immunoassay Plus lot 40860 high and low level, and in-house control, $n = 181$) were 2.7–3.7%. The laboratory participated in an external quality assessment scheme for ferritin organized by Labquality (Finland).

Whole blood selenium was analyzed at Lund University in Sweden by inductive coupled plasma mass spectrometry (ICP-MS; iCAP Q, Thermo Fisher Scientific, Bremen, GmbH) equipped with collision cell with kinetic energy discrimination and helium as collision gas. The detection limit was 3.2 μg/L and the coefficient of variation was 1.5%. The analytical accuracy was verified towards certified reference material; Seronorm Trace elements whole blood L-1 and L-2 (SERO AS, Billingstad, Norway).

Covariates

Data on covariates were obtained from different sources: Maternal age at delivery was obtained from the Medical Birth Registry of Norway. Maternally reported prepregnancy body weight and height for the calculation of body mass index (BMI), maternal education (≤12, 13–16, ≥17 years), parity (previous pregnancies ≥22 weeks: 0, 1, ≥2), maternal chronic illness (asthma, diabetes, inflammatory bowel disease, rheumatic disease, epilepsy, multiple sclerosis or cancer before or during pregnancy [yes/no]), and smoking in pregnancy (average cigarettes/day GW 0–17) was included from questionnaire 1. Energy intake, fiber intake (as a marker of a healthy dietary pattern), and intake of cruciferous vegetables (high in glucosinolates, potent goitrogens) were calculated based on the FFQ.

Selenium status (whole blood selenium <80, ≥80 μg/L), and iron status (plasma ferritin <12, 12–29.9, ≥30 μg/L) were measured in the blood samples in mid-pregnancy, and creatinine concentration was measured in the urine samples. Gestational age at blood and urine sampling was estimated based on the ultrasound examination, which took place the same day as the sampling and, if missing, on the first day of last menstruation.

Ethics

MoBa is conducted according to the guidelines laid down in the Declaration of Helsinki and written informed consent was obtained from all participants. MoBa has obtained a license from the Norwegian Data Inspectorate. The current study was approved by The Regional Committee for Medical Research Ethics South East Norway 2014/2211.

Statistics

Statistical analyses were performed in STATA (version 15.0; Stata Corp., College Station, TX). The package postrcspline for STATA was used for graphing of flexible models (41).

In total, 4.8% of the women had missing values on one or more of the covariates: pre-pregnancy BMI ($n = 54$, 1.9%), maternal education ($n = 62$, 2.1%), gestational age at sampling ($n = 10$, 0.3%), and whole blood selenium ($n = 18$, 0.6%). Missing values in covariates were imputed using multiple imputation by chained equations in STATA, and 20 imputed datasets were generated for analyses.

Associations between continuous exposures and outcomes were modelled flexibly by use of restricted cubic splines

(three knots). Covariates were included in the models based on previous knowledge and a directed acyclic graph (Supplementary Fig. S3).

Linear regression was used to model associations with continuous outcomes. To obtain close to normal distribution of the outcomes, we used the square root of TSH, and we excluded the highest percentile of FT4 and FT3. Logistic regressions were used to model the odds of antibody positivity. Adjusted models included the following covariates: maternal age, education, parity, pre-pregnancy BMI, fiber intake, smoking in pregnancy, plasma ferritin, whole blood selenium, and gestational age at sampling. Urinary creatinine was included in models with UIC ~ Cr as exposure, energy intake in models with iodine from food as exposure, and IVF in models with supplement use as exposure (supplement use was more commonly reported in IVF pregnancies).

Possible interaction effects were explored for iodine supplement use, dichotomous variables of whole blood selenium (<80 μg/L) and plasma ferritin (<20 μg/L), intake of cruciferous vegetables (>75th percentile), and smoking in pregnancy (yes/no). The potential interactions were explored by including an interaction term with iodine from food in the spline models.

p-Values are reported for overall associations between continuous exposures and outcomes by testing the coefficients of all spline transformations equal to zero. The tests for nonlinearity were performed by testing the coefficient of the second spline transformations equal to zero. All statistical tests were performed on the imputed datasets ($n=20$) and adjusted for random effects of person clusters since some women participated with more than one pregnancy ($n=9$). Results are reported including robust 95% confidence intervals. A p-value <0.05 was considered statistically significant. Sensitivity analyses included repeating analyses on thyroid hormones and TSH, but (1) excluding antibody positive women, or (2) including the upper first percentile for outcomes FT3 and FT4.

To explore potential effects of iodine status on population-specific reference ranges for TSH and thyroid hormones, we used quantile regression regressing the 2.5th and 97.5th percentile by categories of UIC, UIC ~ Cr, and by categories of iodine intake (restricted to nonusers of iodine supplements) in participants who were not TPOAb-positive (>4.11 IU/mL, cutoff suggested by manufacturer) and not IVF treated.

Results

Characteristics of the study population are shown in Table 1. A more detailed overview of population characteristics by exposure level is provided in Supplementary Table S1.

Iodine intake and UIC

Median habitual iodine intake from food based on the FFQ was 121 μg/day. Four percent had a calculated iodine intake from food reaching the WHO recommended intake of 250 μg/day for pregnant women, and 30% reaching the recommended intake of 150 μg/day for nonpregnant women. The low intake was reflected in a low median UIC in supplement nonusers (59 μg/L, $n=1738$). In this subgroup, iodine from food according to the FFQ correlated weakly with

Table 1. Descriptive Characteristics of the Study Population ($N=2910$ Pregnancies)

Study sample/pregnancies, *n*	2910
Maternal age at delivery (years), mean (SD)	30.3 (4.2)
Gestational age at sampling (weeks), mean (SD)	18.5 (1.3)
Prepregnancy BMI (kg/m^2), mean (SD)	23.9 (4.0)
Parity, %	
0	52
1	33
2 or more	14
Maternal education, %	
≤12 years	26
13–16 years	47
>16 years	25
Other/missing	2.1
Married/cohabitant, %	98.4
In vitro fertilization, %	2.2
Smoking in pregnancy, %	
Occasionally	14
Daily	3.8
Chronic illness, %	8.9
Household income, %	
Low	26
Medium	43
High	29
Missing	1.9
Iodine from food (μg/day), median (IQR)	121 (90, 160)
UIC (μg/L), median (IQR)	68 (35, 116)
Urinary creatinine (g/L), median (90% range)	0.76 (0.17, 1.92)
UIC (μg/g creatinine), median (IQR)	91 (61, 139)
UIC ~ Cr (μg/L), median (IQR)	74 (55, 105)
UIC, %	
≥150 μg/L (sufficient[a])	14
0–150 μg/L (insufficient[a])	86
0–100 μg/L	68
0–50 μg/L	37
Plasma FT4 [pmol/L], median [95% range]	12.6 [10.3, 15.7]
Plasma FT3 [pmol/L], median [95% range]	4.9 [4.0, 6.0]
Plasma TSH [mU/L], median [95% range]	1.2 [0.4, 2.9]
Plasma TPOAb positive,[b] %	8.1
Plasma TgAb positive,[b] %	7.9
Plasma ferritin (ng/mL), median (IQR)	33 (20, 56)
Empty iron stores (P-Fe <12 ng/mL), %	9.0
Low iron stores (P-Fe 12–29.9 ng/mL), %	35
Whole blood selenium (μg/L), median (IQR)	102 (89, 117)
Low selenium (<80 μg/L), %	10

[a]Population median UIC <150 μg/L is the recommended cutoff for defining inadequate iodine intake in pregnancy by the World Health Organization.

[b]Antibody positivity was defined as values above the 92nd percentile (>6.6 IU/mL for TPOAb and >7 IU/mL for TgAb).

IQR, interquartile range; SD, standard deviation; TgAb; thyroglobulin antibodies; TPOAb, thyroid peroxidase antibodies; UIC, urinary iodine concentration; UIC ~ Cr, UIC adjusted for creatinine.

spot UIC measured in μg/L (Spearman correlation $r = 0.20$, $p < 0.001$), with UIC measured in μg/g creatinine (Spearman $r = 0.30$, $p < 0.001$), and with UIC adjusted for creatinine by the residual method (UIC ~ Cr) (Spearman $r = 0.28$, $p < 0.001$). The association between iodine from food and UIC in nonusers of iodine supplements is shown in Figure 2. Habitual iodine intake explained 3.9% of the variation in UIC ($p < 0.001$) when regressing log iodine from food on log UIC.

The UIC differed according to reported use of iodine-containing supplements (Supplementary Fig. S4). In participants reporting supplement use at the time of sampling (GW 17–20, $n = 580$) median UIC was 98 μg/L, significantly higher than in those not using supplements (median 59 μg/L, $p < 0.001$). There was no data available on the dosage of current intake of iodine from supplements, but the most frequently reported supplements contained ~150 μg per recommended daily dose.

Iodine intake and thyroid function and autoimmunity

Habitual iodine intake from food calculated by the FFQ was not significantly associated with any of the outcome measures (Figs. 3 and 4 and Supplementary Fig. S5), but UIC was inversely associated with FT4 and FT3 (Fig. 3). UIC ~ Cr lower than roughly 100 μg/L was associated with an increase in mean FT3. Excluding thyroid antibody positive women (TPOAb and/or TgAb >92nd percentiles) did not change the results (Supplementary Fig. S6). Results from crude models are included in Supplementary Figures 7 and 8.

The results did not differ when taking into account iron status, selenium status, or smoking during pregnancy. Only smoking during pregnancy was associated with a lower FT4 and higher FT3 (both $p < 0.001$). Intake of cruciferous vegetables was generally low (median: 18 g/day, 90 percent range: 2–56 g/day) and was not associated with the thyroid function parameters.

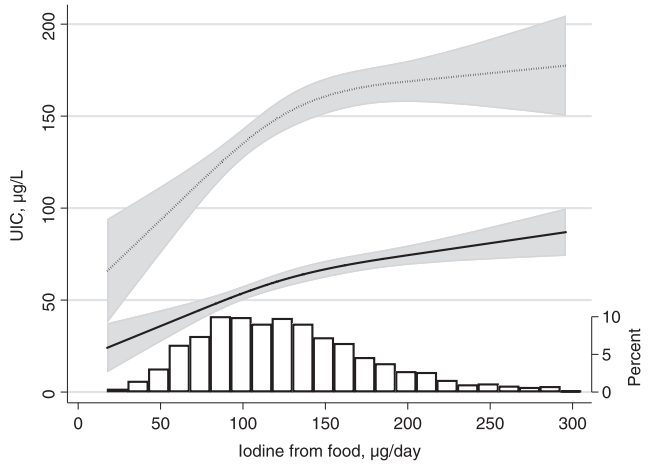

FIG. 2. Association between iodine from food (measured by the food frequency questionnaire covering gestational weeks 0–22) and spot UIC measured at mean gestational week 18.5 (SD 1.2) in nonusers of iodine supplements ($n = 1738$). The solid line represents the estimated median, the dotted line shows the 90th percentile, and shaded areas are the 95% confidence intervals. The predicted values were estimated by quantile regression adjusting for calculated energy intake. The histogram illustrates the distribution of iodine intake from food. UIC, urinary iodine concentration.

Population-specific reference ranges for TSH, FT4, and FT3

Table 2 shows the 2.5th and 97.5th percentiles for plasma TSH, FT4, and FT3 after excluding TPOAb-positive and IVF pregnancies. There was no evidence of any association between either iodine intake or UIC and reference ranges of plasma TSH, FT4 or FT3 (Table 3 and Supplementary Table S2). However, even the groups with the highest iodine intakes had inadequate iodine intake according to the WHO criteria (i.e., median UIC <150 μg/L). Additionally excluding remaining TgAb-positive women ($n = 147$) did not change the results (Table 2).

Based on the reference ranges determined in this subgroup (listed in Table 2), 3.2% of the total study population had subclinical hypothyroidism (high TSH and normal FT4), 0.1% had overt hypothyroidism (high TSH and low FT4), 2.3% had isolated hypothyroxinemia (low FT4 and normal TSH), 2.3% had subclinical hyperthyroidism (low TSH and normal FT4), and 0.1% had overt hyperthyroidism (low TSH and high FT4 or FT3). The prevalence of thyroid antibody positivity was 11.4% by our population-based cutoffs at >92nd percentile (i.e., TPOAb >6.6 IU/mL or TgAb >7 IU/mL).

Iodine from supplements and thyroid function

Forty percent reported use of iodine-containing supplements in gestational week 0–24, and 20% reported use at the time of sampling (GW 17–20). Current iodine supplement use (yes/no) was not significantly associated with the outcomes.

Information on timing of iodine supplement use was specified by 74% of the users of iodine-containing supplements in pregnancy. Of these, 40% ($n = 347$) reported first use in weeks 1–26 before conception, 37% ($n = 323$) in first trimester, and 22% ($n = 194$) in gestational weeks 13–20. Measures of thyroid parameters by timing of supplement use are shown in Figure 5 and Supplementary Figure S9. A recent initiation of supplement use (after GW 12) was associated with a lower mean FT4 (beta = –0.21, $p = 0.027$), and also lower FT3 and higher TSH, but not statistically significant (beta –0.05, $p = 0.17$ and beta 0.02, $p = 0.27$) compared with nonuse of supplements. There was also a tendency of a higher risk of hypothyroxinemia (i.e., plasma FT4 < 10.3 pmol/L, the population specific reference range) (odds ratio 2.09, $p = 0.089$). Results from crude models are included in Supplementary Figure S10.

Discussion

In this mildly to moderately iodine deficient pregnant population, we show that a higher UIC was associated with a slightly lowered FT4 concentration and that a low UIC, from a cutoff point of roughly 100 μg/L, was associated with an elevated FT3. Furthermore, we show that reference ranges for TSH, FT4 or FT3 did not differ by UIC or by calculated habitual iodine intake. A recent introduction of an iodine containing supplement was associated with a lower thyroid function, whereas more long-term use was not (i.e., use initiated before conception or early in the pregnancy).

Calculated iodine intake and UIC

Calculated habitual iodine intake from food in nonusers of supplements explained only 4% of the variation in UIC,

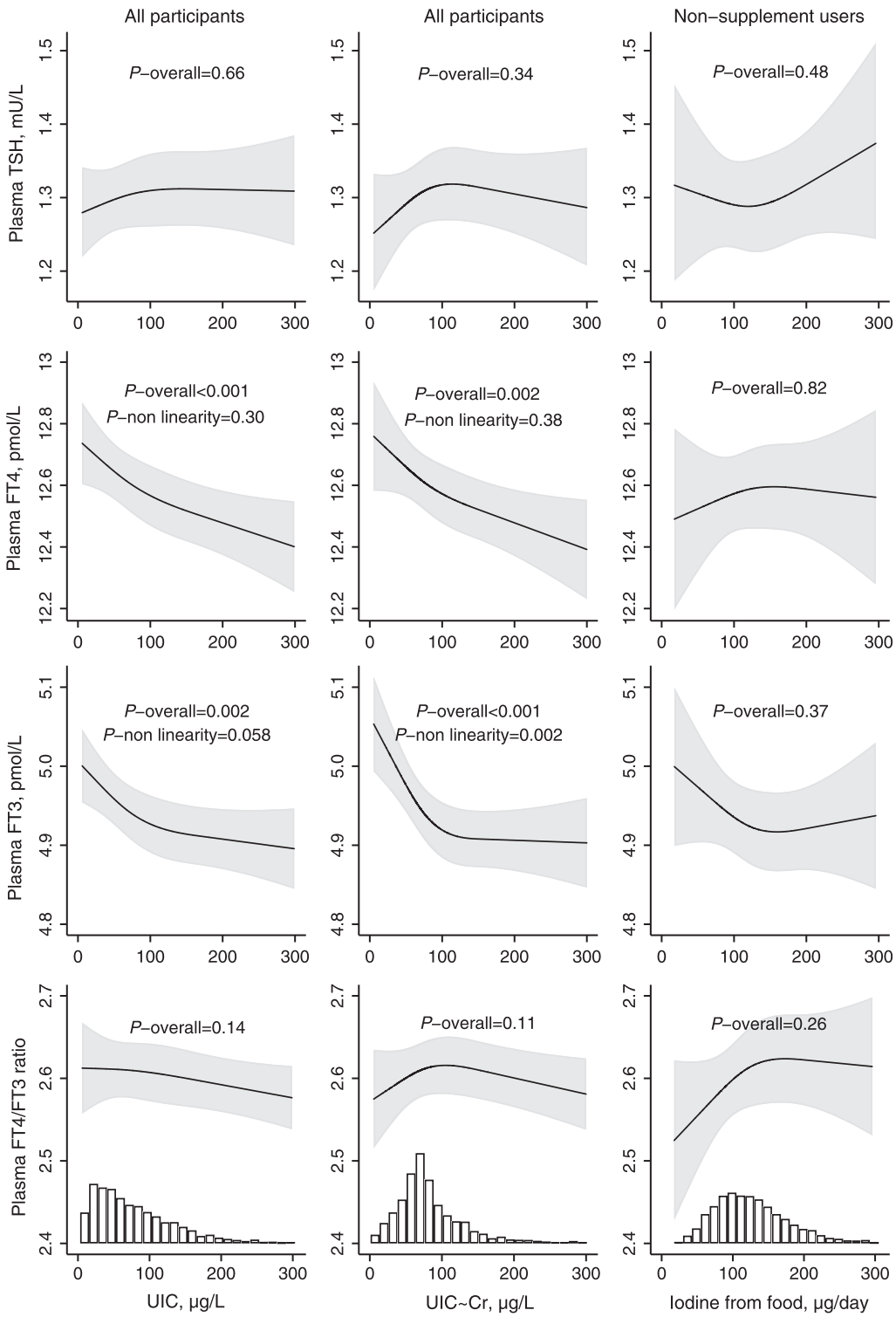

FIG. 3. Adjusted associations between UIC (column 1; $n = 2900$), creatinine-adjusted UIC (column 2; $n = 2900$), and iodine from food (column 3, restricted to nonusers of iodine supplement; $n = 1730$) and TSH, FT4, and FT3. For TSH, the geometric mean was modelled (by square root transformation), and for FT3 and FT4, the highest first percentile was excluded to obtain ~normal distribution. Including the upper first percentile of FT3 and FT4 did not change the results (Supplementary Fig. S5). FT3, free triiodothyronine.

which highlights the restricted value of a spot UIC as a measure of habitual iodine intake at an individual level. However, both the median UIC and the variance in UIC increased with calculated habitual iodine intake (Fig. 2), affirming that the MoBa FFQ provides a valid measure of the habitual iodine intake as reported previously (36). The increase in median UIC started to level off at about 150 μg/L (equivalent to the 70th percentile), indicating overreporting of food intake at higher levels. Median UIC remained below 100 μg/L over the whole range of calculated iodine intakes by the FFQ, thus the whole

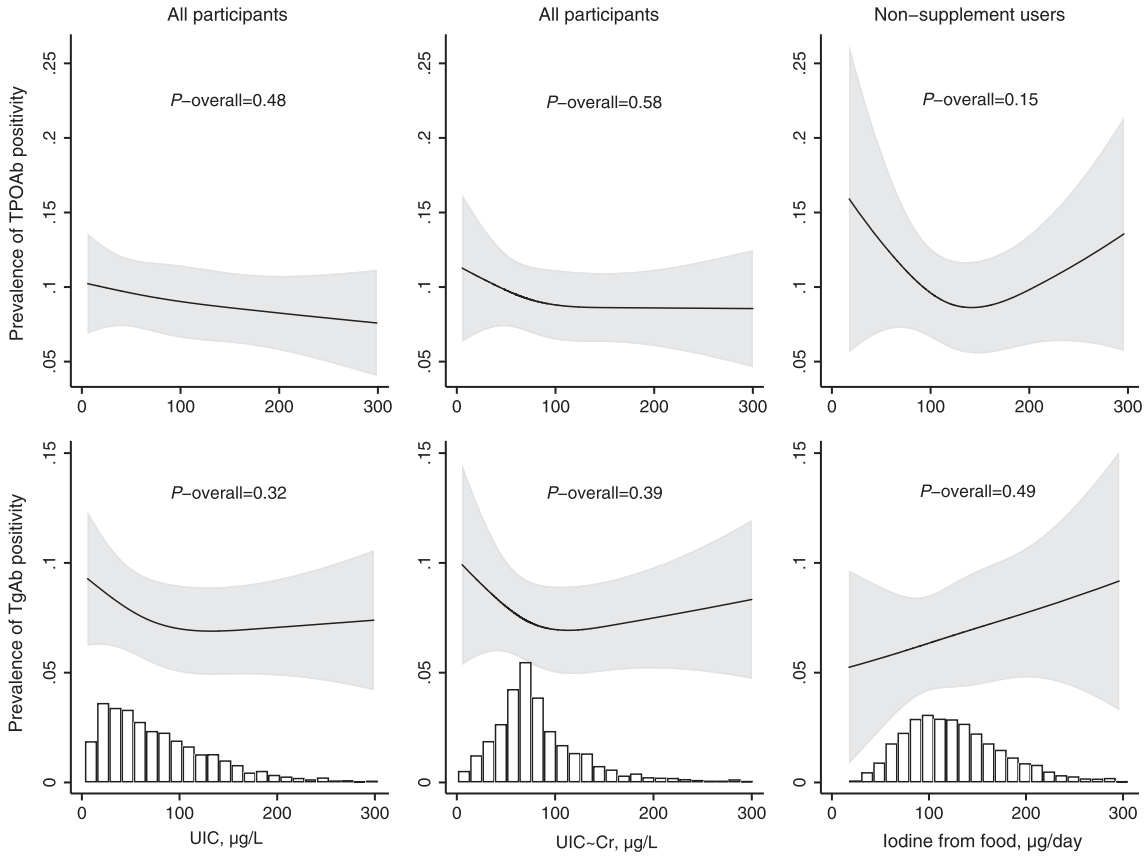

FIG. 4. Prevalence of thyroid antibody positivity by UIC (column 1; $n = 2900$), creatinine-adjusted UIC (column 2; $n = 2900$), and by iodine from food (column 2, restricted to nonusers of iodine supplements; $n = 1730$), adjusted models. Antibody positivity was defined as antibody plasma concentration >92nd percentile.

group of supplement nonusers in this study had an insufficient habitual iodine intake in pregnancy (i.e., population median UIC <150 μg/L). Even the women reporting current use of iodine-containing supplements had median UIC <100 μg/L. Explanations for the low UIC in iodine supplement users might be that the supplements were not consumed daily by all users, or that supplemental iodine was trapped in the thyroid to refill depleted iodine stores. The latter was indicated in a recent study in Bangladesh in pregnant women with comparable median UIC as in our supplement nonusers (42). Introducing a supplement containing 250 μg iodine/day did not result in an increase in UIC.

Iodine intake and thyroid function

In ID, a range of effective autoregulatory mechanisms are triggered in the thyroid and other tissues to maintain euthyroidism. This involves a preferential secretion of T3 over T4, whereas an elevated TSH is rarely seen in populations with mild to moderate ID (43). In our study, we did observe a higher FT3 when habitual iodine intake from food was below $\sim$150 μg/day (reflected in a UIC below $\sim$100 μg/L), but the trends in the FT4/FT3 ratio did not reach statistical significance.

Our findings indicate that short-term iodine availability (measured by UIC) might have a partially different impact on thyroid hormones compared to long-term iodine intake (calculated by the FFQ). For FT3, our findings, both for UIC and for habitual iodine intake from food, indicate that a low iodine intake (below $\sim$150 μg/day) is associated with a higher plasma FT3 (Fig. 3). This is in line with observations in both animal and human studies and reflects the autoregulatory mechanisms to preserve iodine in response to decreased availability of iodine (44). A preferential production of T3 to T4 saves one iodine atom per hormone produced, and at the same time secures availability of most maternal tissues to T3, the active form of thyroid hormone. However, the fetal brain may be vulnerable to lower maternal T4 since T3 in the fetal brain is predominantly generated locally from maternal T4 during early pregnancy (44). Several studies show that maternal hypothyroxinemia may result in suboptimal fetal brain development, and that this may particularly be the case before the onset of fetal thyroid hormone production at mid pregnancy (45,46).

We observed that UIC was inversely associated with FT4, which is opposite to what one might expect, and also opposite to what was indicated for iodine intake by the FFQ (Fig. 3). We hypothesize that this finding might reflect that thyroid hormone production is inhibited during more acute higher iodine availability during pregnancy via a mechanism similar to that of the overt Wolff-Chaikoff effect. Interestingly, these associations were already present within the range of what is considered optimal and safe iodine intake. These results could indicate that pregnant women with mild to moderate ID who increase their iodine intake during pregnancy are more prone to having a (temporary) low FT4. This is supported by

Table 2. The 2.5th and 97.5th Percentiles (Reference Range) of TSH, FT4, and FT3 in TPOAb Negative Pregnant Women

	Reference population[a]	*Also excluding TgAb-positive*[b]
Plasma TSH, mU/L		
n	2577	2430
2.5th percentile [95% CI]	0.39 [0.36, 0.44]	0.41 [0.36, 0.45]
Median [95% CI]	1.19 [1.17, 1.22]	1.19 [1.16, 1.22]
97.5th percentile [95% CI]	2.70 [2.62, 2.80]	2.70 [2.60, 2.79]
Plasma FT4, pmol/L		
n	2576	2429
2.5th percentile [95% CI]	10.3 [10.2, 10.4]	10.3 [10.2, 10.4]
Median [95% CI]	12.6 [12.5, 12.6]	12.5 [12.5, 12.6]
97.5th percentile [95% CI]	15.6 [15.3, 16.0]	15.6 [15.3, 16.0]
Plasma FT3, pmol/L		
n	2577	2430
2.5th percentile [95% CI]	4.00 [3.97, 4.03]	4.00 [3.97, 4.03]
Median [95% CI]	4.90 [4.87, 4.92]	4.90 [4.87, 4.93]
97.5th percentile [95% CI]	6.00 [5.91, 6.10]	6.00 [5.91, 6.10]

Mean gestational week, 18.5; SD, 1.3.
[a]Population characteristics: singleton pregnancies, not TPOAb positive (i.e., TPOAb <4.11 IU/mL), not current user of thyroid medication or thyroid disrupting medication, and not *in vitro* fertilization.
[b]Additionally excluding TgAb positive (>4.11 IU/mL according to manufacturer cutoff), $n = 147$.
FT3, free triiodothyronine; FT4, free thyroxine; TSH, thyrotropin.

our finding that a recent introduction of iodine-containing supplements (GW 13 or later) was associated with lower FT4, whereas longer term use, was not (Fig. 5). Furthermore, a similar effect was indicated in two longitudinal non-randomized studies exploring the impact of iodine supplement initiated early in pregnancy on thyroid function in mild to moderately ID populations by Moleti *et al.* (median UIC $\sim 60\ \mu$g/L in nonsupplemented women) (21,47). Also, Rebagliato *et al.* reported increased risk of elevated TSH in pregnant women taking supplements containing 200 μg iodine per day or more compared to 0–100 μg/day (22). Contrary to this, no difference was seen in a recent RCT of iodine supplementation in pregnancy by Gowachirapant *et al.* in a mildly ID population (median UIC 131 μg/L at baseline) (23). Interestingly, using data from MoBa, we have previously reported that maternal iodine supplement use initiated in the first trimester was associated with more behavior problems in 3 year old children (3) and increased risk of ADHD diagnosis in 6–14 year old children (4). These associations could be explained by a temporary lower thyroid hormone production due to an acute higher iodine availability in this vulnerable window of neurodevelopment. Taken together, these results indicate that mild- to moderate ID should optimally be prevented *before* pregnancy since initiating supplement use in pregnancy might be too late and may have adverse effects on thyroid function.

Several nutrients and nonnutrients are known to affect thyroid function (8,48). We were not able to detect effect modification on the association of iodine intake with thyroid function by either low iron or selenium status, smoking, or intake of cruciferous vegetables. Iron is essential for the activity of thyroid peroxidase in the production of thyroid hormones. Selenium is an integral part of selenoenzymes protecting the thyroid from excess H_2O_2 produced in thyroid hormone synthesis. Selenium is also incorporated in deiodinase enzymes crucial for regulation of thyroid hormone activity and thus action in target-tissues. Cruciferous vegetables and smoking both contribute with goitrogenic compounds inhibiting iodine uptake in the thyroid. In our study population, the prevalence of smoking, selenium deficiency, and the intake of cruciferous vegetables were all low limiting the ability to explore potential effect modifications. Therefore, we cannot conclude that such effects were not present as this study was probably underpowered to detect such interactions. Some studies have indicated that ID populations might be more vulnerable to thyroid disrupting substances (48), and that concurrent iron and/or selenium deficiency might have synergistic effects with ID on thyroid function (49).

Strengths and limitations

The present study has several limitations. Most importantly, the participants in this study had an insufficient iodine intake regardless of their reported food and supplement intake (i.e., median UIC $\geq 150\mu$g/L, see Fig. 2 and Table 1). Thus, we might lack an iodine sufficient group for comparison. However, the cutoff of 150 μg/L for defining inadequacy may be too high. In the Nordic countries, the recommended iodine intake for pregnant women of 175 μg/d is only 60% the WHO recommendation of 250 μg/d (14), corresponding to a median UIC $\geq 105\ \mu$g/L. Nevertheless, median UIC was below 100 μg/L even in the participants with the highest reported iodine intake from food (Fig. 2) as well as in current supplement users. Participants who reported use of iodine-containing supplements all time periods from 6 months before pregnancy probably had adequate iodine status, but they were few ($n = 138$).

The cross-sectional design is a limitation preventing us to study potential intra-individual changes in thyroid function parameters by short-term iodine availability. In addition, the observational design means that residual confounding cannot be ruled out. This study included a selected sample of highly motivated mothers with offspring with low prevalence of language delay and no autism (Fig. 1). Increased risk of

Table 3. The 2.5th and 97.5th Percentiles of TSH, FT4, and FT3 by Measures of Iodine Status (Adjusted Models)

	UIC, μg/L					
	<30	*30–59.9*	*60–99.9*	*100–150*	*>150*	p-*Value*[a]
n	507	625	629	444	372	
UIC, median (IQR), μg/L	20 (14, 25)	44 (37, 51)	78 (69, 88)	121 (110, 135)	195 (167, 260)	
Plasma TSH, mU/L						
2.5th percentile [95% CI]	0.48 [0.39, 0.58]	0.40 [0.32, 0.49]	0.37 [0.29, 0.45]	0.39 [0.29, 0.49]	0.36 [0.25, 0.46]	0.36
97.5th percentile [95% CI]	2.54 [2.32, 2.76]	2.57 [2.36, 2.77]	2.82 [2.61, 3.02]	2.76 [2.52, 3.00]	2.87 [2.60, 3.13]	0.16
Plasma FT4, pmol/L						
2.5th percentile [95% CI]	10.4 [10.2, 10.6]	10.6 [10.4, 10.7]	10.4 [10.2, 10.6]	10.4 [10.2, 10.7]	10.2 [10.0, 10.5]	0.29
97.5th percentile [95% CI]	15.9 [14.9, 16.9]	15.6 [14.7, 16.5]	16.2 [15.3, 17.1]	15.2 [14.2, 16.3]	15.2 [14.0, 16.4]	0.58
Plasma FT3, pmol/L						
2.5th percentile [95% CI]	4.14 [4.04, 4.24]	4.08 [3.98, 4.17]	4.07 [3.98, 4.16]	4.05 [3.94, 4.15]	4.02 [3.90, 4.15]	0.62
97.5th percentile [95% CI]	6.08 [5.89, 6.26]	5.90 [5.73, 6.06]	5.92 [5.75, 6.10]	5.97 [5.77, 6.17]	5.77 [5.55, 5.98]	0.30
	Creatinine-adjusted UIC (UIC ~ Cr), μg/L					
	<45	*45–69.9*	*70–89.9*	*90–130*	*>130*	p-*Value*[a]
n	428	700	580	459	410	
UIC ~ Cr, median (IQR), μg/L	30 (20, 38)	60 (53, 65)	78 (73, 83)	105 (97, 116)	172 (143, 227)	
Plasma TSH, mU/L						
2.5th percentile [95% CI]	0.47 [0.36, 0.58]	0.40 [0.32, 0.48]	0.38 [0.29, 0.47]	0.39 [0.29, 0.49]	0.41 [0.30, 0.51]	0.80
97.5th percentile [95% CI]	2.56 [2.31, 2.82]	2.62 [2.42, 2.81]	2.77 [2.56, 2.99]	2.78 [2.54, 3.02]	2.78 [2.53, 3.04]	0.56
Plasma FT4, pmol/L						
2.5th percentile [95% CI]	10.6 [10.4, 10.8]	10.3 [10.2, 10.5]	10.5 [10.3, 10.6]	10.4 [10.2, 10.6]	10.3 [10.1, 10.5]	0.36
97.5th percentile [95% CI]	16.0 [14.8, 17.2]	15.5 [14.6, 16.4]	16.2 [15.3, 17.2]	15.6 [14.6, 16.7]	15.3 [14.2, 16.5]	0.72
Plasma FT3, pmol/L						
2.5th percentile [95% CI]	4.14 [4.01, 4.28]	4.09 [3.99, 4.20]	4.06 [3.94, 4.17]	4.08 [3.95, 4.21]	4.02 [3.89, 4.16]	0.77
97.5th percentile [95% CI]	6.05 [5.80, 6.29]	5.95 [5.77, 6.13]	5.97 [5.77, 6.17]	5.84 [5.62, 6.06]	5.82 [5.58, 6.05]	0.61
	Iodine from food, μg/day (restricted to supplement nonusers)					
	<100	*100–150*	*>150*			p-*Value*[a]
n	523	562	457			
Iodine intake, median (IQR), μg/day	77 (62, 89)	123 (111, 135)	183 (163, 217)			
Plasma TSH, mU/L						
2.5th percentile [95% CI]	0.49 [0.40, 0.59]	0.41 [0.32, 0.49]	0.43 [0.33, 0.53]			0.20
97.5th percentile [95% CI]	2.70 [2.35, 3.05]	2.78 [2.46, 3.09]	2.61 [2.24, 2.99]			0.74
Plasma FT4, pmol/L						
2.5th percentile [95% CI]	10.3 [10.1, 10.6]	10.5 [10.3, 10.7]	10.5 [10.2, 10.7]			0.47
97.5th percentile [95% CI]	16.1 [15.1, 17.0]	15.4 [14.6, 16.3]	15.4 [14.3, 16.4]			0.35
Plasma FT3, pmol/L						
2.5th percentile [95% CI]	4.06 [3.93, 4.19]	4.07 [3.95, 4.19]	4.08 [3.94, 4.22]			0.94
97.5th percentile [95% CI]	6.03 [5.82, 6.24]	5.88 [5.71, 6,06]	5.90 [5.70, 6.11]			0.30

Data from adjusted models: patients with TPOAb positive (TPOAb >4.11 IU/mL) and current users of thyroid medication or thyroid disrupting medication, and women who have undergone *in vitro* fertilization were excluded.

Values are from quantile regression and adjusted for maternal age, prepregnancy BMI, gestational age at sampling, urinary creatinine (when UIC ~ Cr is the exposure), and energy intake (when iodine from food is the exposure). Results from crude models are reported in Supplementary Table S2.

[a]*p*-Value from quantile regression.

language delay was previously documented in children of mothers with low iodine intake in MoBa (3), thus potentially, the prevalence of mothers with altered thyroid function due to ID might be lower in our study sample. Substantial measurement error of the exposure variables in measuring actual iodine intake probably also contributed to an attenuation of the associations. Although the total number of participants was large, there was limited power to study potential effect modifications by iron status, selenium status, smoking, and goitrogens in cruciferous vegetables.

Strengths of the study include the relatively large number of participants, the substantial variation in iodine intake, having two different measures of iodine intake (i.e., one for habitual intake and one for short-term intake) and the extensive data collection allowing us to adjust for many confounding factors.

Clinical relevance and implications

Overall, the findings in this study indicate that a habitual iodine intake below approximately 150 μg/day is associated with changes in maternal thyroid hormone concentrations in pregnancy but not with significant differences in the reference range limits. Many studies have demonstrated that the

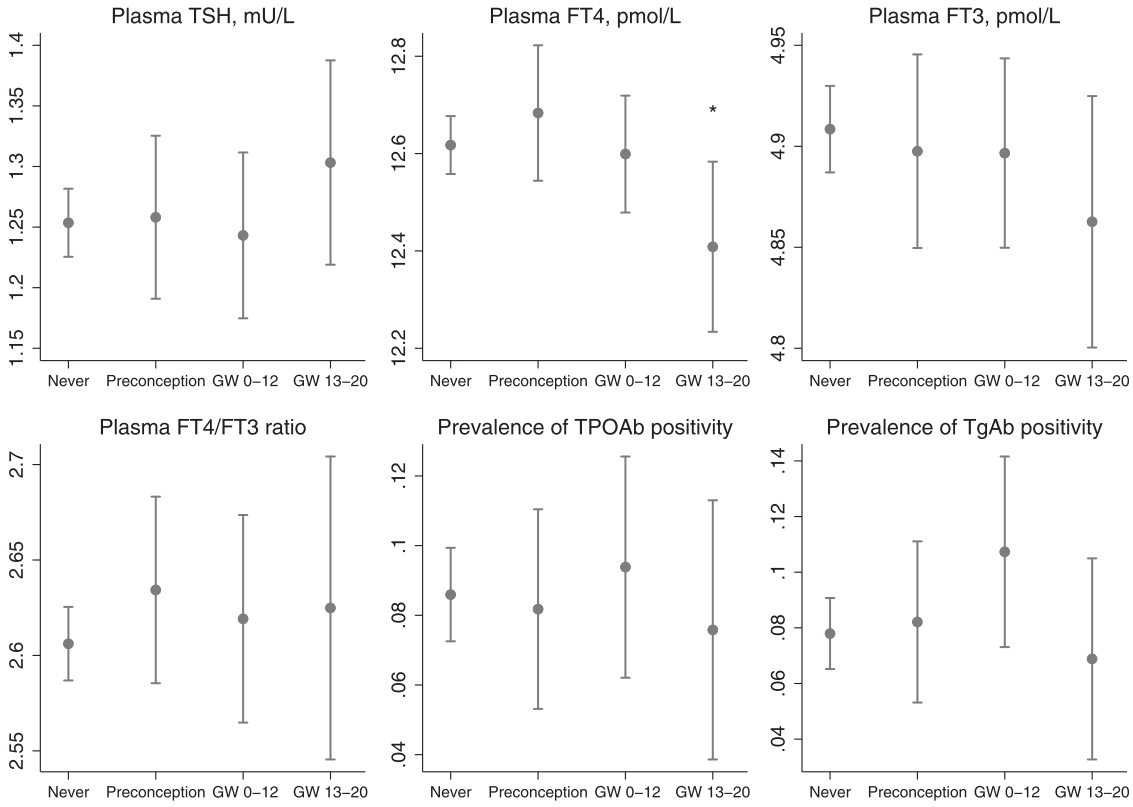

FIG. 5. Timing of initiation of iodine-containing supplements in pregnancy and measures of maternal thyroid function, adjusted models. Timing was categorized as: no reported supplement use (never; $n = 1738$), first use in weeks 1–26 before conception (preconception; $n = 347$), first use during first trimester (GW 0–12; $n = 323$), and first use during second trimester (GW 13–20; $n = 194$). Participants who reported use of iodine-containing supplements in the FFQ but did not report timing of use ($n = 308$) were not included in this analysis. For TSH, the geometric mean was modelled (by square root transformation), and for FT3 and FT4 the highest first percentile was excluded to obtain $\sim$normal distribution. Crude models are included in Supplementary Fig. S10, and models excluding antibody positive women in Supplementary Fig. S9. *$p < 0.05$. GW, gestational week.

developing fetus might be vulnerable to maternal thyroid hormone supply, particularly in the first half of pregnancy (45). Only 30% of the MoBa women reached an iodine intake from food sources of 150 μg/day, which is equivalent to the recommended intake for nonpregnant women.

Current international guidelines recommend that gestational thyroid function reference ranges are calculated in an iodine-sufficient population. To our knowledge, there are no studies that have shown that thyroid function reference ranges during pregnancy differ according to iodine status. Our results indicate that mild iodine deficiency is not a determinant of thyroid function reference ranges during pregnancy and that valid thyroid function reference ranges can be ascertained also in populations classified as being mildly to moderately iodine deficient. However, this finding needs to be confirmed in other studies before changing the guidelines.

We also found that initiating iodine supplement use in pregnancy in a population with mild to moderate ID diets was associated with lower FT4, which may be harmful for the developing child. Thus, this study provides further supporting evidence to the recommendation by the WHO that strategies to prevent iodine deficiency should target the *whole* population, and in particular all women of childbearing age, so that an adequate iodine status is secured well before conception. Today, there is a lack of evidence to support recommending iodine supplements for pregnant women in mild to moderately iodine deficient populations (5,20).

Conclusion

All in all, the results indicate that mild to moderate iodine deficiency is associated with thyroid hormone levels in pregnancy. However, the changes were small, suggesting that normal reference ranges can be determined based on data also from mildly iodine deficient populations, but this must be confirmed in other studies. Introducing an iodine-containing supplement might temporarily inhibit thyroid hormone production and/or release, thus ID should ideally be prevented *before* conception.

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Author Disclosure Statement

M.H. Abel is employed by a Norwegian dairy company (TINE SA), and she participates in this project as an industrial PhD student financed partly by the dairy company and partly by The Research Council of Norway. This project is designed, owned, and administered by The Norwegian Institute of Public Health and analysis of the data follow from protocol. All results of analyses in the project are to be published regardless of the results. The dairy company supports the study to raise awareness on the importance of iodine and to gain more knowledge about the potential health effects of milk in the Norwegian diet. Apart from M.H.A., no one from the dairy company has been involved in the study, and in itself, the company had no direct influence on the analysis and interpretation of the results. All remaining authors declare no competing financial interests.

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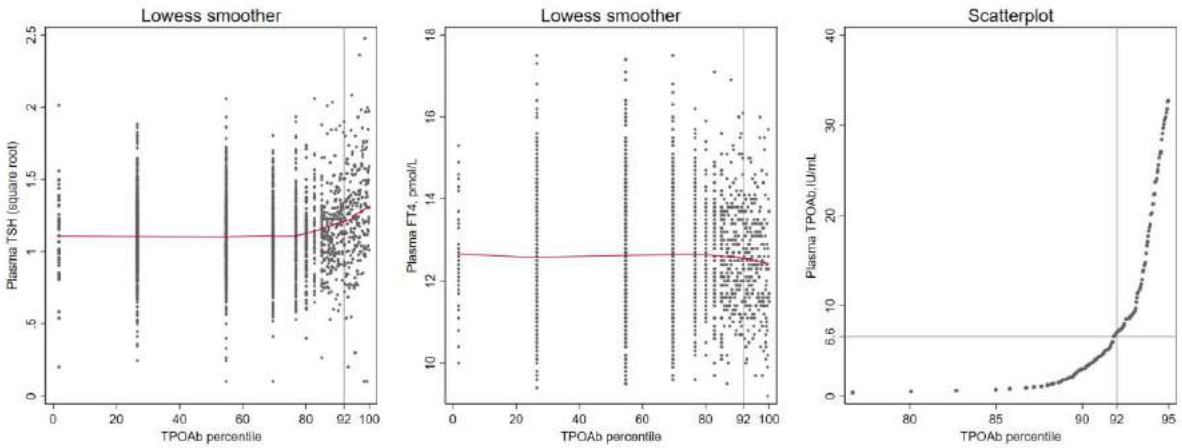
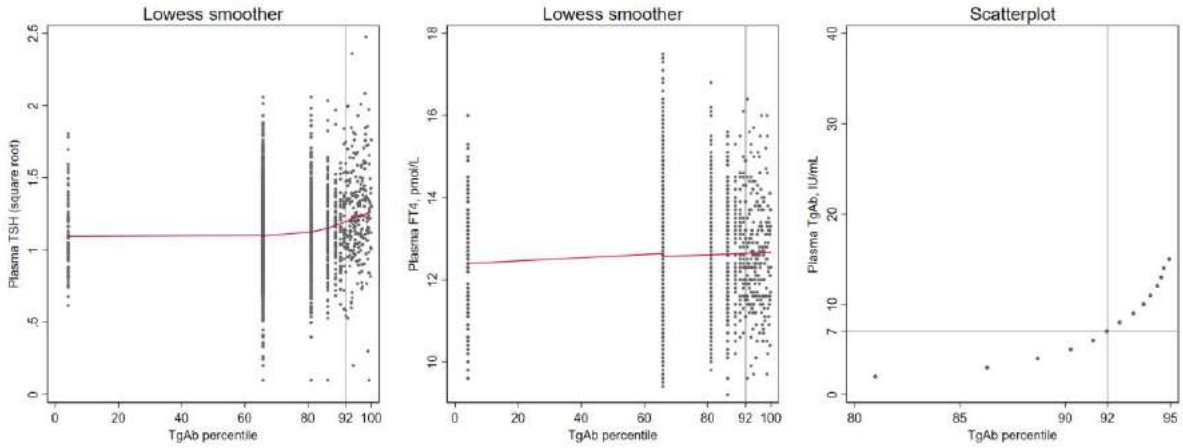
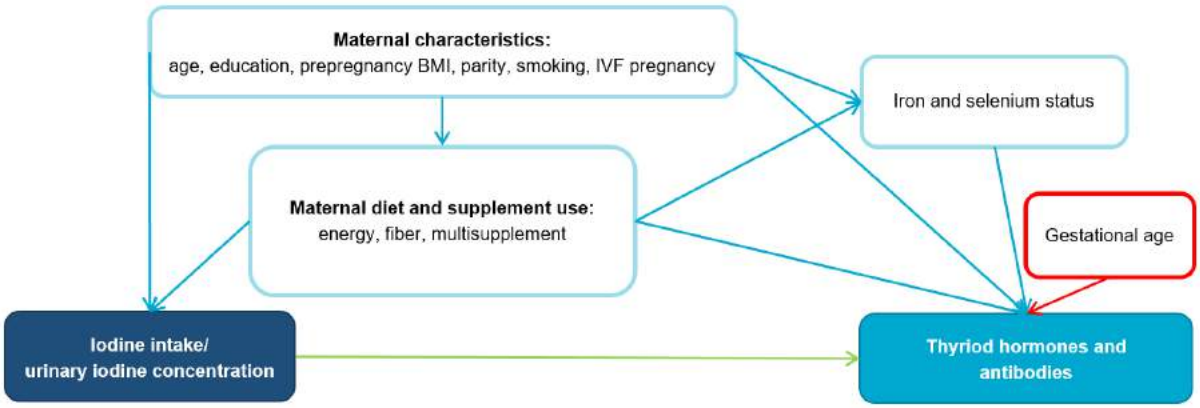


Supplemental Figure 1 Association between plasma TPOAb and geometric mean of TSH (left graph) and FT4 (middle graph) (n=2900). TSH was square root transformed and the upper 1 percentile of FT4 were excluded to obtain close to normal distribution. At the 92nd percentile of TPOAb there was a clear increase in mean square root TSH (p-overall <0.001 incl. adjustment for gestational age at sampling, maternal age, bmi, parity and smoking), but not with FT4 (adj. p-overall=0.19). This cutoff was used to define TPOAb positivity and corresponded to a plasma concentration of TPOAb equal to 6.6 IU/ml (right graph). The lowess smoothers applied in the graphs had a bandwidth of 0.8.



Supplemental Figure 2 Association between plasma TgAb and geometric mean of TSH (left graph) and FT4 (middle graph) (n=2900). TSH was square root transformed and the upper 1 percentile of FT4 were excluded to obtain close to normal distribution. At the 92nd percentile of TgAb there was a clear increase in mean square root TSH (p-overall <0.001 incl. adjustment for gestational age at sampling, maternal age, bmi, parity and smoking), but not with FT4 (adj. p-overall=0.70). This cutoff was used to define TgAb positivity and corresponded to a plasma concentration of TgAb equal to 7.0 IU/ml (right graph). The lowess smoothers applied in the graphs had a bandwidth of 0.8.

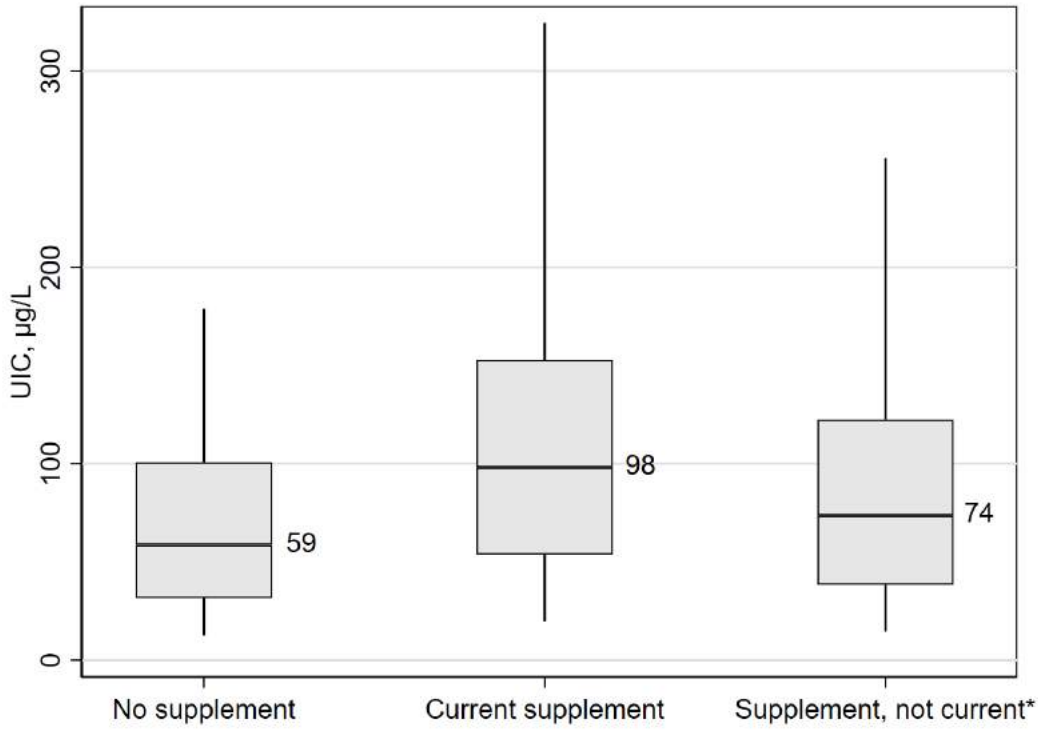


Supplemental Figure 3 Conceptual model (simplified directed acyclic diagram (DAG)) The association between iodine intake/urinary iodine concentration and thyroid hormones and antibodies. Gestational age at sampling is a determinant of the outcome (marked in red).

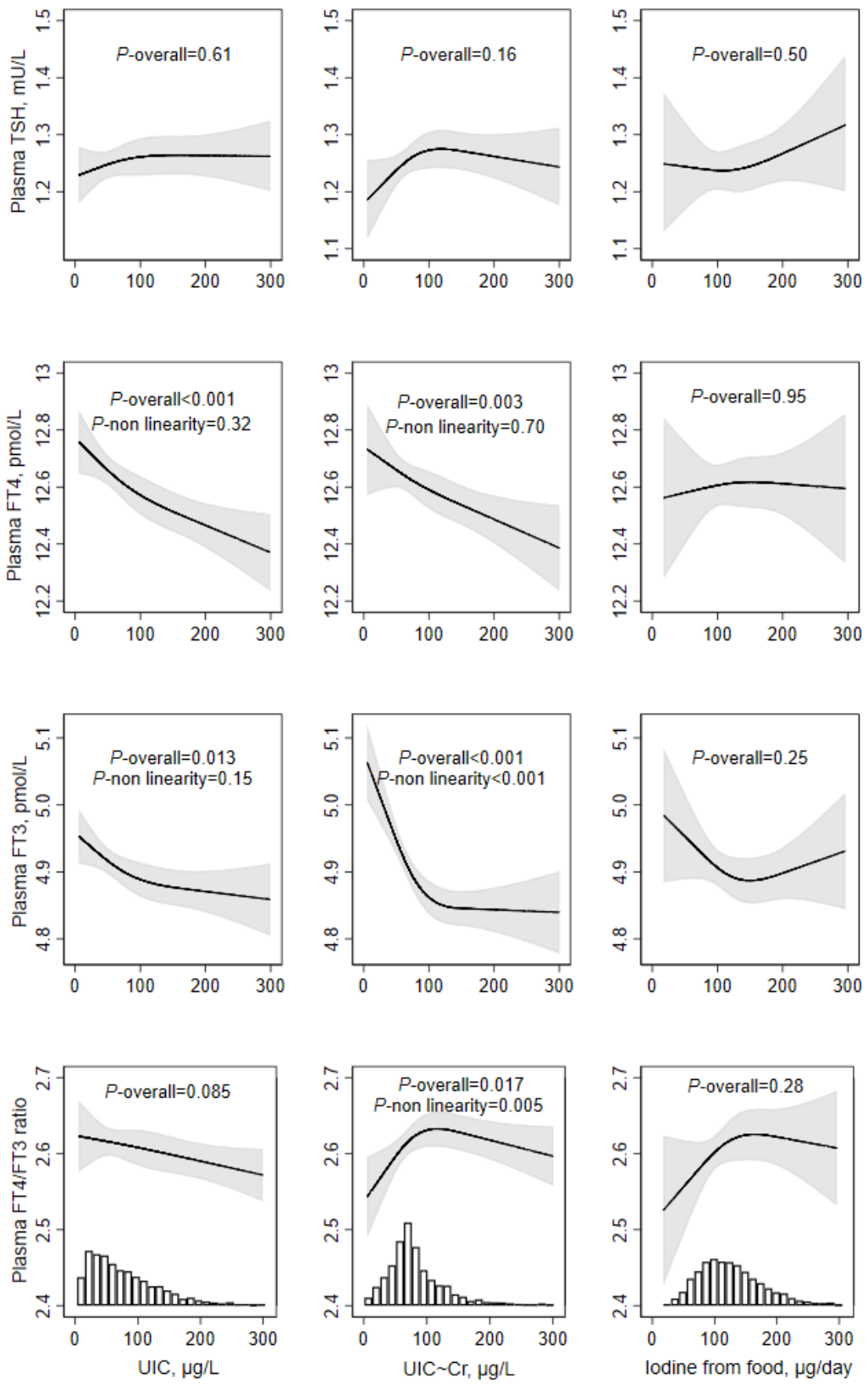
Supplemental Table 1 Descriptive characteristics of the study population (n=2910 pregnancies)

	Iodine from food, non-supplement users			Iodine supplement use in pregnancy (week 0-24)			All	MoBa[1]
	<100 µg/day	100-150 µg/day	>150 µg/day	No	Yes	Current use[2]		
Study sample/pregnancies, n (%)	584 (20)	625 (21)	529 (18)	1738 (60)	1172 (40)	580 (20)	2910 (100)	101,784
Maternal age at delivery, mean (SD), years	30.1 (4.3)	30.7 (4.1)	30.4 (4.4)	30.4 (4.2)	30.1 (4.2)	30.2 (4.1)	30,3 (4.2)	30.2 (4.6)
Gestational age at sampling, mean (SD), weeks	18.5 (1.3)	18.5 (1.1)	18.5 (1.3)	18.5 (1.2)	18.5 (1.4)	18.6 (1.6)	18.5 (1.3)	-
Pre-pregnancy BMI, mean (SD), kg/m^2	24.0 (3.9)	24.1 (4.3)	23.7 (3.7)	23.9 (4.0)	23.9 (3.9)	24.0 (4.0)	23.9 (4.0)	24.0 (4.3)
Parity, %								
0	53	47	43	48	60	63	52	47
1	34	36	39	36	29	26	33	36
2 or more	13	17	19	16	11	11	14	18
Maternal education, %								
≤12 y	27	23	30	26	25	24	26	32
13-16 y	46	47	46	47	47	46	47	41
>16 y	24	28	21	25	26	27	25	25
Other/missing	2.9	1.8	3.0	2.5	1.5	2.1	2.1	2.1
Married/cohabitant, %	98.1	98.7	97.0	98.0	99.0	99.3	98.4	96.1
In vitro fertilization, %	0.9	1.0	3.0	1.6	3.1	3.5	2.2	2.6
Smoking in pregnancy, %								
Occasionally	13	15	14	14	13	13	14	17
Daily	3.3	3.5	4.2	3.6	3.9	3.1	3.8	5.7
Chronic illness, %	11.5	6.9	6.0	8.2	10.0	10.9	8.9	10.2
Household income, %								
Low	28	23	28	26	26	24	26	29
Medium	42	44	45	43	43	44	43	41
High	29	32	24	28	30	30	29	28
Missing	1.7	1.9	2.8	2.1	1.6	1.7	1.9	3.1
Iodine from food, median (IQR), µg/day	77 (62, 88)	123 (111, 135)	183 (163, 216)	121 (88, 160)	121 (91, 160)	124 (91, 162)	121 (90, 160)	121 (89, 161)[3]
UIC, median (IQR), µg/L	48 (26, 85)	60 (31, 101)	72 (41, 115)	59 (32, 101)	85 (44, 139)	98 (54, 153)	68 (35, 116)	-
Urinary creatinine, median (90% range) g/L	0.79 (0.18, 2.10)	0.76 (0.16, 1.81)	0.78 (0.19, 1.95)	0.77 (0.17, 1.93)	0.74 (0.17, 1.92)	0.71 (0.17, 1.91)	0.76 (0.17, 1.92)	-
UIC, median (IQR), µg/g creatinine	66 (43, 97)	81 (57, 115)	97 (69, 132)	80 (54, 115)	113 (75, 184)	140 (88, 223)	91 (61, 139)	-
UIC~Cr, median (IQR), µg/L	59 (39, 76)	70 (51, 88)	76 (61, 102)	68 (49, 89)	88 (65, 131)	104 (72, 149)	74 (55, 105)	-
UIC, %								-
≥150 µg/L (sufficient[4])	7	10	12	10	21	26	14	-
0-150 µg/L (insufficient[4])	93	90	88	90	79	74	86	-
0-100 µg/L	82	74	68	75	58	51	68	-
0-50 µg/L	52	42	34	43	28	23	37	-
Plasma FT4, median (95% range), pmol/L	12.6 (10.3, 16.0)	12.6 (10.3, 15.4)	12.6 (10.1, 15.7)	12.6 (10.2, 15.4)	12.5 (10.3, 16.0)	12.5 (10.1, 16.2)	12.6 (10.3, 15.7)	-
Plasma FT3, median (95% range), pmol/L	4.9 (4.0, 6.1)	4.9 (4.0, 5.9)	4.9 (4.1, 6.0)	4.9 (4.0, 6.0)	4.9 (4.0, 6.1)	4.9 (4.0, 6.2)	4.9 (4.0, 6.0)	-
Plasma TSH, median (95% range), mU/L	1.2 (0.4, 3.0)	1.2 (0.4, 2.9)	1.2 (0.4, 2.7)	1.2 (0.4, 2.8)	1.2 (0.4, 2.9)	1.2 (0.4, 2.7)	1.2 (0.4, 2.9)	-
Plasma TPOAb positive[5], %	8.9	7.4	9.3	8.5	7.6	8.8	8.1	-
Plasma TgAb positive[5], %	7.0	7.7	9.0	7.9	8.0	8.5	7.9	-
Plasma ferritin, median (IQR), ng/mL	34 (19, 54)	33 (20, 58)	29 (19, 50)	32 (19, 54)	34 (22, 58)	35 (23, 60)	33 (20, 56)	-
Empty iron stores (P-Fe<12 ng/mL), %	11.5	9.8	11.0	10.7	6.4	5.7	9.0	-
Low iron stores (P-Fe 12-29.9 ng/mL), %	33	35	40	36	34	34	35	-
Plasma selenium, median (IQR), µg/L	99 (86, 114)	102 (88, 115)	100 (87, 114)	100 (87, 114)	106 (93, 121)	106 (94, 121)	102 (89, 117)	-
Low plasma selenium (<80 µg/L), %	14.7	9.0	14.4	12.6	6.4	4.9	10.1	-

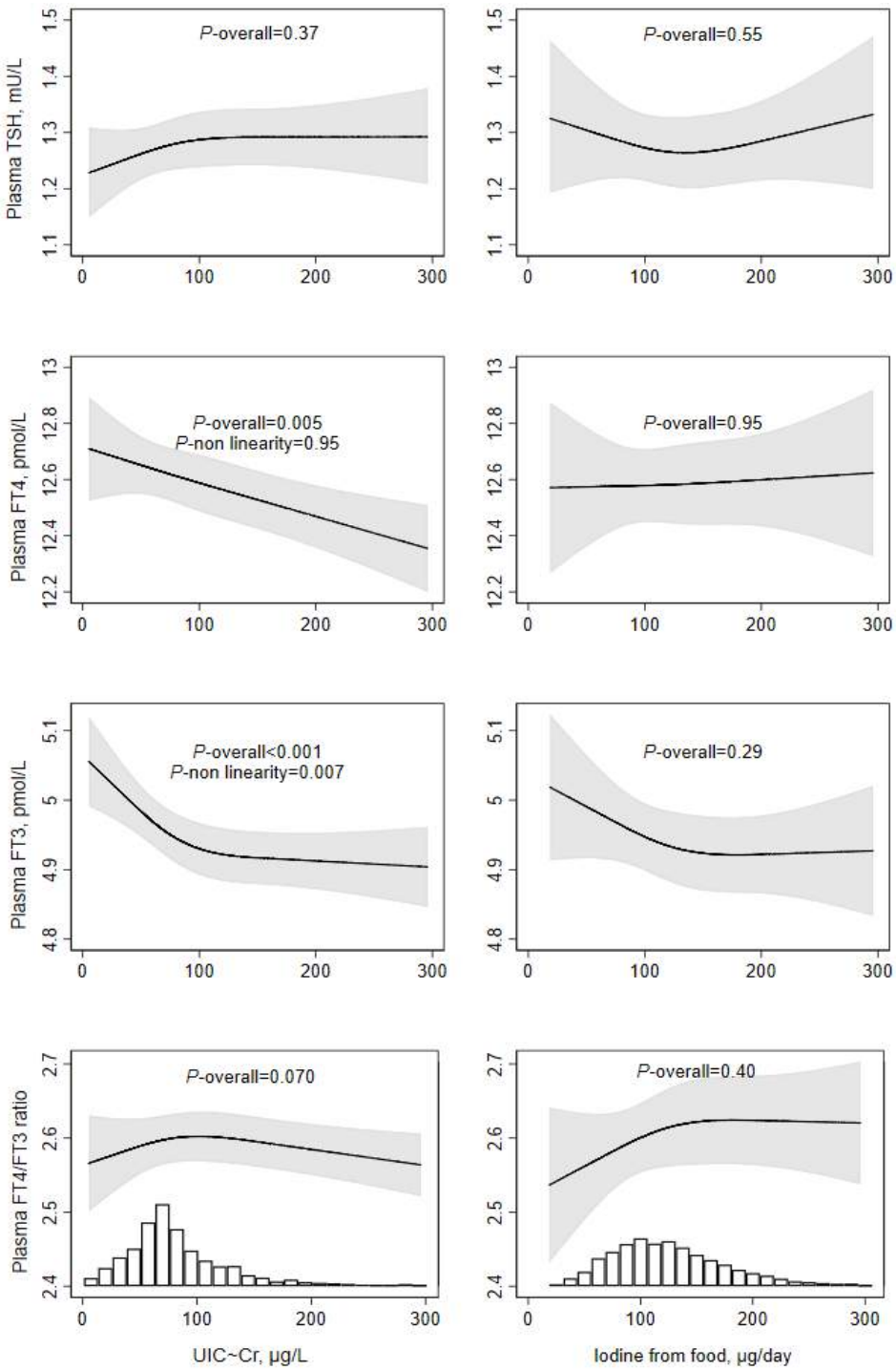
[1] Includes all pregnancies in the Norwegian Mother and Child Cohort Study with information on background characteristics from questionnaire 1.[2] Reported use of iodine-containing supplement in gestational week 17-20. [3] In singleton pregnancies with data from the food frequency questionnaire (n=83,721). [4] Population median UIC<150µg/L is a recommended cutoff for defining inadequate iodine intake in pregnancy by the WHO. [5] Antibody positivity was defined as values above the 92-percentile (>6.6 IU/mL for TPOAb and >7 IU/mL for TgAb).



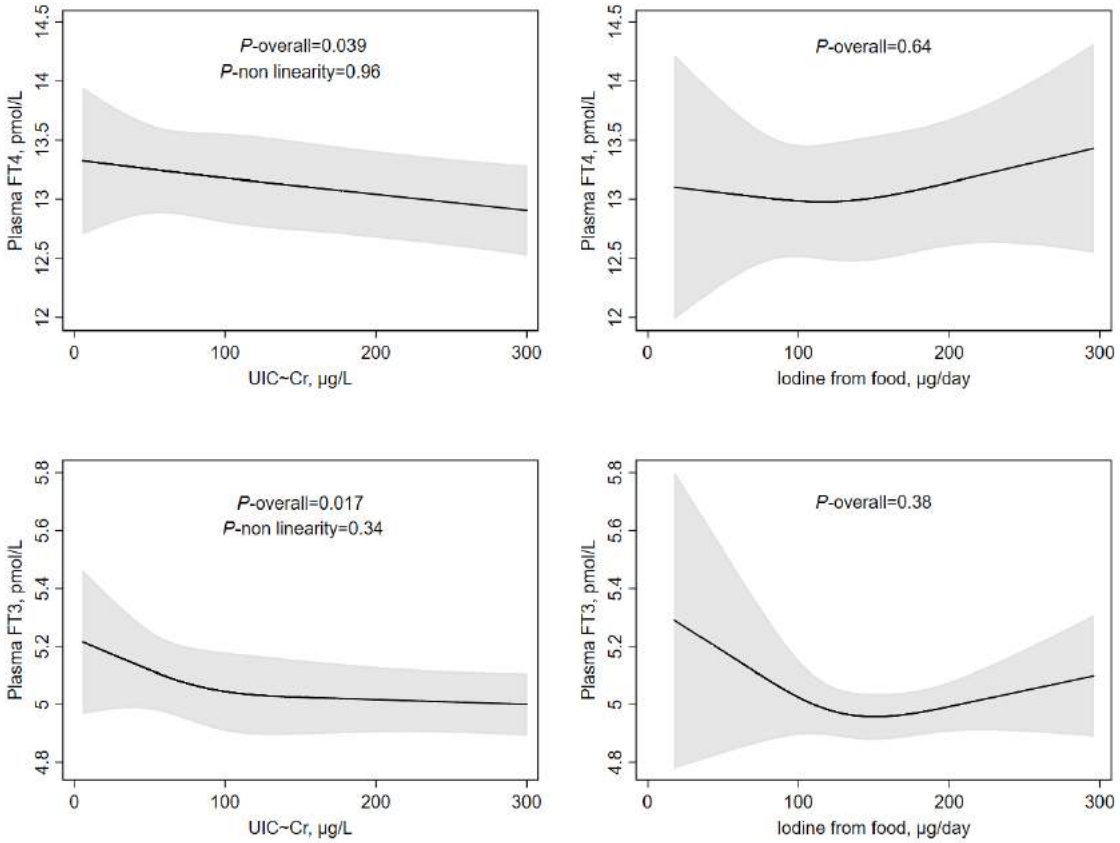
Supplemental Figure 4 Spot urinary iodine concentration (UIC) by reported use of iodine-containing supplements. Groups shown are non-supplement users (n=1738), current supplement users (gestational week 17-20, n=580), and *women who reported use during first half of pregnancy, but not in gestational week 17-20 (n=592). UIC differed significantly between the three groups (Mann-Whitney U-test, $p<0.001$) indicating that some of the women who reported use of supplements, but not current use, were actually current users. The boxes illustrate the median and IQR, and the whiskers illustrate the 90% range.



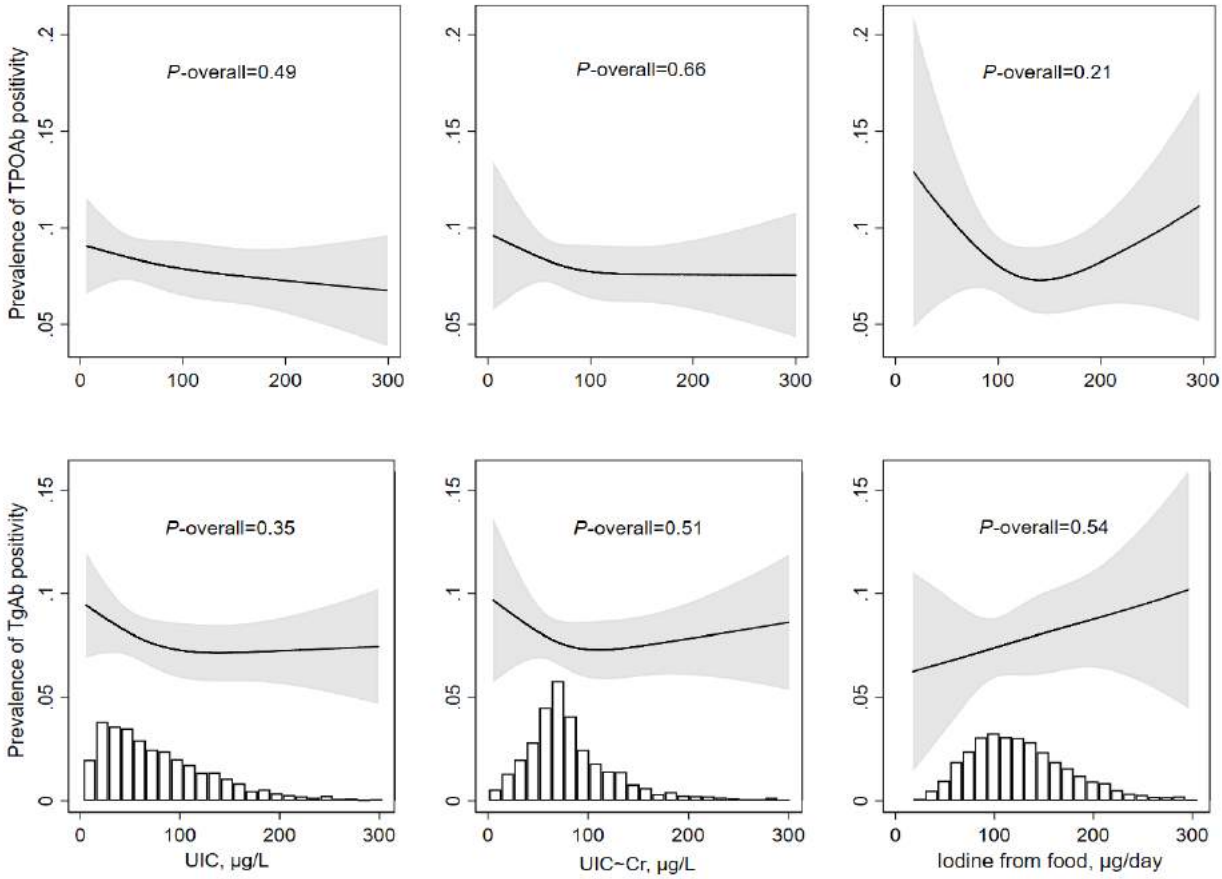
Supplemental Figure 5 Crude associations between UIC (column 1, n=2900), UIC~Cr (column 2, n=2900), and iodine from food (column 3, restricted to non-users of iodine supplement, n=1730) and thyroid hormones. Models with UIC~Cr as exposure were adjusted for urinary creatinine, and models with iodine from food as exposure were adjusted for energy intake. All models were adjusted for random effect of person clusters. For TSH, the geometric mean was modelled (by square root transformation), and for FT3 and FT4 the highest one percentile was excluded to obtain ~normal distribution. Missing values for BMI (2%), education (2%), selenium (0.6%), and gestational age at sampling (0.3%) were imputed.



Supplemental Figure 6 Associations of creatinine-adjusted urinary iodine concentration (UIC~Cr, column 1, n=2900), and iodine from food (column 2, restricted to non-users of iodine supplement, n=1730) with thyroid hormones **excluding women with thyroid antibody positivity** (either TPOAb and/or TgAb >92 percentile). Models were adjusted for maternal age, bmi, parity, education, chronic illness, smoking, fiber intake, gestational age at sampling, plasma ferritin, plasma selenium, and for random effect of person clusters. Models with UIC~Cr as exposure were additionally adjusted for urinary creatinine. Models with iodine from food as exposure were additionally adjusted for energy intake. For TSH, the geometric mean was modelled (by square root transformation), and for FT3 and FT4 the highest one percentile was excluded to obtain ~normal distribution. Missing values for BMI (2%), education (2%), plasma selenium (0.6%), and gestational age at sampling (0.3%) were imputed.



Supplemental Figure 7 Associations of creatinine-adjusted urinary iodine concentration (UIC~Cr, column 1, n=2900), and iodine from food (column 2, restricted to non-users of iodine supplement, n=1730) with thyroid hormones **including women with highest percentile of FT3 and FT4.** In the results reported in the paper, they were excluded to obtain a closer to normal distribution. Including them does not change the shape of the associations. Models were adjusted for maternal age, bmi, parity, education, chronic illness, smoking, fiber intake, gestational age at sampling, plasma ferritin, plasma selenium, and for random effect of person clusters. Models with UIC~Cr as exposure were additionally adjusted for urinary creatinine. Models with iodine from food as exposure were additionally adjusted for energy intake. Missing values for BMI (2%), education (2%), plasma selenium (0.6%), and gestational age at sampling (0.3%) were imputed.



Supplemental Figure 8 Estimated prevalence of thyroid antibody positivity by UIC (column 1, n=2900), creatinine-adjusted UIC (column 2, n=2900), and by iodine from food (column 2, restricted to non-users of iodine supplements, n=1730) based on **crude models**. Antibody positivity was defined as antibody plasma concentration > 92 percentile. Models with UIC~Cr as exposure were adjusted for urinary creatinine. Models with iodine from food as exposure were adjusted for energy intake. All models were adjusted for random effect of person clusters. Missing values for BMI (2%), education (2%), selenium (0.6%), and gestational age at sampling (0.3%) were imputed.

Supplemental Table 2 Population-based reference ranges of FT4 and TSH by measures of iodine status (crude models)[1]

UIC, µg/L

	<30	**30-59.9**	**60-99.9**	**100-150**	**>150**	***P*-value[2]**
n	507	625	629	444	**372**	
UIC, median (IQR), µg/L	20 (14, 25)	44 (37, 51)	78 (69, 88)	121 (110, 135)	195 (167, 260)	
Plasma FT4, pmol/L						
2.5th percentile (95% CI)	10.4 (10.2, 10.6)	10.4 (10.2, 10.6)	10.1 (9.9, 10.3)	10.3 (10.0, 10.6)	10.1 (9.8, 10.4)	0.20
Median (95% CI)	12.6 (12.5, 12.7)	12.7 (12.6, 12.8)	12.7 (12.6, 12.8)	12.5 (12.4, 12.6)	12.4 (12.3, 12.5)	0.005
97.5th percentile (95% CI)	16.2 (15.3, 17.1)	15.5 (14.7, 16.3)	16.0 (15.2, 16.8)	15.4 (14.4, 16.4)	15.0 (13.9, 16.1)	0.43
Plasma FT3, pmol/L						
2.5th percentile (95% CI)	4.00 (3.90, 4.10)	4.10 (4.00, 4.19)	4.00 (3.91, 4.10)	4.00 (3.89, 4.11)	4.00 (3.88, 4.12)	0.49
Median (95% CI)	4.90 (4.86, 4.94)	4.90 (4.87, 4.93)	4.90 (4.87, 4.93)	4.90 (4.86, 4.94)	4.90 (4.86, 4.94)	0.99
97.5th percentile (95% CI)	6.00 (5.78, 6.22)	6.10 (5.90, 6.30)	6.20 (6.00, 6.40)	6.10 (5.86, 6.34)	5.80 (5.54, 6.06)	0.19
Plasma TSH, mU/L						
2.5th percentile (95% CI)	0.49 (0.41, 0.57)	0.41 (0.34, 0.48)	0.36 (0.29, 0.43)	0.39 (0.30, 0.48)	0.36 (0.26, 0.46)	0.17
Median (95% CI)	1.20 (1.14, 1.26)	1.15 (1.10, 1.20)	1.19 (1.14, 1.24)	1.23 (1.17, 1.29)	1.23 (1.16, 1.30)	0.30
97.5th percentile (95% CI)	2.59 (2.37, 2.81)	2.53 (2.33, 2.73)	2.73 (2.54, 2.93)	2.80 (2.56, 3.04)	2.80 (2.54, 3.06)	0.31

Creatinine-adjusted UIC (UIC~Cr), µg/L

	<45	**45-69.9**	**70-89-9**	**90-130**	**>130**	***P*-value[2]**
n	428	700	580	459	410	
UIC~Cr, median (IQR), µg/L	30 (20, 38)	60 (53, 65)	78 (73, 83)	105 (97, 116)	172 (143, 227)	
Plasma FT4, pmol/L						
2.5th percentile (95% CI)	10.5 (10.2, 10.8)	10.2 (10.0, 10.4)	10.3 (10.1, 10.6)	10.2 (10.0, 10.5)	10.3 (10.1, 10.6)	0.49
Median (95% CI)	12.6 (12.4, 12.7)	12.6 (12.5, 12.8)	12.5 (12.4, 12.7)	12.6 (12.4, 12.7)	12.4 (12.3, 12.6)	0.27
97.5th percentile (95% CI)	15.9 (14.9, 17.0)	15.3 (14.6, 16.1)	16.2 (15.3, 17.0)	15.4 (14.5, 16.4)	15.3 (14.3, 16.3)	0.56
Plasma FT3, pmol/L						
2.5th percentile (95% CI)	4.20 (4.07, 4.33)	4.10 (4.00, 4.20)	4.00 (3.89, 4.11)	4.00 (3.88, 4.12)	4.00 (3.87, 4.13)	0.77
Median (95% CI)	5.00 (4.94, 5.05)	4.90 (4.85, 4.94)	4.90 (4.85, 4.95)	4.90 (4.85, 4.95)	4.80 (4.75, 4.85)	<0.001
97.5th percentile (95% CI)	6.12 (5.88, 6.35)	6.05 (5.87, 6.22)	6.06 (5.86, 6.25)	5.93 (5.72, 6.15)	5.81 (5.58, 6.05)	0.34
Plasma TSH, mU/L						
2.5th percentile (95% CI)	0.47 (0.36, 0.58)	0.40 (0.31, 0.48)	0.38 (0.29, 0.48)	0.38 (0.28, 0.48)	0.42 (0.31, 0.53)	0.81
Median (95% CI)	1.15 (1.09, 1.22)	1.15 (1.10, 1.20)	1.20 (1.14, 1.25)	1.25 (1.19, 1.32)	1.23 (1.16, 1.29)	0.070
97.5th percentile (95% CI)	2.50 (2.26, 2.74)	2.63 (2.45, 2.81)	2.78 (2.59, 2.98)	2.83 (2.61, 3.05)	2.77 (2.54, 3.00)	0.21

Iodine from food[3], µg/day

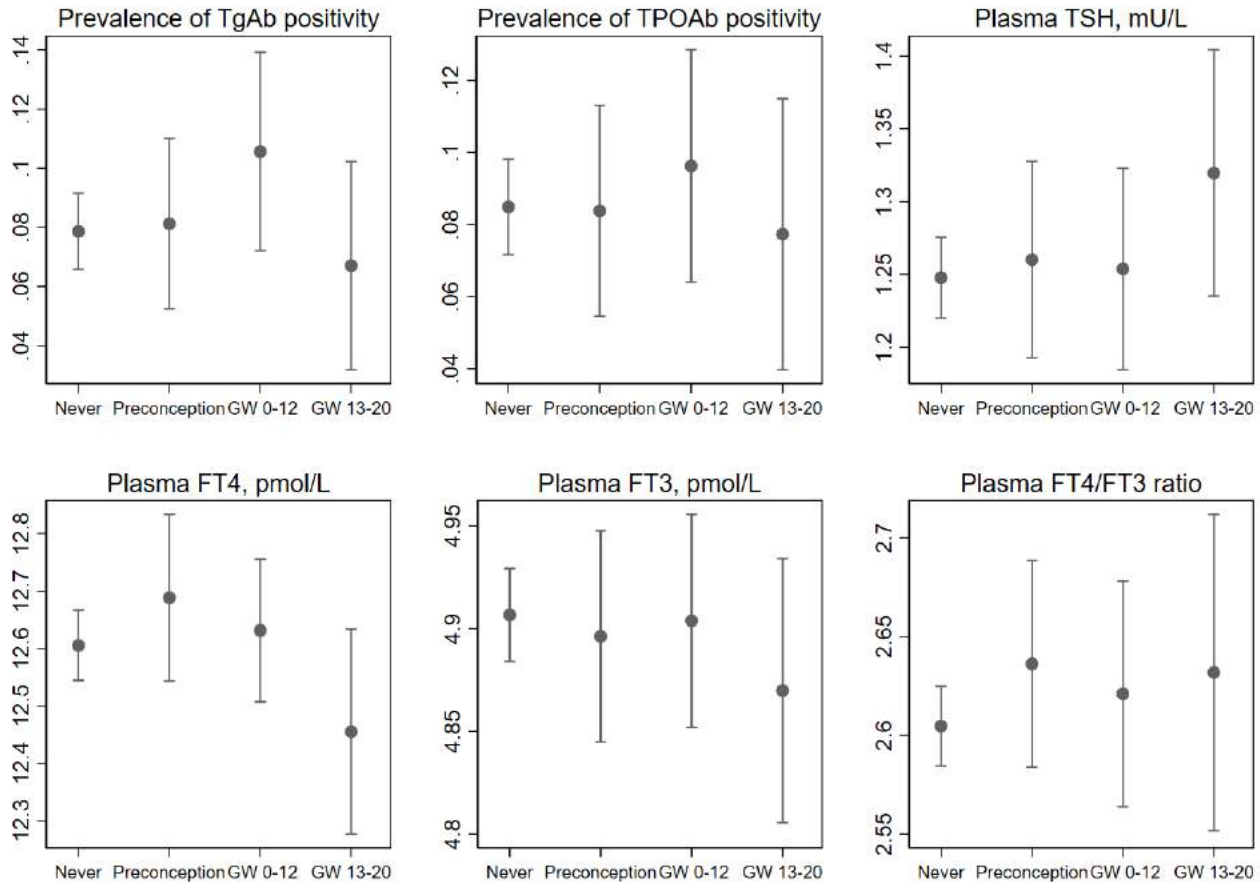
	<100	**100-150**	**>150**	***P*-value[2]**
n	523	562	457	
Iodine intake, median (IQR), µg/day	77 (62, 89)	123 (111, 135)	183 (163, 217)	
Plasma FT4, pmol/L				
2.5th percentile (95% CI)	10.3 (10.0, 10.6)	10.3 (10.0, 10.6)	10.2 (9.8, 10.5)	0.77
Median (95% CI)	12.6 (12.5, 12.8)	12.6 (12.4, 12.7)	12.5 (12.3, 12.6)	0.30
97.5th percentile (95% CI)	16.1 (15.1, 17.1)	15.5 (14.6, 16.4)	15.0 (13.9, 16.1)	0.40
Plasma FT3, pmol/L				
2.5th percentile (95% CI)	4.10 (4.00, 4.20)	4.00 (3.91, 4.09)	4.10 (3.99, 4.21)	0.24
Median (95% CI)	4.90 (4.85, 4.95)	4.90 (4.85, 4.95)	4.90 (4.84, 4.96)	0.99
97.5th percentile (95% CI)	6.05 (5.83, 6.26)	5.89 (5.69, 6,08)	6.04 (5.81, 6.28)	0.45
Plasma TSH, mU/L				
2.5th percentile (95% CI)	0.46 (0.34, 0.57)	0.41 (0.31, 0.51)	0.44 (0.32, 0.56)	0.81
Median (95% CI)	1.19 (1.12, 1.23)	1.18 (1.12, 1.23)	1.20 (1.14, 1.27)	0.84
97.5th percentile (95% CI)	2.68 (2.34, 3.02)	2.76 (2.46, 3.07)	2.51 (2.15, 2.88)	0.59

Values are from quantile regression (crude models) and only adjusted for urinary creatinine (UIC~Cr models) or energy intake (iodine from food models).

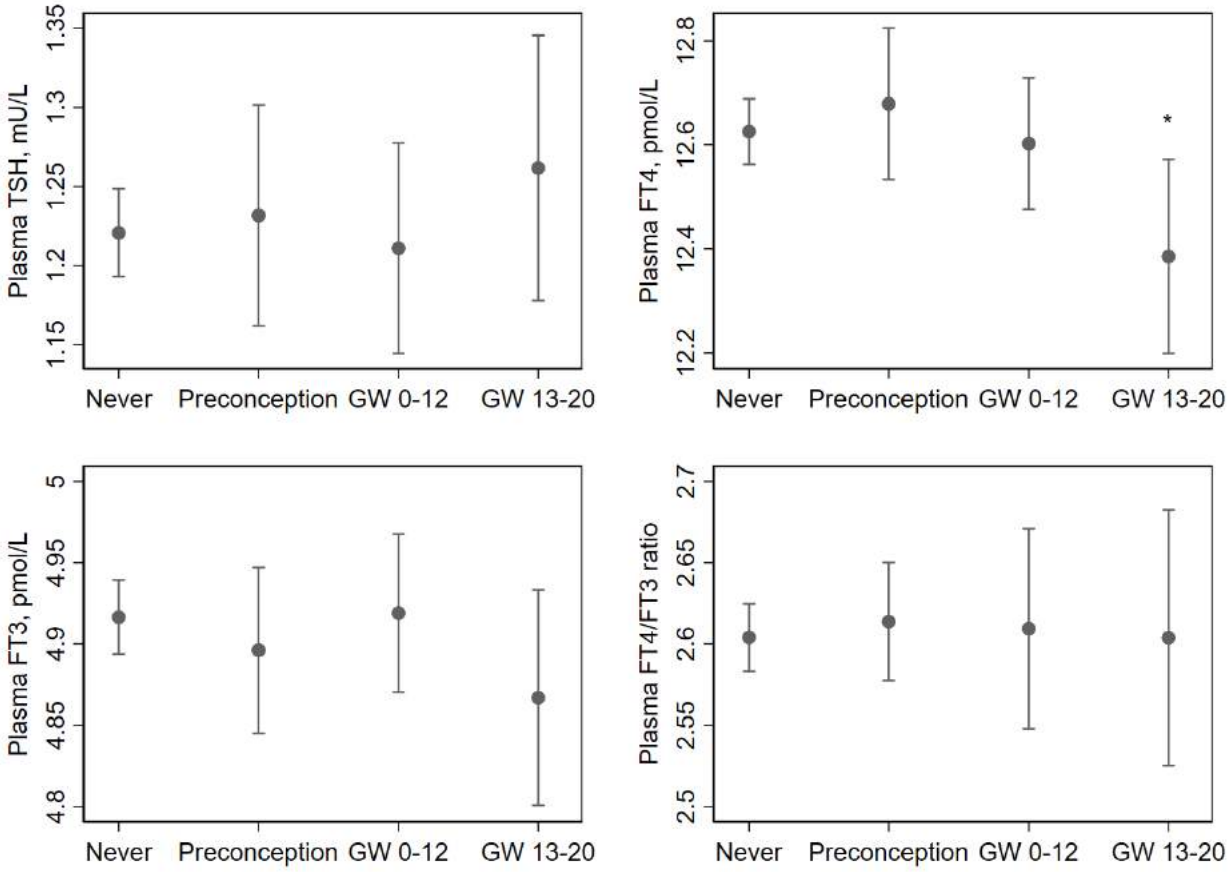
[1] Excluded: TPOAb positive (TPOAb>4.11 IU/mL), current users of thyroid medication or thyroid disrupting medication, and in vitro fertilization.

[2] *P*-value from quantile regression

[3] Iodine from food in non-users of iodine supplements



Supplemental Figure 9 Timing of initiation of iodine-containing supplements in pregnancy and measures of maternal thyroid function, **crude models.** Timing was categorized as: no reported supplement use (Never, n=1738), first use in week 1-26 before conception (Preconception; n=347), first use in first trimester (GW 0-12, n=323), and first use in second trimester (GW 13-20, n=194). Participants who reported use of iodine-containing supplements in the food frequency questionnaire, but did not report timing of use (n=308) were not included in this analysis. For TSH, the geometric mean was modelled (by square root transformation), and for FT3 and FT4 the highest one percentile was excluded to obtain ~normal distribution. Models were only adjusted for random effect of person clusters. Missing values for BMI (2%), education (2%), selenium (0.6%), and gestational age at sampling (0.3%) were imputed.



Supplemental Figure 10 Timing of initiation of iodine-containing supplements in pregnancy and measures of maternal thyroid function **excluding participants with thyroid antibody positivity** (either TPOAb and/or TgAb >92 percentile) (adjusted models). Timing was categorized as: no reported supplement use (Never, n=1738), first use in week 1-26 before conception (Preconception; n=347), first use in first trimester (GW 0-12, n=323), and first use in second trimester (GW 13-20, n=194). Participants who reported use of iodine-containing supplements in the FFQ, but did not report timing of use (n=308) were not included in this analysis. For TSH, the geometric mean was modelled (by square root transformation), and for FT3 and FT4 the highest one percentile was excluded to obtain ~normal distribution. Models were adjusted for maternal age, pre-pregnancy bmi, parity, education, IVF, chronic illness, smoking, fiber intake, gestational age at sampling, plasma ferritin, whole blood selenium, and for random effect of person clusters. Missing values for BMI (2%), education (2%), selenium (0.6%), and gestational age at sampling (0.3%) were imputed.
* Women who had recently started with supplements (gestational week 13-20) had significantly lower plasma FT4 (estimated difference in mean to non-users of supplement: -0.24 95% CI: (-0.44, -0.04), p=0.017).

PAPER 4

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ORIGINAL CONTRIBUTION

Language delay and poorer school performance in children of mothers with inadequate iodine intake in pregnancy: results from follow-up at 8 years in the Norwegian Mother and Child Cohort Study

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Abstract

Purpose Some studies indicate that mild-to-moderate iodine deficiency in pregnant women might negatively affect offspring neurocognitive development, including previous results from the Norwegian Mother and Child Cohort study (MoBa) exploring maternally reported child development at age 3 years. The aim of this follow-up study was to investigate whether maternal iodine intake in pregnancy is associated with language and learning at 8 years of age.

Methods The study sample includes 39,471 mother–child pairs participating in MoBa with available information from a validated food frequency questionnaire covering the first half of pregnancy and a questionnaire on child neurocognitive development at 8 years. Multivariable regression was used to explore associations of iodine intake from food and supplements with maternally reported child outcomes.

Results Maternal iodine intake from food less than ~ 150 µg/day was associated with poorer child language skills (p-overall = 0.013), reading skills (p-overall = 0.019), and writing skills (p-overall = 0.004) as well as poorer school test result in reading ($p < 0.001$), and increased likelihood of the child receiving special educational services (p-overall = 0.042) (in non-iodine supplement users). Although significant, differences were generally small. Maternal use of iodine supplements in pregnancy was not significantly associated with any of the outcomes.

Conclusions Low habitual iodine intake in pregnant women, i.e., lower than the recommended intake for non-pregnant women, was associated with mothers reporting poorer child language, school performance, and increased likelihood of special educational services. We found no indications of benefits or harm of using iodine-containing supplements in pregnancy. Initiating use in pregnancy might be too late.

Keywords Iodine · Pregnancy · Dietary supplements · Neurodevelopment · Norwegian Mother and Child Cohort Study · MoBa

Abbreviations

EAR	Estimated average requirement
FFQ	Food frequency questionnaire
GW	Gestational week
ID	Iodine deficiency

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MoBa The Norwegian Mother and Child Cohort Study
UIC Urinary iodine concentration

Introduction

Iodine is an essential micronutrient incorporated into the thyroid hormones thyroxine (T4) and triiodothyronine (T3). Thyroid hormones are vital in the regulation of early brain development, and the developing foetus is vulnerable to low maternal T4, especially before the foetal thyroid starts to function in gestational week (GW) 18–20 [1]. Iodine status is an important determinant of risk for thyroid disorders and, as a result, abnormal thyroid hormone concentrations [2–4]. In mild-to-moderate iodine deficiency (ID), a range of autoregulatory mechanisms are triggered in the maternal thyroid to maintain the production and release of the hormones [5]. To conserve iodine, more T3 is released and less T4. The resulting maternal hypothyroxinaemia (i.e., low/suboptimal T4) may reduce foetal supply of the hormones since the direct transfer of maternal T3 over the placenta is extremely low [1].

Globally, ID is one of the most prevalent nutrient deficiencies, common in both low and high income countries [6]. Substantial efforts have been made to eradicate ID, and today the number of countries with severe ID populations has declined [7]. Still, mild-to-moderate ID is prevalent in many regions, and particularly in pregnant women, as the recommended intake in pregnancy is higher. The iodine requirement increases in pregnancy due to a higher production of thyroid hormones, an increase in renal iodide clearance, and trans-placental iodine transfer [8, 9]. In 2007, the World Health Organisation (WHO) reported that an estimated 50% of the population in continental Europe remains mildly iodine deficient [10].

Results from animal studies and observational human studies, including the Norwegian Mother and Child Cohort Study (MoBa), indicate that maternal mild-to-moderate ID might affect thyroid function in pregnancy [11], and negatively affect foetal neurodevelopment including reduced IQ, school performance, language delay, and behaviour problems [1, 12–17]. When children do not reach their full developmental potential due to ID, this is not only important for the children's future, but also costly at a societal level [18].

Although studies indicate negative effects, there is little knowledge about what is the optimal iodine intake to secure an adequate iodine status in pregnant women. Thus, different recommendations for daily intake during pregnancy exist. In the Nordic countries, 175 µg/day is recommended [19], whereas the European Food Safety Authority recommendation is 200 µg/day [20], and the WHO recommendation is 250 µg/day [21]. Additionally, studies show conflicting results as to whether iodine supplement use initiated in pregnancy is beneficial or potentially harmful in mild-to-moderate ID populations [22]. Iodine supplements can reduce the risk of thyroid enlargement due to ID, but some studies, including results from MoBa, indicate that a sudden increase in iodine intake may temporary inhibit thyroid hormone production and cause lower availability of T4 to the foetus [11, 23, 24]. In MoBa, we observed an increased risk of child ADHD symptoms and diagnosis at age 6–13 years in children of mothers who had initiated use of iodine-containing supplements in the first trimester [13], but no effect on language and motor skills at age 3 years [12].

In this follow-up of the MoBa-children, the main aim was to investigate associations between maternal iodine intake in pregnancy (from food or from supplements) and child language and learning at age 8 years. A second aim was to evaluate current iodine status in a subsample of 300 8-years-old children.

Materials and methods

Subjects and design

This study is based on the Norwegian Mother and Child Cohort Study (MoBa) conducted by the Norwegian Institute of Public Health [25]. Women pregnant in their first trimester were recruited from all over Norway during the years 1999–2008 and were asked to answer questionnaires (in Norwegian) at regular intervals during pregnancy and after birth. More than 99% of participants are of Caucasian origin. Pregnancy and birth records from the Medical Birth Registry of Norway are linked to the MoBa database [26]. The women consented to participation in 41% of the pregnancies. The cohort now includes 114,500 children, 95,200 mothers and 75,200 fathers. The current study is based on version 10 of the quality-assured data files released for research in 2017 and includes participants with data from the 8-year questionnaire ($n = 39{,}471$). A flow chart of inclusion is illustrated in Fig. 1. A subsample of 8-years-old MoBa-children living in Oslo and the surrounding areas were recruited for an EU-project [27] and contributed with urine samples for analysis of UIC ($n = 279$).

Exposure variable: iodine intake

The MoBa FFQ [28] was specifically designed for the MoBa study and in use from 2002. It is a semi-quantitative questionnaire designed to capture dietary habits and use of dietary supplements during the first half of pregnancy

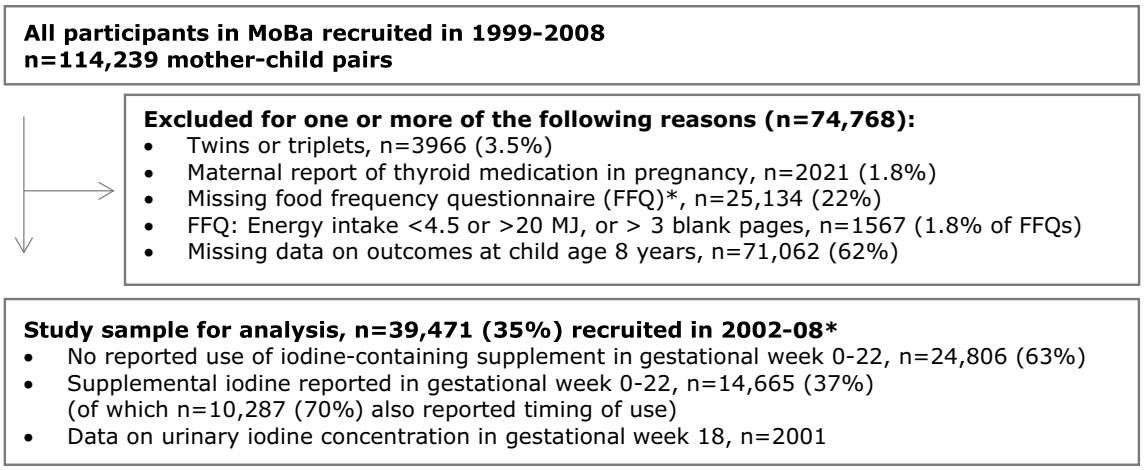

Fig. 1 Flow chart of inclusion. *The MoBa food frequency questionnaire was introduced in March 2002

[29] and was completed by participating women around GW 22. It included questions about intake of 255 food items or dishes. The average intake of specific foods and nutrients in GW 0–22 were calculated based on standard Norwegian portion sizes, the Norwegian food composition table, analyses of Norwegian food samples [30, 31], and data on the content of more than 1000 food supplements collected from suppliers [32]. There is limited data on dosage of supplements since the wording of the question on supplement use in the MoBa FFQ was unclear as to which time period it referred to (i.e., average intake in GW 0–22, intake only when using, or current use in GW 22). Therefore, we decided not to use the dosage of supplement as an exposure variable, but instead included it as a dichotomous variable (use/no use in GW 0–22).

As reported previously [12], the MoBa FFQ has been shown to be a valid tool for ranking pregnant women according to high and low intakes of energy, nutrients and foods [33]. Iodine was validated separately and iodine intake by the FFQ, including supplemental iodine, agreed well with the reference methods 24-h UIE and 4 days weighed-food diary, triangular validity coefficient for total iodine intake by the FFQ was 0.62 (95% CI 0.46, 0.77) [34, 35].

Timing of iodine supplement use was reported in the general questionnaires answered in GW 17 and GW 30. Timing of the first reported use was coded in four categories (never, week 0–26 before pregnancy, GW 0–12 and GW 12–22).

Blood and spot-urine samples were collected at the routine ultrasound examination offered free of charge to all Norwegian women in GW 18, and urinary iodine concentration (UIC) was measured for a subsample of the women ($n=2001$) as an alternative measure of iodine intake.

UIC was also measured in a subgroup of 8-year-old children in MoBa residing in the Oslo area ($n=279$, year of sampling 2014–2015) in a urine sample where equal volumes of an evening urine and the following morning urine were mixed.

Outcome variables: child language and learning

Outcomes were based on the MoBa questionnaire completed by the mothers at child age 8 years. The questionnaire was distributed to all participants in MoBa by mail and the response rate was 38%.

Maternally reported language skills

A standardized score was calculated based on items from The Children's communication checklist-Short (CCC-S), a brief version of the CCC-2 [36]. The full CCC-2 is as effective as a standardized assessment in identifying children with clinically significant language impairment [37]. The CCC-S contains 13 items that best discriminated typically developing children from peers with language impairment in the validation study [38], with high degrees of internal consistency (Cronbach's $\alpha=0.80$, this sample) and a significant correlation between CCC-S and CCC-2 total scores in the standardization sample, Pearson's $r=0.88$. Each item provides an example of language behaviour in everyday contexts and covers speech, vocabulary, grammar and discourse.

Maternally reported child reading and writing skills

Standardized scores were calculated based on three items on reading skills: (1) reads simple stories aloud, with ease, when asked, (2) identifies all lowercase and uppercase printed letters of the alphabet, (3) reads and understands texts suitable for 7–8 years olds, and two questions on writing skills: (1) writes simple information/messages at least

three sentences long, (2) writes reports, papers, or essays at least one page long; may use computer. May make small errors in spelling or sentence structure. Items were selected from the written sub-scale of the Vineland Adaptive Behaviour Scale-II [39]. Mothers answered "yes", "partially", or "not yet" which was coded as 0, 1, and 2.

Mandatory mapping tests in reading and mathematics in first and/or second grade in school

Performance tests in reading, writing, and mathematics are conducted in first and second grade. The tests map the ability to write letters, recognize written letters, identify spoken letters, combine sounds, write words, read words and read sentences. The mathematics test maps the ability to count, to compare numbers, to rank numbers, to recognize sequences of numbers, to count forward and backward from a given number, to split a number into two other numbers, to solve textual assignments and to add two numbers. Mothers were asked if they were informed by the teacher about their child's performance on the mapping tests. Alternative answers were "masters subject well", "must work more but teacher is not concerned", "teacher is concerned", or "don't know/not discussed with teacher".

Special education

Child granted special education in school (extra educational services) due to disabilities or learning difficulties was reported by the mother (yes/no).

Covariates

Covariates were included in the models based on previous knowledge and directed acyclic graphs (DAGs). Data on covariates were obtained from different sources: maternal age and child sex was obtained from the Medical Birth Registry of Norway. Maternally reported pre-pregnancy body weight and height for the calculation of body mass index (BMI), maternal education (≤ 12, 13–16, ≥ 17 years), parity (previous pregnancies ≥ 22 weeks: 0, 1, ≥ 2), maternal chronic illness [asthma, diabetes, inflammatory bowel disease, rheumatic disease, epilepsy, multiple sclerosis or cancer before or during pregnancy (yes/no)], bilingual parent(s) (yes/no), and use of a folic acid supplement within the interval from 4 weeks before to 8 weeks after conception (yes/no) were included from questionnaire 1. Energy intake, fibre intake (as a marker of a healthy dietary pattern), and intake of the omega-3 fatty acids EPA and DHA were calculated based on the FFQ. Information on smoking in pregnancy was obtained from questionnaire 1 and, if available, questionnaires 3 (GW 30) and 4 (child's age 6 months) (three categories: no reported smoking in pregnancy, reported occasional smoking or stopped smoking before GW 12, and daily smoking at any time in pregnancy and had not stopped smoking before GW 12). Maternal history of reading or writing difficulties (yes/no) was retrieved from the 8 years questionnaire. Paternal history of reading and writing difficulties is not reported in MoBa. Child ADHD symptoms was defined as the mean score on 18 items from the ADHD Rating Scale included in the 8-year questionnaire.

Laboratory procedures

UIC was determined at the National Institute for Health and Welfare in Helsinki, Finland by inductively coupled plasma–mass spectrometry using an Agilent 7800 ICP-MS system (Agilent Technologies Inc, Santa Clara, CA, USA). The limit of quantification was 2 µg/L and the linearity was excellent up to 1500 µg/L ($r = 0.99$). Coefficient variation was 2–3%.

Statistics

Statistical analyses were performed in STATA (version 15.0; Stata Corp., College Station, TX).

In total, 4.2% of the women had missing values on pre-pregnancy BMI ($n = 956$, 2.4%), maternal education ($n = 839$, 2.1%), or on all covariates from questionnaire 1 ($n = 107$, 0.3%) including parity, marital status, chronic illness, bilingual parent(s), and folic acid supplement use. Missing values for covariates were imputed using multiple imputation by chained equations in STATA, and 20 imputed datasets were generated for analyses.

Associations were estimated by generalized linear regression (gamma family) for the continuous outcomes (language-, reading-, and writing scores), logistic regression for the dichotomous outcome special education, and ordered logistic regression for the ordinal outcomes (mandatory school mapping tests in reading and mathematics). Associations between iodine from food and the outcomes were modelled flexibly by restricted cubic splines (four knot positions, at percentiles 5, 35, 65, and 95). All models were adjusted for random effects of sibling clusters since some women participated with more than one pregnancy.

Adjusted models included the following covariates based on a causal diagram (Supplementary Figure S1, Online Resource 1): maternal age, education, parity, pre-pregnancy BMI, energy intake, fibre intake, and smoking in pregnancy. Models with continuous outcomes also included the following covariate(s) to increase the precision of the estimates: child sex, bilingual parent(s) (for the language outcome), and maternal history of reading/writing difficulty (for read/write scores). To isolate the effect of long-term/habitual iodine intake, we restricted analysis on associations between iodine intake from food and outcomes to participants who had not

reported any use of supplemental iodine in gestational week 0–22.

The potential impact of iodine supplement use was explored by including an interaction-term between iodine from food (modelled by restricted cubic splines) and (1) any supplement use in GW 0–22 and (2) timing of first reported use (never, initiation 1–26 weeks before conception, gestational week 0–12, or gestational week 13–22). If interactions were not significant, iodine from food was not included in the final models. Adjusted models on supplement use additionally included the covariates total intake of the n-3 fatty acids EPA and DHA (from food and dietary supplements), and maternal use of folic acid supplements within the interval from 4 weeks before to 8 weeks after conception.

Associations between maternal UIC (in µg/L) and child outcomes were also explored in multivariable regression models.

p values are reported for overall associations between continuous exposures and outcomes (testing H0: no association) by testing the coefficients of all spline transformations equal to zero. The tests for non-linearity were performed by testing the coefficients of the second and third spline transformations equal to zero. Potential interactions were explored by testing all interaction coefficients equal to zero. All statistical tests were performed on the imputed datasets ($n = 20$).

Results are reported including robust 95% confidence intervals (CI). A p value < 0.05 was considered statistically significant. Sensitivity analysis included adjusting for score on 18 items from the ADHD symptoms checklist to see if the associations were also independent of effect on ADHD symptom score at age 8 years reported in a previous publication [13].

Results

Characteristics of the study population are shown in Table 1, and calculated iodine intake and UIC by background characteristics are shown in Supplementary Table S1 (Online Resource 1). At a group level, maternal iodine intake in pregnancy was insufficient according to WHO guidelines (i.e., iodine intake was < 250 µg/day and median UIC < 150 µg/L) [21], and also according to EFSA and Nordic guidelines [19, 20]. The calculated median iodine intake from food was 122 µg/day (IQR 89, 161 µg/day), and median UIC (mean GW 18.5 (SD 1.3)) was 67 µg/L. Women who reported use of iodine-containing supplements at the time of UIC sampling had higher UIC (median 95 µg/L vs. 59 µg/L in non-users, $p < 0.001$). Median iodine intake from food differed only slightly by background characteristics, and did not differ between supplement users (37%) and non-supplement users (63%), between women with and without UIC measurements, or between responders and non-responders to the questionnaire at 8 years (Table 1). The major dietary sources of iodine were milk/yoghurt (47%), other dairy products (13%), and lean fish (14%). Drinking water only contributed with 2%, egg with 4%, fatty fish with 4%, and all other foods with the remaining 17%. Food sources of iodine by categories of iodine intake from food are shown in Supplementary Figure S2 (Online Resource 1). Iodine supplement use was more commonly reported in nulliparous (43%), higher educated (40%), and high-income households (40%) than in primi- or multiparous, less highly educated and lower-income groups (all $p < 0.001$), but the differences were generally small. UIC was higher in supplement users ($p < 0.001$), but did not differ significantly by any other background characteristic (Supplementary Table S1, Online Resource 1). The Spearman correlation between iodine intake by the FFQ and UIC (in µg/g creatinine) was $r = 0.31$ (95% CI 0.26, 0.36).

UIC measured in the subsample of 8-year-old MoBa-children ($n = 279$) indicated adequate iodine intake in the children (median UIC: 110 µg/L, IQR: 79, 155 µg/L). According to WHO criteria [21], median UIC > 100 µg/L is characterized as adequate in school-age children.

In our study sample, 6.9% of the 8 year olds were granted special education at school. According to the mother, 28% had suboptimal or low score on the mandatory mapping test in school in reading, and 18% had suboptimal or low score in mathematics. All outcome measures were correlated or partially overlapping. Maternally reported child language skills was correlated with reading skills (Spearman $r = 0.26$, $p < 0.001$) and writing skills (Spearman $r = 0.29$, $p < 0.001$). Among children receiving special educational services, 85% had suboptimal scores on the mapping tests in reading (81%) and/or mathematics (56%). We have previously reported significant associations of maternal iodine intake with child ADHD-symptoms reported in the same 8-year questionnaire [13]. ADHD-symptoms correlated with child language skills (Spearman $r = 0.35$, $p < 0.001$), and 31% of children with special education scored > 1.5 SD on ADHD symptoms. Venn diagrams further illustrating overlaps between outcomes are provided in Supplementary Figures S3–S6 (Online Resource 1).

We have previously reported that maternal iodine intake was associated with child language skills at age 3 years [12]. Eighty-one percent of the participants in the current sample were also included in the 3-year sample. Compared to those with normal language skills at 3 years (96%), children with mild to moderate language delay at 3 years (3.1%) scored on average 1.2 SD higher on the language score (CCC-S) at 8 years (95% CI 1.1, 1.3), and those with severe language delay at 3 years (0.7%) scored 2.8 SD higher (95% CI 2.3,

Table 1 Descriptive characteristics of the study population ($n = 39{,}471$ mother–child pairs) by maternal iodine intake

	Iodine from food, non-supplement users			Iodine supplement use in pregnancy (week 0–22)		All	Whole MoBa[a]
	< 100 µg/day	100–150 µg/day	> 150 µg/day	No	Yes		
Study sample, *n* (%)	8096 (21)	9126 (23)	7584 (19)	24,806 (63)	14,665 (37)	39,471 (100)	101,784
Maternal age at delivery, mean (SD), years	30.5 (4.4)	30.8 (4.3)	30.6 (4.5)	30.6 (4.4)	30.6 (4.4)	30.6 (4.4)	30.2 (4.6)
Pre-pregnancy BMI, mean (SD), kg/m^2	24.1 (4.2)	23.8 (4.0)	23.9 (4.0)	23.9 (4.0)	23.7 (4.1)	23.8 (4.1)	24.0 (4.3)
Parity, %							
0	46	42	41	43	54	47	47
1	37	37	37	37	32	35	36
2 or more	17	20	22	19	13	17	18
Maternal education, %							
≤ 12 years	27	24	27	26	22	24	32
13–16 years	43	45	46	45	45	45	41
> 16 years	28	29	25	27	31	29	25
Other/missing	2.5	1.9	2.1	2.2	2.1	2.1	2.1
Married/cohabitant, %	97.1	97.5	96.9	97.2	96.9	97.1	96.1
Smoking in pregnancy, %							
Occasionally	15	14	14	14	15	14	17
Daily	4.1	3.4	4.0	3.8	3.0	3.5	5.7
Chronic illness, %	11.1	8.7	8.4	9.4	10.8	9.9	10.2
Household income, %							
Low	24	24	27	25	23	24	29
Medium	41	42	43	42	41	42	41
High	33	32	27	31	34	32	28
Missing	2.3	2.1	2.9	2.4	2.1	2.3	3.1
Bilingual parent(s), %	9.8	9.5	7.6	9.0	10.8	9.7	10.8
Maternal history of reading/writing difficulties (%)	6.2	4.8	5.4	5.4	5.4	5.6	–
Child sex boy (%)	51.4	51.1	51.5	51.3	50.2	50.9	51.2
Iodine from food, median (IQR), µg/day	78 (63, 89)	123 (111, 136)	182 (163, 212)	122 (90, 161)	121 (89, 160)	122 (89, 161)	121 (89, 161)[b]
UIC, median (IQR), µg/L[c]	47 (26, 85)	62 (33, 105)	70 (39, 116)	59 (32, 101)	83 (43, 138)	67 (35, 115)	–
Urinary creatinine, median (90% range) g/L[c]	0.75 (0.17, 2.00)	0.76 (0.16, 1.88)	0.72 (0.21, 1.89)	0.75 (0.17, 1.92)	0.72 (0.16, 1.87)	0.74 (0.17, 1.90)	–
UIC, median (IQR), µg/g creatinine[c]	69 (44, 97)	83 (58, 115)	99 (72, 132)	83 (56, 115)	116 (75, 187)	91 (63, 139)	–
Iodine supplement (%)	0	0	0	0	100	37	37[b]

Table 1 (continued)

	Iodine from food, non-supplement users			Iodine supplement use in pregnancy (week 0–22)		All	Whole MoBa[a]
	< 100 µg/day	100–150 µg/day	> 150 µg/day	No	Yes		
Folic acid supplement (%)[d]	71	71	68	70	84	75	69
Omega 3 supplement (%)	79	81	82	81	80	80	79[b]
Maternal energy intake, median (IQR), MJ	8.0 (6.9, 9.3)	9.2 (8.1, 10.6)	11.0 (9.6, 12.8)	9.3 (7.9, 11.0)	9.3 (7.9, 11.0)	9.3 (7.9, 11.0)	9.4 (7.9, 11.1)

[a]Includes all pregnancies in the Norwegian Mother and Child Cohort Study with information on background characteristics from questionnaire 1

[b]In singleton pregnancies with available data from the food frequency questionnaire ($n = 83{,}721$)

[c]In a subsample of $n = 2001$ with data on urinary iodine and creatinine in gestational week 18

[d]Use of a folic acid supplement within the interval from 4 weeks before to 8 weeks after conception from questionnaire 1

3.2). Higher scores at 8 years indicated poorer language skills.

Iodine from food and outcomes

Low maternal iodine intake from food was associated with the child having poorer skills in language, reading, and writing, and increased likelihood of special education (Figs. 2, 3). There was also a tendency for a lower performance in mathematics (Fig. 3b), but the association was not statistically significant ($p = 0.083$). Combined, the spline graphs indicated that habitual iodine intakes lower than about 150 µg/day was associated with lower child performance. Tabular results of the estimated associations are provided in Table 2.

Sensitivity analyses showed that associations were somewhat attenuated when adjusting for ADHD-symptoms at 8 years (Supplementary Figures S9 and S10, Online Resource 1), but remained largely the same (language skills and writing skills were no longer significant).

Maternal UIC in mid pregnancy (measured in a subgroup, $n = 2001$) was not significantly associated with any of the outcomes (Supplementary Figures S11 and S12, Online Resource 1), and adjustment for urinary creatinine to adjust for hydration level did not change the results (results not shown).

Iodine from supplements

There was no evidence of any associations between maternal use of iodine-containing supplements and child outcomes (Supplementary Table 2, Online Resource 1). Interaction with iodine from food was tested, but there was no indication of interaction for any of the outcomes. Iodine intake from food did not differ by supplement use (including by timing of supplement use).

Discussion

The main finding in this study is that low maternal habitual iodine intake (i.e., lower than ~ 150 µg/day from food) was associated with poorer maternally reported child performance, i.e., the child having poorer skills in language, reading, and writing, and also increased likelihood of the child receiving special education services. This finding is alarming and corroborate the previous findings in MoBa of increased risk of maternally reported language delay at child age 3 years [12]. Further, we did not detect any association between maternal iodine supplement use and child outcomes.

Iodine intake from food and outcomes

Our findings suggest that impairments associated with mild-to-moderate ID in pregnancy persist although iodine intake is adequate in childhood (indicated by the subgroup median child UIC > 100 µg/L). Experimental animal studies have demonstrated that mild gestational ID can cause permanent neurological alterations in the developing brain [1]. Our findings are also supported by an observational study in Australia ($n = 228$, median gestational UIC: 81 µg/L) where children of mothers with gestational UIC < 150 µg/L had poorer educational outcomes (spelling, grammar and English-literacy) at age 9 years than peers whose mothers did not have mild or moderate gestational ID (i.e., UIC ≥ 150 µg/L), even though the children were iodine sufficient in childhood [15]. A follow-up study demonstrated that the reduced educational performance persisted also at 15 years of age [16]. Bath et al. found that children of mothers with a suboptimal

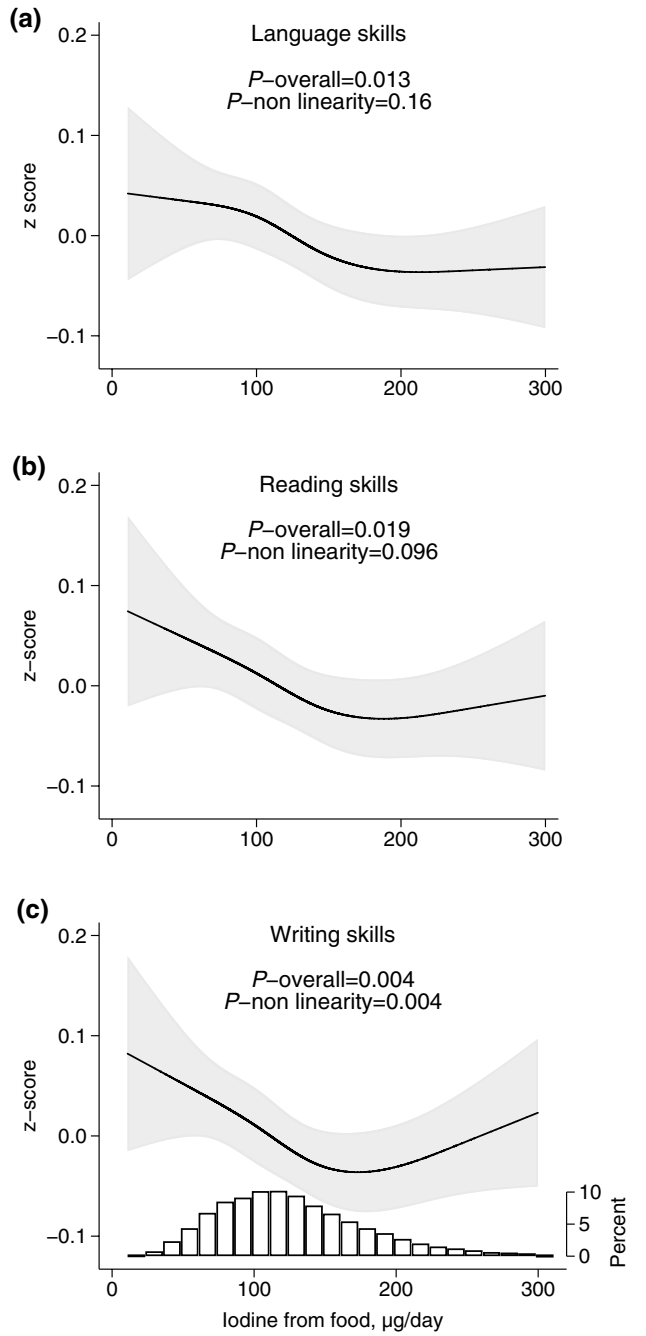

Fig. 2 Maternal iodine intake from food (in non-supplement users) and child skills in language (**a**, $n = 24{,}643$), reading (**b**, $n = 19{,}492$), and writing (**c**, $n = 19{,}483$), adjusted models. Higher z scores indicate poorer skills. The histogram in **c** represents the distribution of iodine intake. For crude models, see Supplementary Figure S7 (Online Resource 1)

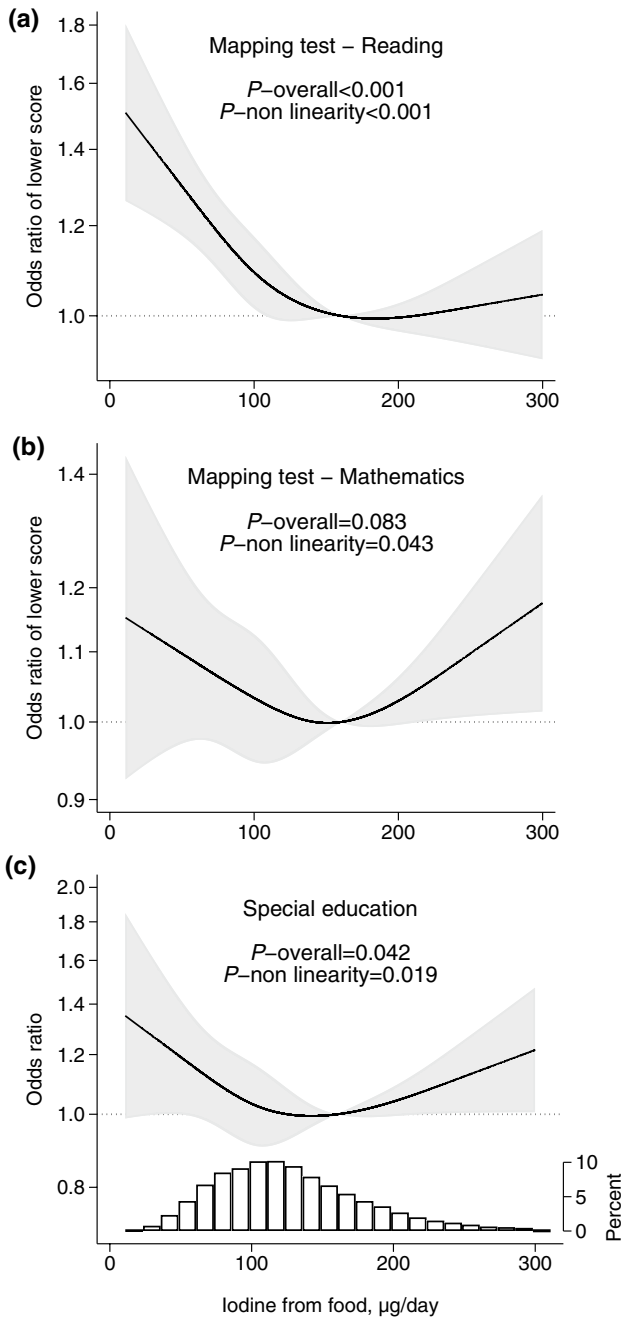

Fig. 3 Maternal iodine intake from food (in non-supplement users) and child school outcomes at age 8 years, adjusted models. Higher odds ratio indicate poorer test results (**a, b**) and increased likelihood of receiving special educational services (**c**). The reference level (OR = 1) was set to 160 µg/day. Sample size was $n = 24{,}309$ for mapping tests in reading (**a**), $n = 23{,}527$ for mathematics (**b**), $n = 24{,}806$ for special education (**c**). The histogram in **c** represents the distribution of iodine intake. For crude models, see Supplementary Figure S8 (Online Resource 1)

UIC in pregnancy (< 150 µg/g creatinine) had a lower IQ and reading ability at child age 8–9 years in a UK cohort ($n = 1040$, median gestational UIC: 91 µg/L) [14]. However, limitations of these studies were that spot-UIC was the only measure of maternal iodine intake, and that the exposure was not modelled continuously. As a consequence, optimal

Table 2 Iodine intake from food in pregnancy and language and learning at age 8 years in non-supplement users, adjusted models

Iodine intake (µg/day)	Language skills[a]	Reading skills[a]	Writing skills[a]	Mapping test Reading[b]	Mapping test Mathematics[b]	Special education
	n	*n*	*n*	*n*	*n*	*n/N* (%)
	24,643	19,492	19,483	24,309	23,527	1756/24,806 (7.1%)
	Std. beta (95% CI)	Std. beta (95% CI)	Std. beta (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
25	0.07 (0.00, 0.13)	0.09 (0.02, 0.17)	0.11 (0.03, 0.18)	1.43 (1.23, 1.66)	1.13 (0.94, 1.36)	1.29 (1.00, 1.67)
50	0.06 (0.01, 0.11)	0.08 (0.03, 0.13)	0.09 (0.03, 0.14)	1.30 (1.18, 1.43)	1.10 (0.97, 1.24)	1.19 (1.00, 1.42)
75	0.06 (0.02, 0.09)	0.06 (0.02, 0.10)	0.07 (0.03, 0.11)	1.18 (1.10, 1.28)	1.06 (0.97, 1.16)	1.10 (0.96, 1.25)
100	0.04 (0.01, 0.08)	0.04 (0.00, 0.08)	0.05 (0.01, 0.08)	1.09 (1.01, 1.17)	1.03 (0.95, 1.13)	1.03 (0.91, 1.17)
125	0.03 (0.01, 0.05)	0.02 (0.00, 0.04)	0.02 (0.00, 0.05)	1.04 (0.99, 1.08)	1.01 (0.96, 1.07)	1.00 (0.92, 1.08)
160 (ref)	0	0	0	1	1	1
200	−0.01 (−0.02, 0.00)	0.00 (−0.02, 0.01)	0.00 (−0.01, 0.02)	1.00 (0.97, 1.03)	1.03 (1.00, 1.06)	1.04 (0.99, 1.09)
225	−0.01 (−0.03, 0.01)	0.00 (−0.03, 0.03)	0.02 (−0.01, 0.04)	1.01 (0.96, 1.06)	1.06 (1.00, 1.12)	1.08 (1.00, 1.16)
250	−0.01 (−0.04, 0.03)	0.01 (−0.04, 0.05)	0.03 (−0.01, 0.07)	1.02 (0.94, 1.10)	1.10 (1.01, 1.20)	1.12 (1.00, 1.26)
300	−0.01 (−0.07, 0.05)	0.02 (−0.06, 0.10)	0.06 (−0.01, 0.13)	1.04 (0.92, 1.19)	1.18 (1.01, 1.36)	1.22 (1.01, 1.47)
p overall	$p=0.013$	$p=0.019$	$p=0.004$	$p<0.001$	$p=0.083$	$p=0.042$
p non-linearity	$p=0.16$	$p=0.096$	$p=0.004$	$p<0.001$	$p=0.043$	$p=0.019$

Results are from multivariable regression analysis adjusting for confounders and for random effects of sibling clusters

[a]Standardized beta > 0 indicate poorer skills

[b]Odds ratio > 1 indicate increased risk of attaining poorer test results

intake was not explored and many participants may have been misclassified as deficient/not deficient.

We included a spot-UIC in mid pregnancy as an alternative measure of habitual iodine intake since data on maternal UIC was available for $n=2001$ pregnant women. The null findings for UIC and outcomes (Supplementary Figures S11–S12, Online Resource 1) did not support our results that maternal habitual iodine intake was associated with the outcomes, but the seemingly conflicting results might have several explanations. Firstly, a single spot-UIC is a poor measure of habitual iodine intake at an individual level [40]. König et al. found that a minimum of ten repeated spot-UICs are needed to reliably estimate individual iodine intake [41]. Secondly, the subsample with UIC measurements was not a random sample of MoBa participants. They were selected from highly dedicated participants with complete datasets of questionnaires and biological samples up to child age 3 years, and the sample did not include children with language difficulties, or those with suspected or diagnosed autism. This reduces the power to identify potential associations with language and learning outcomes. In fact, we found that in this subsample, association curves between calculated iodine intake by the FFQ and outcomes were different from the total sample, and were more in agreement with the UIC findings (Supplementary Figures S11–S12, Online Resource 1).

We have previously reported that low maternal iodine intake was associated with child ADHD symptoms in the same population of 8-years-old MoBa-children [13]. In sensitivity analyses, we found that adjusting for maternally reported ADHD symptoms at child age 8 years did not change the results markedly (Supplementary Figures S9–S10, Online Resource 1), demonstrating that the associations between iodine intake with child language and learning were partly independent of score on ADHD-symptoms.

Iodine intake from supplements

We have previously reported that women in MoBa who initiated use of iodine-containing supplements in the first trimester gave birth to children with more behaviour problems at age 3 [12] and 8 years [13] and increased risk of ADHD diagnosis by age 6–14 years [13]. However, iodine supplement use was not associated with child language- or motor skills at 3 years [12]. In the present study, there was no significant association between maternal use of iodine-containing supplements and child language and learning at 8 years. Summarized, results from MoBa are consistent in demonstrating no beneficial effects of iodine supplement use in pregnancy, but inconclusive as to whether it can be potentially harmful. Initiating use in pregnancy might be too late to compensate for a depleted maternal iodine store. The "jury is still out" on this question for areas of mild-to-moderate ID as recently concluded in a Cochrane review [22]. Nevertheless, the optimal strategy to prevent impairments

caused by ID is to secure an adequate iodine status well before conception for all women of childbearing age [42].

Strengths and limitations

Strengths of this study include the large size, prospective- and population-based design, and the substantial data collection allowing to control for a range of confounders. Additionally, in Norway there is a large variation in habitual iodine intake since only milk and salt-water fish are important food sources of iodine in the Norwegian diet [43]. Consequently, we could study a wide range of iodine intake with flexible modelling techniques and explore potential non-linear associations. The multiple outcomes on child language and learning included results on mapping tests and whether the child was granted special education. These outcomes are more objective than the subjective evaluation of the child's language, reading and writing skills evaluated and reported by the mother which may include substantial measurement error. Child language skills were also measured in the 3-year questionnaire [12], and ideally, we would have modelled this repeated outcome longitudinally. Unfortunately this was not feasible since the instruments used were not the same at ages 3 and 8, and consequently did not measure the exact same dimensions.

Calculating iodine intake by an FFQ is inaccurate, both due to a natural variation of iodine content of food, limited quality of the data on food iodine content, the FFQ not covering all possible food and drink alternatives, deliberate or undeliberate misreporting, and an FFQ being a survey format requiring some literacy skills to complete correctly. Nevertheless, the previous validation study showed acceptable agreement between the MoBa FFQ calculations, 24 h UIC, and a 4 days weighed-food dairy [35]. Additionally, since there are few food sources of iodine in Norway, a person's iodine intake is largely dependent on individual food choices and can be easier to estimate than many other nutrients. In the FFQ, women were asked to report their habitual food intake since the beginning of pregnancy. The results of this study might reflect not only iodine intake during pregnancy but also habitual iodine intake prior to pregnancy and thus maternal iodine stores.

The low participation rate in MoBa (41%) is a concern. Women participating in MoBa are more often nulliparous, non-smokers, older and better educated than non-participants [44]. These are attributes generally associated with healthier diet and lifestyles, and yet both UIC and calculated iodine intake confirmed insufficient iodine intake, and there is little reason to expect that non-participants would have higher iodine intake than participants. Loss to follow-up is another concern, and only 35% of recruited women completed the 8-year questionnaire. Loss to follow-up is inevitable and commonly leads to selection bias and loss of statistical power [45]. In this study, iodine intake from food and iodine supplement use did not differ between responders and non-responders to the 8 years questionnaire. Still, we cannot exclude that loss to follow-up have influenced the results. Finally, as in all epidemiological studies, residual confounding might still be present due to the observational design, and thus we cannot make causal inference based on the results.

Clinical relevance and implications

Although the estimated effects of mild-to-moderate ID in this study are small, the high prevalence of insufficient maternal iodine intake worldwide indicates that a substantial proportion of children might not reach their full developmental potential as a consequence of maternal mild-to-moderate ID. In MoBa, 69% had a calculated iodine intake from food < 150 µg/day which was associated with poorer child outcomes. In Europe, two-thirds of countries that have assessed iodine nutrition in pregnant women have reported inadequate intakes [46]. The cost of insufficient iodine nutrition is high both at the individual and societal level [18], whereas the cost of salt iodization, the primary strategy recommended by the WHO, is low [42]. Our findings support the current recommendations by the WHO that it is of vital importance to secure sufficient iodine intake of > 150 µg/day in women of childbearing age [21].

In Norway, the health authorities have now, since 2017, initiated several actions to prevent ID. The national guidelines for a healthy diet now specifies that three portions of dairy products per day can contribute to secure an adequate intake of iodine and calcium. They recommend iodine supplements for women of childbearing age, pregnant, and lactating women who have a low milk/yoghurt intake. In addition, they are currently evaluating strategies to increase iodine intake by salt iodization.

Conclusions

A low habitual iodine intake in pregnant women, i.e., lower than the recommended daily intake for non-pregnant women, was associated with mothers reporting slightly poorer child language, school performance, and increased likelihood of special educational services at child age 8 years. We found no indications of benefits or harm of using iodine-containing supplements in pregnancy. Initiating use in pregnancy might be too late.

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Author contributions All authors contributed in designing the study; MHA performed the statistical analysis with support from IHC and ALB; MHA wrote the first draft of the manuscript; ALB had primary responsibility for the final content; all authors read and approved the final manuscript.

Compliance with ethical standards

Ethical approval MoBa is conducted according to the guidelines laid down in the Declaration of Helsinki and written informed consent was obtained from all participants. MoBa has obtained a license from the Norwegian Data Inspectorate. The current study was approved by The Regional Committee for Medical Research Ethics South East Norway 2014/2211.

Conflict of interest The first author of this paper is employed by a Norwegian dairy company (TINE SA), and she participates in this project as an industrial Ph.D.-student financed partly by the dairy company and partly by The Research Council of Norway. This project is designed, owned and administered by The Norwegian Institute of Public Health and analysis of the data follow from protocol. All results of analysis in the project are to be published regardless of the results. The other authors have no conflicts of interest.

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Supplementary material to:

Abel MH[123] , Brandlistuen RE[1], Caspersen IH[1], Aase H[1], Torheim LE[2], Meltzer HM[1], Brantsæter AL[1*] Language delay and poorer school performance in children of mothers with inadequate iodine intake in pregnancy - results from follow up at 8 years in the Norwegian Mother and Child Cohort Study.

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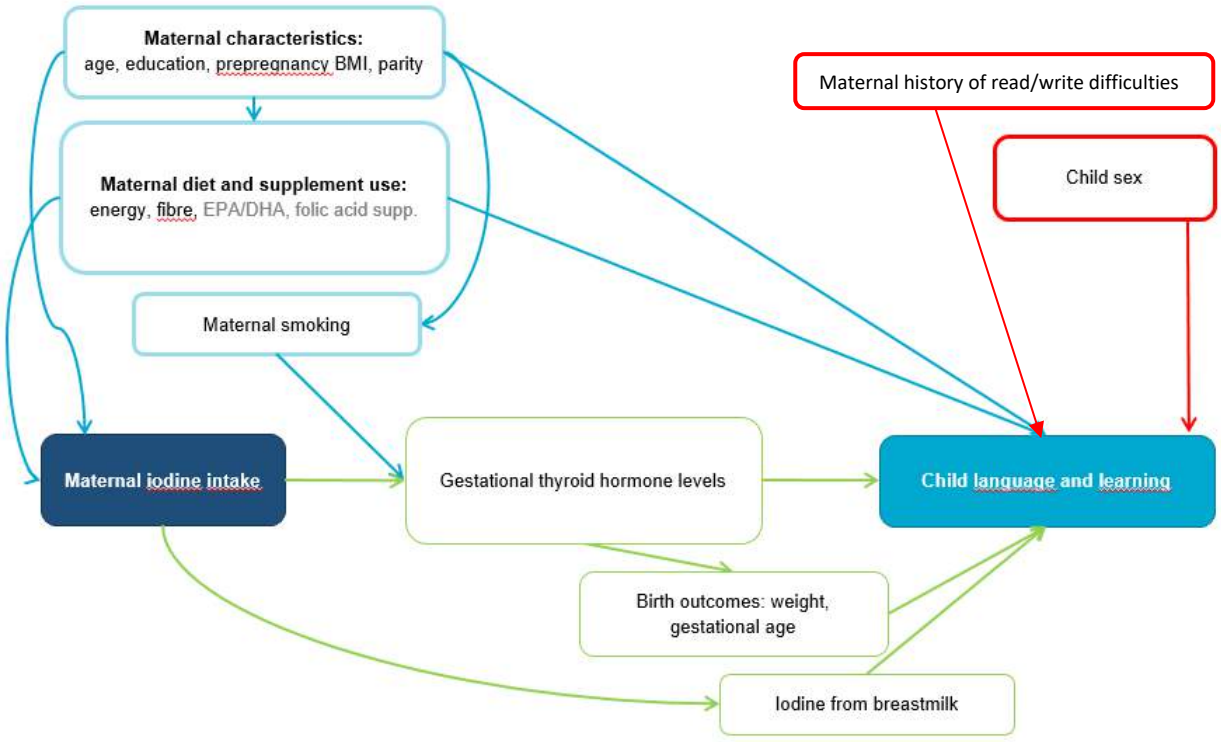
Supplementary Figure S1 Conceptual model (simplified directed acyclic diagram (DAG))



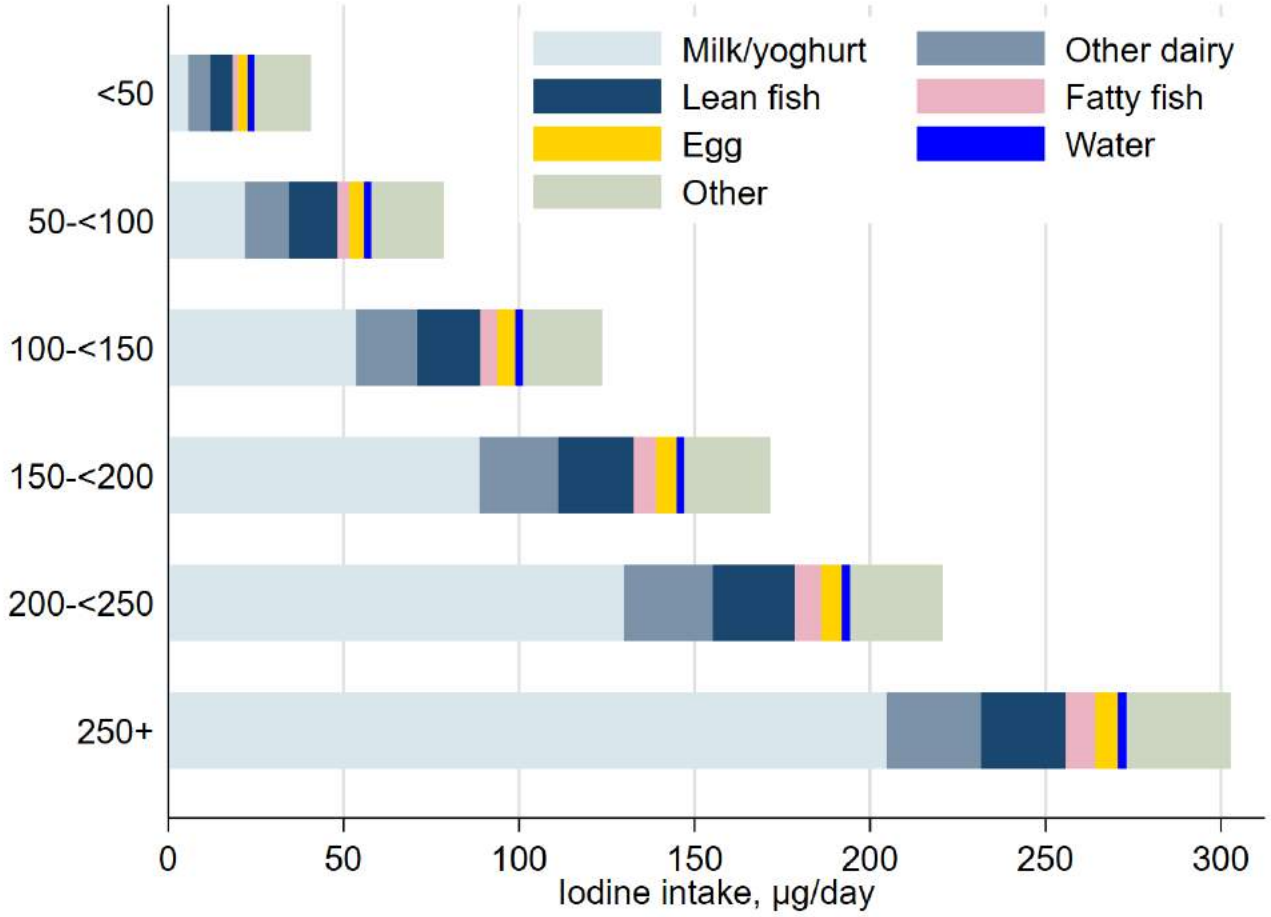
Association between maternal iodine intake and child language and learning. Potential causal pathways are illustrated in green and confounding pathways in blue. Child sex and maternal history of reading and writing difficulties are important determinants of the outcome marked in red. Intake of the n-3 fatty acids EPA and DHA and reported use of folic acid supplements were only included as confounders when iodine from supplements was the exposure.

Supplementary Table S1 Iodine exposures by characteristics of the study population (n=39,471 mother-child pairs)

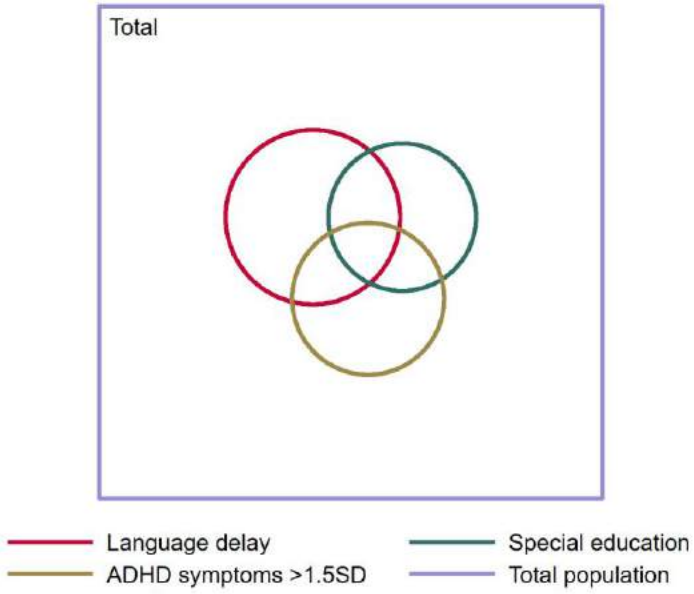
	Study population	Iodine from food	Iodine supplement	UIC [a]
		median (IQR) µg/day	GW 0-22 %	median (IQR) µg/L
Study sample, n (%)	39,471 (100)	122 (89, 161)	37	67 (35, 115)
Maternal age at delivery, mean (SD), years	30.6 (4.4)			
<25	8.0	122 (85, 169)	37	66 (39, 107)
25-34	73	121 (89, 160)	37	66 (35, 114)
≥35	19	123 (91, 160)	37	71 (33, 122)
Pre-pregnancy BMI, mean (SD), kg/m^2	23.8 (4.1)			
<18.5	2.7	122 (89, 161)	41	71 (31, 117)
18.5-24.9	66	122 (90, 160)	38	66 (34, 113)
25-30	21	121 (87, 162)	35	70 (33, 118)
>30	8.0	118 (84, 160)	36	76 (45, 122)
Missing	2.4	125 (91, 163)	34	61 (35, 123)
Parity, %				
0	47	118 (87, 158)	43	65 (34, 114)
1	35	122 (90, 160)	34	70 (35, 118)
2 or more	17	128 (95, 168)	29	68 (40, 102)
Missing	0.3	117 (82, 159)	30	-
Maternal education, %				
≤12 y	24	122 (87, 166)	34	69 (40, 116)
13-16 y	45	122 (90, 161)	38	67 (33, 113)
>16 y	29	120 (91, 155)	40	67 (33, 119)
Other/missing	2.1	117 (85, 157)	36	66 (42, 112)
Married/cohabitant, %				
Yes	96.8	122 (90, 160)	37	67 (35, 115)
No	2.9	120 (87, 165)	39	79 (35, 109)
Missing	0.3	118 (82, 159)	30	-
Smoking in pregnancy, %				
Occasionally	14	120 (87, 161)	38	65 (32, 109)
Daily	3.5	120 (88, 166)	32	65 (32, 99)
Chronic illness, %	9.9	116 (83, 156)	41	65 (33, 108)
Household income, %				
Low	24	124 (91, 167)	35	69 (38, 110)
Medium	42	123 (90, 162)	36	66 (34, 119)
High	32	118 (88, 153)	40	69 (34, 114)
Missing	2.3	126 (92, 170)	34	64 (44, 113)
Bilingual parent(s), %	9.7	119 (87, 155)	42	60 (34, 114)
Maternal history of reading/writing difficulties (%)	5.6	118 (85, 161)	38	74 (45, 133)
Child sex boy (%)	50.9	122 (90, 161)	37	66 (34, 116)
Iodine supplement in pregnancy, %				
No	63	122 (90, 161)	0	59 (32, 101)
Yes	37	121 (89, 160)	100	83 (43, 138)
Current user in GW 17-20	18	121 (89, 160)	100	95 (50, 152)
Folic acid supplement (%)	75	121 (89, 159)	42	70 (36, 120)
Omega 3 supplement (%)	80	123 (91, 161)	42	69 (35, 119)
Maternal energy intake, median (IQR), MJ	9.3 (7.9, 11.0)			
Iodine from food, median (IQR), µg/day	122 (89, 161)			
<75	15	61 (51, 68)	37	41 (24, 72) [b]
75-149.9	54	112 (95, 129)	37	59 (31, 100) [b]
≥150	30	186 (165, 220)	37	70 (39, 116) [b]
Urinary creatinine, median (90% range) g/L[a]	0.74 (0.17, 1.90)			
UIC, median (IQR), µg/g creatinine[a]	91 (63, 139)			

[a] Urinary iodine concentration (UIC) was measured in a subsample of n=2001 pregnant women in mean gestational week 18.5 (SD: 1.3).

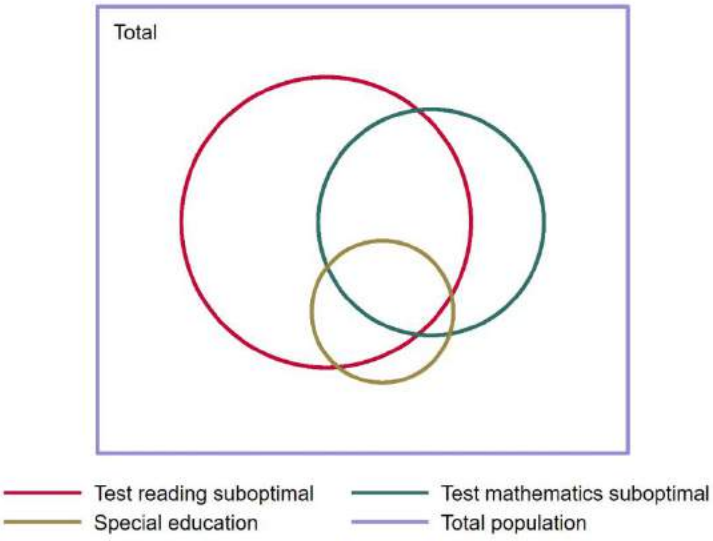
[b] In non-users of iodine-containing supplements (n=1208)



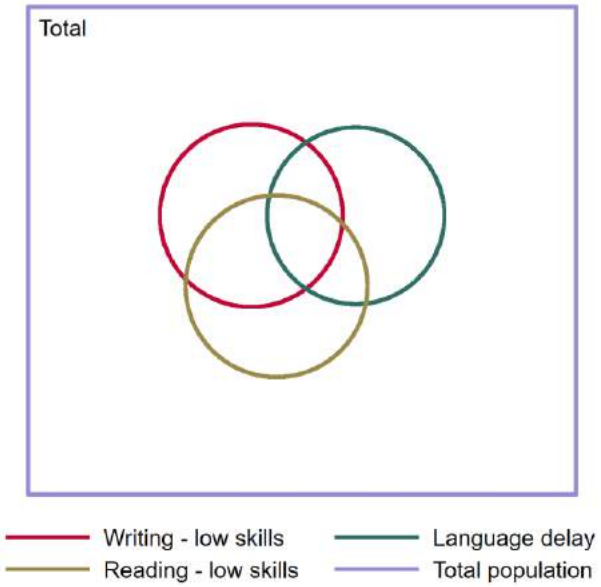
Supplementary Figure S2 The mean contribution to iodine intake by different food sources by categories of total iodine intake from food (n=29,471). The vertical axis show the iodine intake intervals (in µg/day) for the categories.



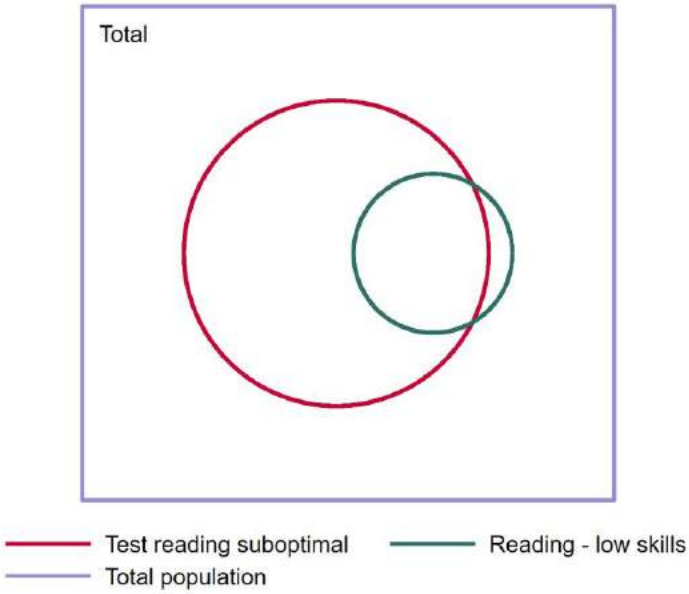
Supplementary Figure S3 Venn diagram illustrating the overlap of children scoring high on ADHD symptoms (18 items from the ADHD rating scale), children that were granted special education in school, and children with language delay (scoring above the 90th percentile on the Children's Communication Checklist, or mother reported language delay as a current health problem).



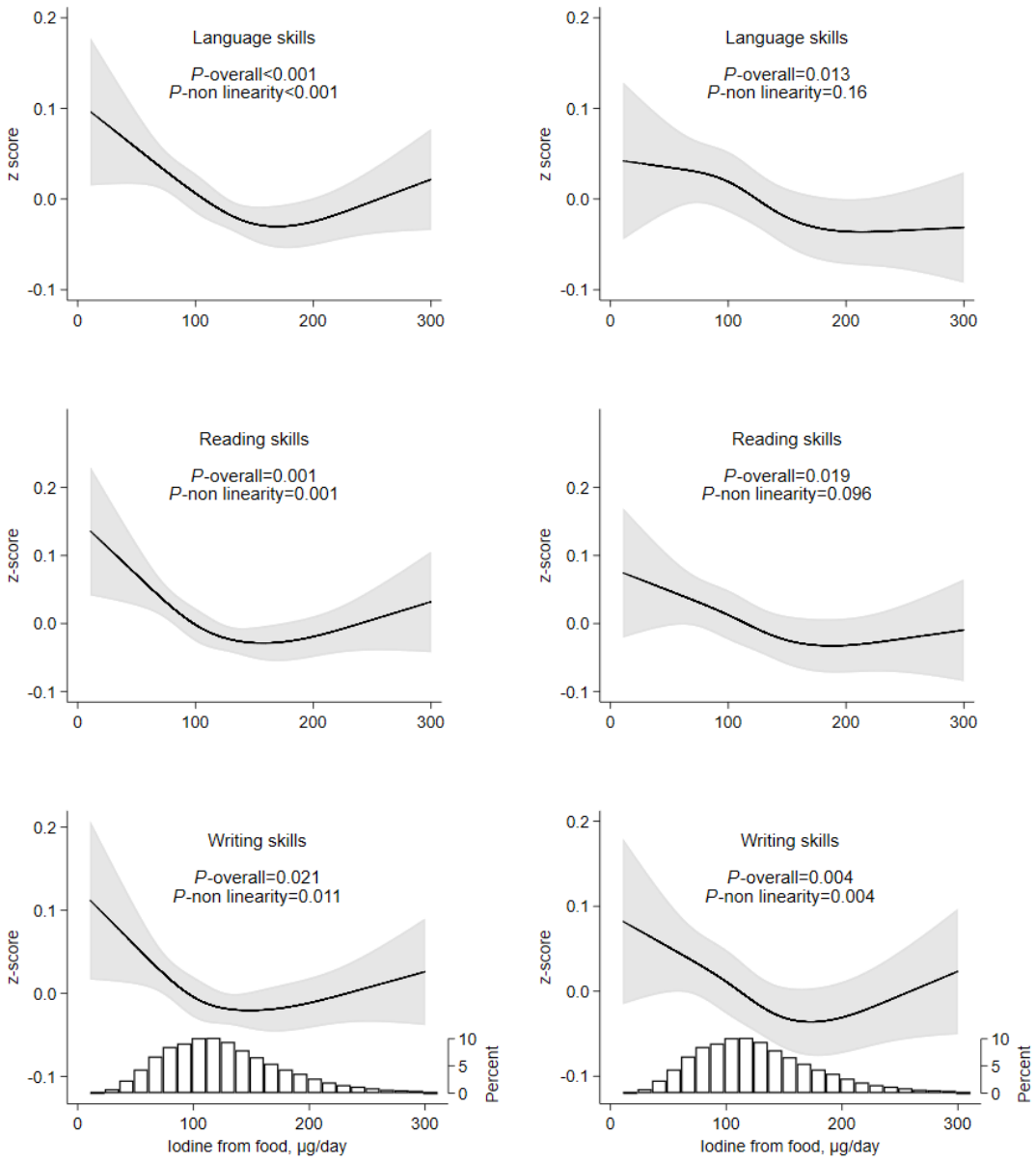
Supplementary Figure S4 Venn diagram illustrating the overlap of children granted special education in school, and children with suboptimal results on the mandatory mapping tests in reading and mathematics.



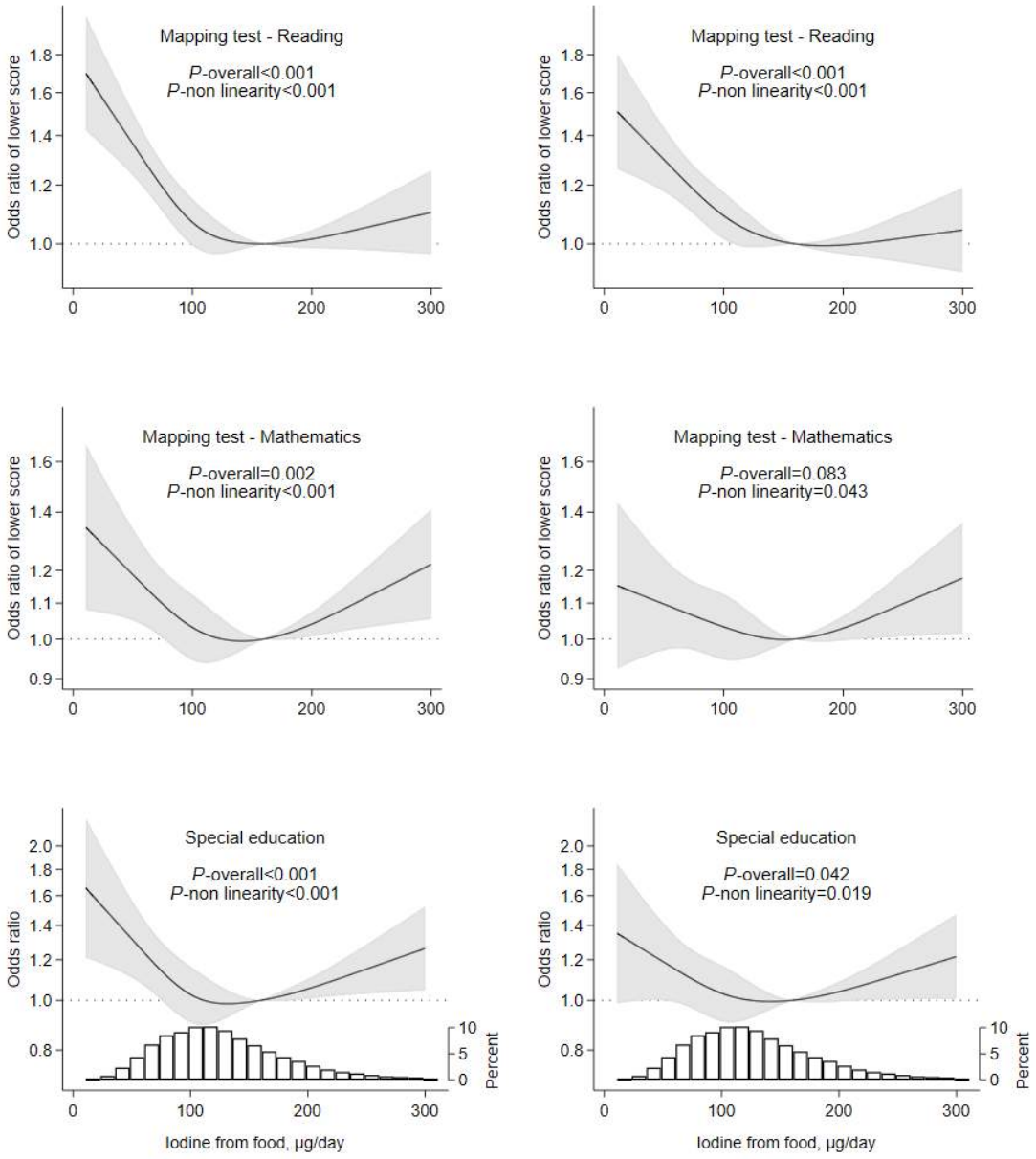
Supplementary Figure S5 Venn diagram illustrating the overlap of children with scores >90th percentile on maternally rated reading and writing skills (higher score for lower skills), and language delay (scoring above the 90th percentile on the Children's Communication Checklist, or mother reported language delay as a current health problem).



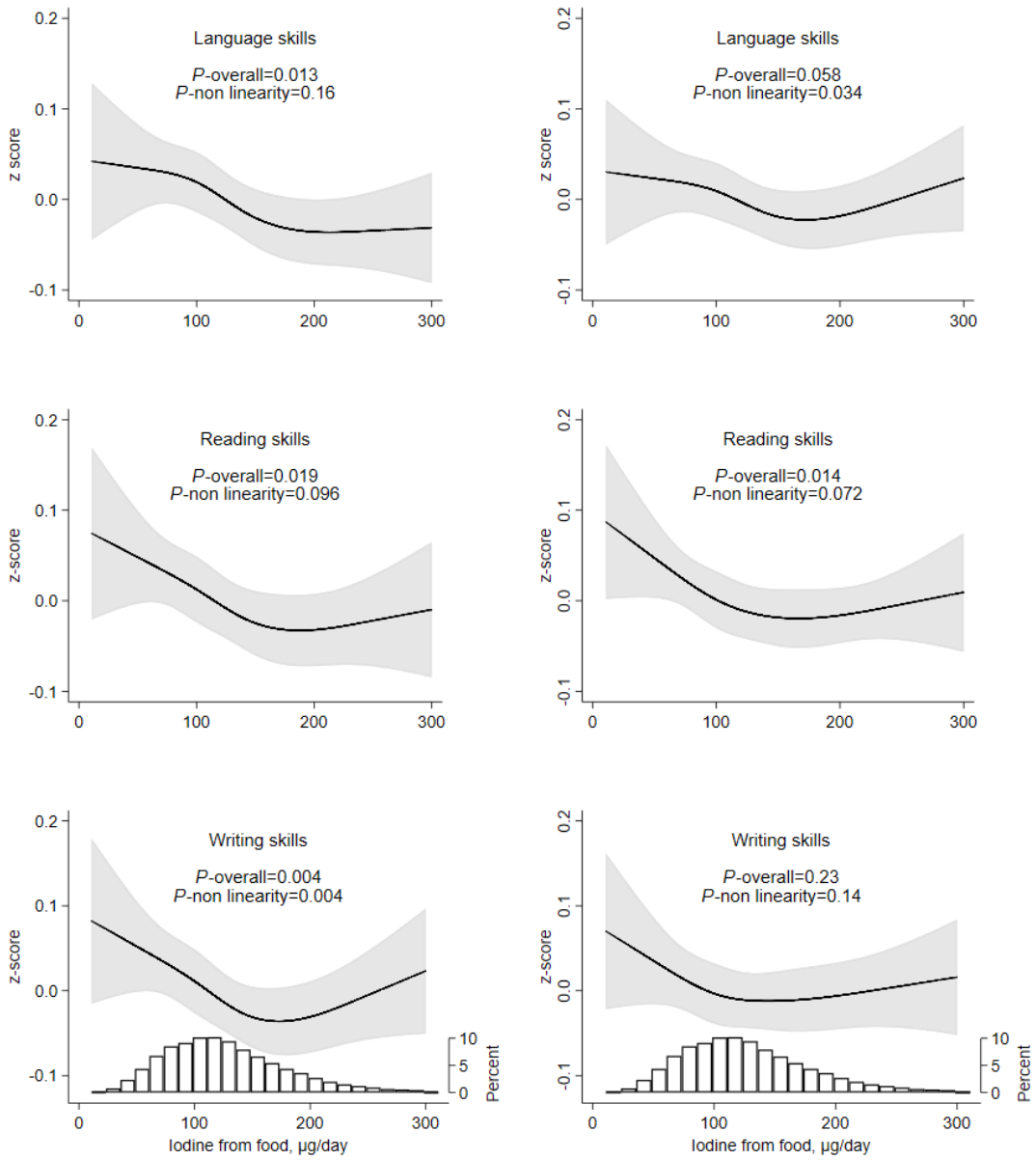
Supplementary Figure S6 Venn diagram illustrating the overlap of children with suboptimal results on the mandatory mapping tests in reading, and children with scores >90th percentile on maternally rated reading and writing skills (higher score for lower skills).



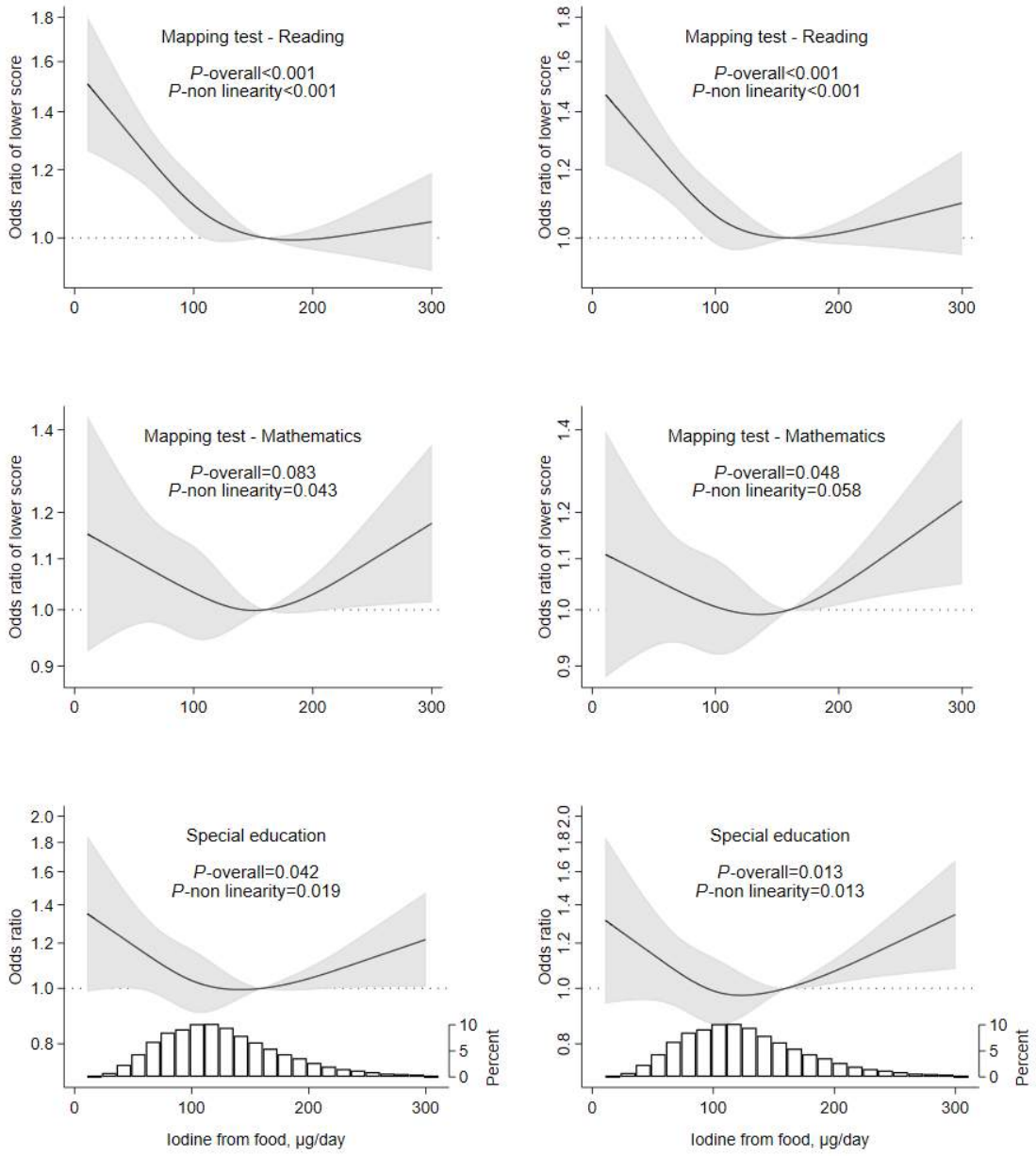
Supplementary Figure S7 Crude (left) and adjusted (right) associations of maternal iodine intake from food (in non-supplement users) and child language (n=24,643), reading (n=19,492), and writing skills (n=19,483) at age 8 years. Higher z-score indicate poorer skills. Associations were modelled flexibly (restricted cubic splines, four knots) and estimated by generalized linear regression. All models were adjusted for energy intake and random effects of sibling clusters. Adjusted models were additionally adjusted for maternal age, education, parity, pre-pregnancy BMI, fibre intake, smoking in pregnancy, child sex, bilingual parent(s) (for the language outcome), and maternal history of reading/writing difficulty (for read/write scores). The histogram represents the distribution of iodine intake from food. Missing data on covariates were imputed (4.2% of participants had missing data for one or more covariate).



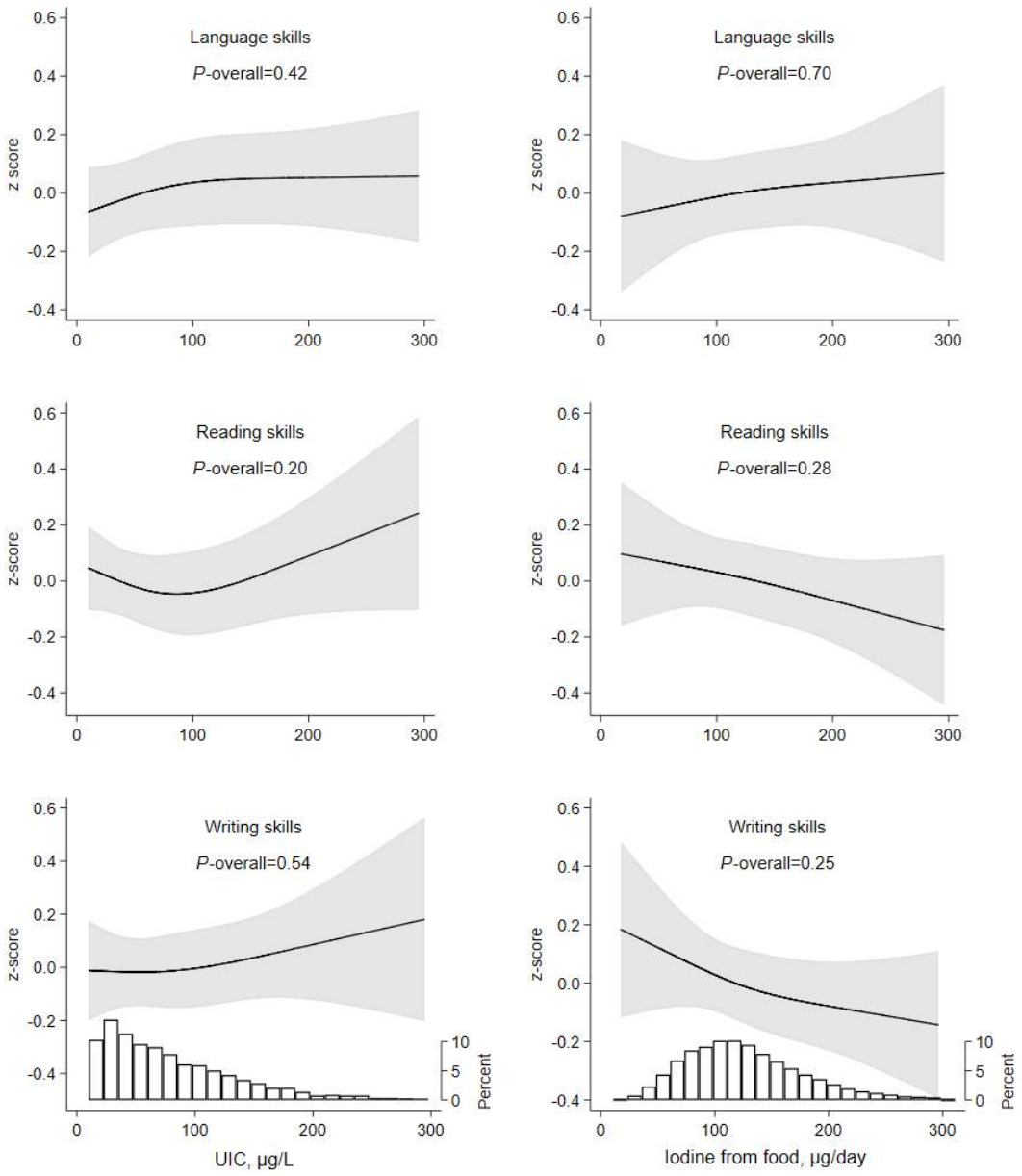
Supplementary Figure S8 Crude (left) and adjusted (right) associations of maternal iodine intake from food (in non-supplement users) and school outcomes at age 8 years. Higher odds ratio indicate poorer test results/increased likelihood of receiving special educational services. The reference level (OR=1) was set to 160 µg/day, the estimated average requirement in pregnancy by the Institute of Medicine. Associations were modelled flexibly (restricted cubic splines, four knots) and estimated by ordered logistic regression for the mapping tests (n=24,309 for reading and n=23,527 for mathematics) and by logistic regression for special education (n=24,806). All models were adjusted for energy intake and random effects of sibling clusters. Adjusted models were additionally adjusted for maternal age, education, parity, pre-pregnancy BMI, fibre intake, and smoking in pregnancy. The histogram represents the distribution of iodine intake from food. Missing data on covariates were imputed (4.2% of participants had missing data for one or more covariate).



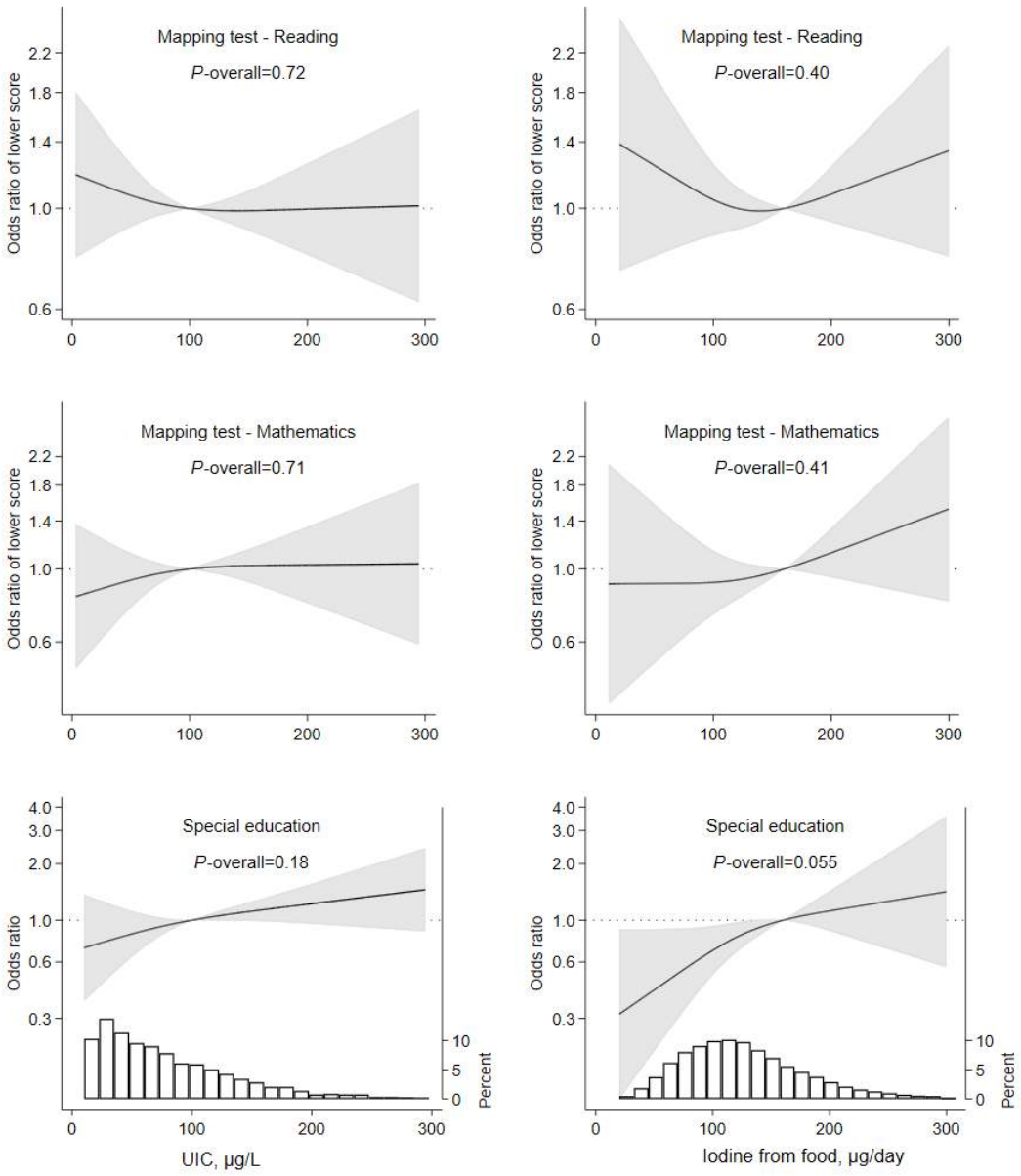
Supplementary Figure S9 Adjusted models (same as in the manuscript) to the left and **models additionally adjusted for maternally reported ADHD symptoms at age 8 years to the right**. Associations of maternal iodine intake from food (in non-supplement users) and child language (n=24,643), reading (n=19,492), and writing skills (n=19,483) at age 8 years. Higher z-score indicate poorer skills. Associations were modelled flexibly (restricted cubic splines, four knots) and estimated by generalized linear regression. All models were adjusted for maternal age, education, parity, pre-pregnancy BMI, energy intake, fibre intake, smoking in pregnancy, child sex, bilingual parent(s) (for the language outcome), maternal history of reading/writing difficulty (for read/write scores), and for random effects of sibling clusters. The histogram represents the distribution of iodine intake from food. Missing data on covariates were imputed (4.2% of participants had missing data for one or more covariate).



Supplementary Figure S10 Associations of maternal iodine intake from food (in non-supplement users) and school outcomes at age 8 years. Adjusted models (same as in the manuscript) to the left and **models additionally adjusted for maternally reported ADHD symptoms at age 8 years to the right**. Higher odds ratio indicate poorer test results/increased likelihood of receiving special educational services. The reference level (OR=1) was set to 160 µg/day, the estimated average requirement in pregnancy by the Institute of Medicine. Associations were modelled flexibly (restricted cubic splines, four knots) and estimated by ordered logistic regression for the mapping tests (n=24,309 for reading and n=23,527 for mathematics) and by logistic regression for special education (n=24,806). Models were adjusted for maternal age, education, parity, pre-pregnancy BMI, energy intake, fibre intake, and smoking in pregnancy. The histogram represents the distribution of iodine intake from food. Missing data on covariates were imputed (4.2% of participants had missing data for one or more covariate).



Supplementary Figure S11 In subsample of non-users of iodine-containing supplements with available data on urinary iodine concentration (mean gestational week mean: 18.5, SD: 1.3): **Maternal urinary iodine concentration (left column), iodine intake from food by the food frequency questionnaire (right column) and maternally reported child skills** (language (n=1158), reading (n=1116), and writing (n=1117)). Higher z-score indicate poorer skills. Models were adjusted for maternal age, education, parity, pre-pregnancy BMI, energy intake, fibre intake, smoking in pregnancy, child sex, bilingual parent(s) (for the language outcome), maternal history of reading/writing difficulty (for read/write scores), and for random effects of sibling clusters. Associations were modelled for complete cases (no missing covariates). The histograms represent the distribution of UIC and of iodine intake from food. Results were similar when using UIC adjusted for hydration status (i.e. UIC in µg/g creatinine), and when also including the iodine supplement users (results not shown).



Supplementary Figure S12 In a subsample of non-users of iodine-containing supplements with available data on urinary iodine concentration (mean gestational week mean: 18.5, SD: 1.3): **Maternal urinary iodine concentration (left column), iodine intake from food by the food frequency questionnaire (right column) and school outcomes** (reading (n=1145), mathematics (n=1110), and special education (n=1162)). Higher odds ratio indicate poorer test results/increased likelihood of receiving special educational services. The reference level (OR=1) was set to 160 µg/day, the estimated average requirement in pregnancy by the Institute of Medicine. Models were adjusted for maternal age, education, parity, pre-pregnancy BMI, energy intake, fibre intake, and smoking in pregnancy. Associations were modelled for complete cases (no missing covariates. The histograms represent the distribution of UIC and of iodine intake from food.). Results were similar when using UIC adjusted for hydration status (i.e. UIC in µg/g creatinine), and when also including the iodine supplement users (results not shown).

Supplementary Table S2 Use of iodine-containing supplements in pregnancy and child language and learning at 8 years

	n	Crude models[a]	Adjusted models[b]
Language skills	39,229	Standardized beta (95% CI)	Standardized beta (95% CI)
Any iodine supplement use GW 0-22	14,586	- 0.02 (-0.04, 0.00)	0.00 (-0.02, 0.02)
First report of iodine supplement			
Never (non-supplement user)	24,643	0 (ref.)	0 (ref.)
Before pregnancy[c]	4076	-0.03 (-0.06, 0.00)	0.00 (-0.03, 0.03)
GW 0-12	3521	-0.04 (-0.07, 0.00)	0.00 (-0.03, 0.03)
GW >12	2642	-0.04 (-0.08, 0.00)	-0.02 (-0.06, 0.01)
Reading skills	31,822	Standardized beta (95% CI)	Standardized beta (95% CI)
Any iodine supplement use GW 0-22	12,330	-0.03 (-0.05, 0.00)	- 0.01 (-0.03, 0.01)
First report of iodine supplement			
Never (non-supplement user)	19,492	0 (ref.)	0 (ref.)
Before pregnancy[c]	3466	-0.05 (-0.08, -0.01)	-0.02 (-0.05, 0.02)
GW 0-12	2976	-0.01 (-0.05, 0.03)	0.02 (-0.02, 0.06)
GW >12	2138	-0.06 (-0.10, -0.02)	-0.04 (-0.08, 0.00)
Writing skills	31,812	Standardized beta (95% CI)	Standardized beta (95% CI)
Any iodine supplement use GW 0-22	12,329	-0.04 (-0.06, -0.01)	- 0.01 (-0.04, 0.01)
First report of iodine supplement			
Never (non-supplement user)	19,483	0 (ref.)	0 (ref.)
Before pregnancy[c]	3467	-0.04 (-0.08, -0.01)	-0.03 (-0.07, 0.00)
GW 0-12	2974	-0.01 (-0.05, 0.02)	0.02 (-0.02, 0.06)
GW >12	2138	-0.05 (-0.09, -0.01)	-0.03 (-0.07, 0.02)
Mapping test - Reading	38,659	Odds ratio (95% CI)	Odds ratio (95% CI)
Any iodine supplement use GW 0-22	14,350	0.93 (0.89, 0.98)	0.98 (0.94, 1.03)
First report of iodine supplement			
Never (non-supplement user)	24,309	1 (ref.)	1 (ref.)
Before pregnancy[c]	4,021	0.96 (0.89, 1.03)	1.02 (0.95, 1.11)
GW 0-12	3461	0.97 (0.90, 1.05)	1.02 (0.94, 1.11)
GW >12	2589	0.88 (0.81, 0.97)	0.91 (0.83, 1.00)
Mapping test – Mathematics	37,433	Odds ratio (95% CI)	Odds ratio (95% CI)
Any iodine supplement use GW 0-22	13,906	0.97 (0.92, 1.03)	0.99 (0.93, 1.04)
First report of iodine supplement			
Never (non-supplement user)	23,527	1 (ref.)	1 (ref.)
Before pregnancy[c]	3887	0.92 (0.84, 1.01)	0.96 (0.88, 1.06)
GW 0-12	3366	1.02 (0.93, 1.12)	1.04 (0.94, 1.14)
GW >12	2509	0.95 (0.85, 1.06)	0.97 (0.87, 1.08)
Special education	39,471	Odds ratio (95% CI)	Odds ratio (95% CI)
Any iodine supplement use GW 0-22	14,665	0.92 (0.85, 1.00)	0.96 (0.88, 1.04)
First report of iodine supplement			
Never (non-supplement user)	24,806	1 (ref.)	1 (ref.)
Before pregnancy[c]	4094	0.89 (0.78, 1.02)	0.95 (0.83, 1.09)
GW 0-12	3540	0.98 (0.85, 1.12)	1.03 (0.89, 1.18)
GW >12	2653	0.85 (0.72, 1.01)	0.87 (0.73, 1.02)

Standardized beta>0 and odds ratio>1 indicate poorer performance or increased likelihood of special education.

[a] All models (including crude models) were adjusted for random effects of sibling clusters.

[b] Models were adjusted for maternal age, BMI, parity, education, smoking in pregnancy, fibre intake, EPA and DHA intake, folic acid supplement within the interval from 4 weeks before to 8 weeks after conception, child sex (only models with continuous outcomes), bilingual parent(s) (only language outcome), and maternal history of reading/writing difficulty (only continuous outcomes for reading and writing skills).

[c] 1-26 weeks before conception

APPENDIX 1

Changes from the original protocol of the project - The ADHD Study

The ADHD Study is a substudy of MoBa, and n=1195 children were recruited between August 2007 and January 2011 at child age 3 years (1). Participants had a high mean score on 11 items on ADHD-symptoms (inattention, impulsivity, and hyperactivity) in the MoBa 3-year questionnaire (i.e. scoring >90 percentile or mother had reported hyperactivity as a current health problem, n=1048) or were randomly selected from MoBa (n=147 controls). Participants in the ADHD study had slightly older mothers, higher educational levels, and they had fewer children compared to all MoBa participants (2). The participation rate was 38% of the invited "cases" and 23% of invited randomly selected controls. Children participated in a 1-day clinical assessment where ADHD symptoms, IQ, working memory, executive functioning, and expressive language were investigated. The interviews and tests were conducted by psychologists, psychiatrists, or by trained psychology students.

In the original protocol for our study, we planned to base one of the research papers on clinically assessed neurodevelopmental outcomes measured in the ADHD study. This plan was abandoned after a thorough evaluation for several reasons:

1) **Sample size and statistical power calculations.** Based on results from our Paper 1 and from the ALSPAC-cohort (3), we estimated that there was not enough statistical power to detect significant associations in the ADHD study even if the study had been based on a random sample of MoBa participants. For example, to detect a 2 point IQ difference between the lowest quartile of maternal iodine intake (i.e. <81 µg/day) vs. higher iodine intake one would need a sample size of n=2300 children with alpha set to 0.05 (i.e. not adjusting for multiple comparison) and power to identify the difference set to 80%. The estimated study sample needed was even higher for ADHD symptoms (~9000 to detect a 0.07 SD difference) and for language score (~47,000 to detect a 0.03 SD difference).
2) **Selection bias.** The ADHD Study participants were a selected sample from MoBa scoring high on ADHD symptoms in the 3-year questionnaire, and there were few controls (only 12%). The items used for recruiting to the ADHD study were partially overlapping with the items used for score on externalizing behaviour problems in Paper 1. In Paper 1, we found that both maternal iodine intake from food and iodine supplement use in pregnancy was

associated with child behaviour problems at 3 year. Thus, maternal iodine status in pregnancy was associated with the likelihood of being recruited to the ADHD study. Indeed, we found that the maternal iodine intake from food was lower in pregnancy in ADHD study participants compared to the whole MoBa (median 108 µg/day vs. median 121 µg/day). The selected sample for the ADHD study not only reduces the variation in the outcomes measures, but it also leads to selection bias. Since the likelihood of being in the study sample was both related to our exposures of interest as well as to the outcomes measured, the selection bias introduced is not possible to estimate or control for. The reduced variation in the measured outcomes would probably contribute to attenuate potential associations.

3) **Too few controls in the ADHD study** made it impossible to perform a case-control design. Also, the control group probably did not represent a random sample of MoBa children because of the low participation rate. The investigators speculated that worried mothers of more difficult children would be more likely to participate in the clinical examination.
4) **The opportunity to explore ADHD diagnosis from NPR as an alternative outcome**. We were contacted by another project group who had access to data on ADHD diagnosis from the NPR (now included in Paper 2). They wanted us to collaborate with them to explore this outcome in relation to maternal iodine nutrition. We decided to prioritize this over the ADHD study-data since we then would have a much larger sample size (n=77,164), and since this did not represent a selected sample from MoBa.

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APPENDIX 2

Mild-to-moderate iodine deficiency (MID) in pregnancy and child neurodevelopment, -results from observational studies in humans[1]

Study	Subjects	Exposures and outcomes	Main results	Comments to the study
Robinson et al. 2018 (1) The United Kingdom The Southampton Women's Survey	**N=654** mother-child pairs Median maternal pre-pregnancy UIC was **108 µg/L**	A spot UIC was collected 2-5 years before conception (mean 3.3), and outcomes were: Offspring IQ (Wechsler Abbreviated Scale of Intelligence) and executive functioning (the Cambridge Neuropsychological Test Automated Battery) at age 6-7 y.	Preconception I/Cr ratio was associated with child IQ [β = 0.13 (95% CI: 0.04, 0.21)/SD, P = 0.003]. Children of mothers who had I/Cr<50µg/g (8.9%) had 0.49 (95% CI: 0.79, 0.18) SD lower IQ score. No association was seen for executive functioning.	A spot urine sample collected years before conception represents a very poor measure of maternal iodine status in pregnancy. Adjusting UIC by creatinine might have introduced other dimensions into the exposure variable causing confounding. Exclusion of pre-term births (GW<37) might have introduced bias since risk of preterm birth might represent a mediator. The non-consistent results of IQ and executive functioning were unexpected, but might be due to low power to detect differences. A strength is that they adjusted for maternal IQ.

[1] The table includes relevant human studies identified through our literature search reporting associations between maternal iodine intake in/before pregnancy (not iodine supplement use specifically) and measures of child neurodevelopment. Studies in severely iodine deficient populations are not included in the table.

Study	Subjects	Exposures and outcomes	Main results	Comments to the study
Murcia et al. 2017 (2) Spain The INMA cohort	**N=1803** mother-child pairs Median UIC (end of first trimester): **123 µg/L** Mean calculated iodine intake (FFQ): 161 µg/day	Exposures were crude UIC and UIC adjusted for creatinine measured in spot-urine samples collected before GW24 (mean 13.5, SD 2), and iodine intake from food (Two FFQs GW 10-13 and 28-32). Child cognitive and motor function was measured by McCarthy Scales of Children's Abilities at mean age 4.8 (SD 0.6) y. Potential confounders were included if they changed effect estimates by ≥10%.	Iodine intake was not associated with cognitive outcomes, but negatively associated with motor score. Crude UIC was not associated with the outcomes, but UIC adjusted for creatinine was. Children of mothers with UIC~Cr<100µg/L had poorer cognitive and fine motor scores (general cognitive score: -3.93 (95% CI: -6.18, -1.69)) compared to UIC~Cr 100-249µg/L.	Median UIC in the study was quite high, thus this may represent an iodine sufficient population. No clear trends were seen for crude UIC and outcomes, and UIC was only significantly associated to the outcomes after adjusting for creatinine. Authors state that urinary creatinine by itself was associated with the outcomes. This might explain why UIC~Cr was also associated. Using the residual method for creatinine adjustment does not remove confounding by creatinine, opposite to what the authors suggest.
Hynes *et al*. 2013 (3) and 2017 (4) Australia (Tasmania) The Gestational Iodine Cohort	Pregnant women (singleton) (**n=228/266**) and their children up to age 14-15y Median maternal UIC **81µg/L** Mean gestational age at urine collection was 24.6 weeks (range 8–41 weeks)	UIC<150µg/L vs. ≥150µg/L (mean of 1-3 spot urine samples in pregnancy) Outcomes: Results on NAPLAN tests at school year 3, 5, 7, and 9: standardized criteria-referenced measures of individual student's performance in literacy (reading, writing, and language conventions [spelling, grammar, and punctuation]). Testing is conducted annually in all schools by the Australian Federal Government. In a subgroup (n=45 aged 13-14y), a more comprehensive language test (CELF-4) and hearing test (CAPD) was performed. Confounders: gestational age at urine collection, maternal age, gestational length, birth weight, sex, maternal education and maternal occupation	UIC<150 (71%) vs. ≥150µg/L (29%): In fully adjusted analysis only spelling remained significant and indicated 10% lower performance while grammar and English literacy were borderline significant. For all but one outcome (numeracy) estimated β's were negative, but p>0.05. For spelling, the difference in performance remained significant from school year 3 to 9 (remained 6% lower in ID group). CELF-4 scores in children of MID mothers were generally lower, but the differences were not significant.	Maternal-, paternal- and pregnancy characteristics were similar in the two exposure groups. Most women had only one spot urine sample taken. They did not collect information on use of iodine supplements in the study. Measures of school performance were consistently lower for all except for one outcome (1/7) in children of MID mothers. Spelling being the only remaining significant outcome after adjusted analysis might be due to the small size of the study (type II error).

Study	Subjects	Exposures and outcomes	Main results	Comments to the study
Ghabassian *et al*, 2014 (5) The Netherlands The Generation R Cohort	**N=1525** pregnant women and their children at age 6y Median maternal UIC **230 µg/L** assessed in GW<18	UIC<150 µg/g creatinine (12% of mothers) vs. ≥150, mean GW 13 (range 6-17) Outcomes: Non-verbal IQ (Snijders-Oomen Niet-verbale intelligentie Test-Revisie) and receptive language (Taaltest voor Kinderen)	In the adjusted models, there were no differences in child IQ score or language scores between low and adequate maternal UIC. Additional adjustment for maternal thyroid parameters (TSH and FT4) did not change the results.	This was an iodine sufficient population and most likely there were few truly iodine deficient in the group with spot UIC<150 µg/g creatinine. Dichotomization of the continuous exposure reduced the statistical power. Adjusting by creatinine may have introduced bias.
Bath *et al.* 2013 (6) The United Kingdom The ALSPAC cohort	Pregnant women (singleton, ≤13 weeks' gestation) (**n=1040**) and their children tested at age 8 and 9 Maternal median UIC **91µg/L** (median 110 µg/g creatinine)	Women's UIE: <150µg/g creatinine (67%) vs. ≥150µg/g (33%) and association with: IQ at 8y: Abbreviated form of the Wechsler Intelligence Scale for Children Reading ability at 9y: Trained psychologists assessed children's reading speed, accuracy, and comprehension with the Neale Analysis of Reading Ability Confounders: Adjusted for 21	UIE<150µg/g creatinine vs. ≥150µg/g: Increased risk of being in the lowest quartile for verbal IQ (OR 1.58, 95% CI 1.09–2.30; p=0.02), reading accuracy (1.69, 1.15–2.49; p=0.007), and reading comprehension (1.54, 1.06–2.23; p=0.02). ID was more strongly associated with a lower IQ cutoff (< 85) (OR 2.69, 95% CI 1.21–5.98). A dose-response relationship was seen when comparing <50 to 50-150 and ≥150µg/g creatinine. Analysis with continuous exposure and outcome variables showed similar result.	A single spot urine sample does not provide a good measure of iodine status at an individual level. High risk of misclassification of iodine status which is further increased by categorization of the variable. They might have (over-)adjusted for covariates in the causal pathway (family adversity index (pregnancy), mothers parenting score (6m), HOME score (6m), low birth weight, preterm birth, maternal depression since birth). However, adjustment for up to 21 potential confounders did not significantly alter the results. There was no information on iodine supplement use, and supplement users were not excluded. However, according to the authors (personal communication), supplemental iodine was not commonly used at the time.

Study	Subjects	Exposures and outcomes	Main results	Comments to the study
Hamza *et al.* 2013 (7) Egypt	**N=100** mother-child pairs (50 children with autism and 50 controls). Child age was 3-8 y. By design, controls could not be iodine deficient. Mean UIC: **83 µg/L** in mothers of autistic children and **152 µg/L** in mothers in control group	This was a cross-sectional study, and maternal- and child spot UIC was collected at child age 3-8y. Maternal and child UIC was assumed to represent indicators of maternal iodine status in pregnancy. Outcomes: Severity of autism (Childhood autism rating scale), child IQ (WISC-III), brain electrical activity (inter-ictal EEG), thyroid function (TSH, FT4, FT3), and thyroid volume (ultrasound).	Maternal UIC and autistic child UIC was highly correlated (r=0.88). Child UIC was inversely correlated with severity of autism in the autistic children (r=-0.94). There were no significant findings for thyroid function or thyroid volume. In autistic children, UIC and FT4 was lower in moderately mentally retarded (IQ 35-49) compared to mild mentally retarded (IQ 50-69).	Maternal and child UIC at child age 3-8 y may represent a poor indicator of maternal iodine status in pregnancy. The extreemely high correlation between child symptoms of autism and child UIC is unlikely to reflect the true association (although it might still be an association). The measurement error of child UIC in reflecting maternal iodine status in pregnancy is large, thus the high correlation must be explained, at least partly, by coincidence. Iodine deficient were excluded from the control group, which made the control group less suitable for comparison of iodine status. There may be reverse causality since severity of autism might affect the diet of the mothers and children (in this case the use of iodized salt).
van Mil *et al.* 2012 (8) The Netherlands The Generation R cohort	Pregnant women (singleton, not TPO-ab+) and their children at 4y (**n=692 pairs**) Median maternal UIC **203µg/L**	Women's spot UIE: <10 percentile (corresponding to range 49-136 µg/g creatinine) vs >136 µg/g) and association with: Executive functioning at 4 y: BRIEF-P (questionnaire) with 5 scales (inhibition, shifting, emotional control, working memory and planning/organization)	Low maternal UIC was associated with higher standardized scores on the problem scales of inhibition [β = 0.05 (95% CI: 0.01, 0.10), p = 0.03] and working memory [β = 0.07 (95% CI: 0.02, 0.12), P = 0.003]. Problems of inhibition were attenuated after adjustment for maternal psychological symptoms Authors stated that prenatal psychological problems was a strong confounder.	A single spot urine sample does not provide a good measure of iodine status at an individual level. High risk of misclassification, especially since the study is conducted in an iodine sufficient area. Thus, the 10% of women who had UIC<136 µg/g creatinine were most likely not truly iodine deficient. They might have (over-)adjusted for covariates that were in the causal pathway or represented indicators of the exposure (prenatal psychological problems, gestational age at birth, birth weight, mode of delivery and Apgar score).

Study	Subjects	Exposures and outcomes	Main results	Comments to the study
Vermiglio *et al.* 2004 (9) Italy, Sicily	**N=27** mother-child pairs (16 from a moderately ID area (A) and **11** from a marginally ID area (B) (UIC in school-aged children in areas A and B: **48 vs. 95µg/L** at the time of pregnancy)	Maternal thyroid function was measured in pregnancy (3 times). Child ADHD screening (DSM-IV-TR) and test of full-scale IQ (WISC-III) was performed at child age 8-10y.	ADHD was diagnosed in 11 of the 16 children from the moderately ID area, none in area B. IQ scores were lower in the children from area A compared to B (mean IQ 92 vs. 110). Hypothyroxinaemia was found in early pregnancy in 8 of the women in area A and only transiently in 1 women in area B. Seven of the 11 children with ADHD were born to mothers who were hypothyroxinaemic in pregnancy. A negative correlation was documentet between maternal FT4 at mid gestation and child IQ.	This is a small (n=27) and non-randomized study comparing dyads from different regions. Other factors might have deviated in addition to iodine status causing residual confounding. There were no measures of iodine status at the individual level. The control group (region B) also had suboptimal iodine status. There were no adjustments for covariates in the analyses.
Aghini Lombardi *et al.* 1995 (10) Italy, Tuscany	**Study 1:** **N=107** schoolchildren from a moderately ID area vs. **N=106** schoolchildren from a close to adequate iodine area (UIC **64 vs. 142 µg/L** in school-aged children). **Study 2:** **N=30** children born before iodine prophylaxis and **N=27** born after (**UIC 32 vs. 109 µg/L**), compared with **N=29 and 27** age-matched controls from an iodine sufficient area.	Three different tests were performed measuring child neurodevelopment: 1) The block design sand coding subtest of the WISC-R measuring the general neuropsychological and cognitive functions 2) Reaction time measuring the efficiency of information processing and nervous transmission mechanisms unrelated to cognitive processes.	There was no difference in performance on the cognitive tests (WISC-R), but children born to mothers who were pregnant in ID areas (or before iodine prophylaxis) had significantly slower reaction times.	This is a relatively small, non-randomized study which is probably underpowered to detect differences in neurocognitive development potentially caused by prenatal MID. Although the researchers applied two different study designs to compare exposed vs. controls, one cannot rule out the possibility of residual confounding by other factors associated with living in the different regions (ID vs. adequate, study 1) or time of birth (before vs. after iodine fortification, study 2). Only group-mean test-scores are reported. They did not control for other factors when testing differences in means between the groups.

Abbreviations: ADHD: attention-deficit/hyperactivity disorder, Cr: creatinine, FT4: Free thyroxine, GW: gestational week, ID: iodine deficiency, IQ: intelligence quotient, MID: mild-to-moderate ID, TSH: Thyroid stimulating hormone, UIC: urinary iodine concentration, UIE: urinary iodine excretion

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