# Maternal thyroid disease in Pregnancy

*Nationwide investigations on the occurrence and outcomes*

Andersen, Stine Linding

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# MATERNAL THYROID DISEASE IN PREGNANCY

NATIONWIDE INVESTIGATIONS ON
THE OCCURRENCE AND OUTCOMES

**BY**
**STINE LINDING ANDERSEN**

DISSERTATION SUBMITTED 2018

AALBORG UNIVERSITY
DENMARK

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## NATIONWIDE INVESTIGATIONS ON THE OCCURRENCE AND OUTCOMES

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Stine Linding Andersen

AALBORG UNIVERSITY
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Aalborg, February 7, 2019.

Lars Hvilsted Rasmussen
Dean

# CURRICULUM VITAE

Stine Linding Andersen obtained her medical degree at Aarhus University in 2011. She was a PhD student in the Department of Endocrinology, Aalborg University Hospital, and obtained her PhD degree at Aalborg University in 2015. Her PhD dissertation included four published papers and was entitled 'Iodine status in pregnant and breastfeeding women. A Danish Regional Investigation'. In relation to her work on iodine and pregnancy, she initiated her work on thyroid disease and pregnancy using Danish nationwide registers. She started her medical specialist training within clinical biochemistry in 2014 in the Department of Clinical Biochemistry, Aalborg University Hospital. This employment facilitated her research interest in the combined use of nationwide register data and measurements of maternal thyroid function in pregnancy. She has contributed with national and international lectures at scientific meetings within thyroidology and clinical biochemistry, and she has received national and international awards for her work on iodine, thyroid and pregnancy. Her research within this field and her medical specialist training in clinical biochemistry are currently still in progress.

# ENGLISH SUMMARY

Thyroid disease in women of reproductive age is mainly of autoimmune origin. Pregnancy initiates a number of physiological changes in the maternal immune system and thyroid function, which influence the occurrence of thyroid disease in and around pregnancy and challenge the interpretation of thyroid function tests. The doctoral dissertation includes a series of nationwide investigations on the occurrence and outcomes of maternal thyroid disease in pregnancy. The findings illustrate that the incidence of maternal hyperthyroidism and hypothyroidism varies considerably in and around pregnancy and that the early pregnancy and the postpartum period may trigger the onset of disease. Another finding in the early pregnancy was the dynamic changes in maternal thyroid function parameters, which possibly influence the definition of reference ranges in pregnancy. Environmental factors have been associated with the development of autoimmune thyroid disease, and studies in the doctoral dissertation indicate that maternal smoking, alcohol intake, body mass index, origin, and iodine intake influence the occurrence of thyroid disease in and around pregnancy. The occurrence of maternal thyroid disease in and around pregnancy was evaluated in the Danish National Birth Cohort, which was established from 1997 to 2003. At that time, four percent of Danish pregnant women had thyroid disease diagnosed and treated before, during or in the years following the pregnancy. When maternal thyroid function was measured in stored blood samples from the early pregnancy, altogether 12% of the pregnant women had some degree of abnormal thyroid function, which was often undetected and untreated. Thyroid hormones are considered crucial developmental factors involved in the regulation of early brain development, and a hypothesis of fetal programming by maternal thyroid disease has been proposed. Experimental studies and observations in humans have shown profound mental and physical disabilities in children exposed to severe and untreated maternal hypothyroidism. On the other hand, the impact of smaller aberrations in maternal thyroid function is less clear. The main finding in the outcome studies included in the doctoral dissertation was an adverse association between severe and undetected maternal hypothyroidism and child intelligence at the age of five years, whereas no consistent associations emerged with smaller deviations in maternal thyroid function. The treatment of choice for thyroid dysfunction in pregnant women is medical treatment. Another outcome investigated in the doctoral dissertation was the risk of severe side effects to the use of antithyroid drugs for the treatment of hyperthyroidism pregnancy. Results raise concern about the use of Methimazole and Carbimazole in early pregnancy, because maternal use of these drugs was associated with a risk of severe birth defects in the offspring. Birth defects were also described after exposure to the other available antithyroid drug, Propylthiouracil, but these malformations were less severe and confined to specific organ systems. The findings challenge the clinical guidance for the management of thyroid disease in pregnant women and the choice of treatment.

# DANSK RESUME

Thyroidea sygdom hos kvinder i fødedygtig alder er primært autoimmunt betinget. Under graviditet sker en række fysiologiske ændringer i kvindens immunsystem og i skjoldbruskkirtlens funktion, hvilket påvirker forekomsten af thyroidea sygdom under og efter en graviditet og vanskeliggør tolkningen af thyroidea analyser. Doktorafhandlingen inkluderer en række nationale studier omhandlende forekomsten og betydningen af thyroidea sygdom hos moderen under graviditet. Fundene i doktorafhandlingen illustrerer, at hyppigheden af nydiagnosticeret for højt stofskifte (hyperthyroidisme) og for lavt stofskifte (hypothyroidisme) hos moderen varierer væsentligt under og efter en graviditet og både i den tidlige graviditet og i årene efter en graviditet kan ses en øget forekomst. Et andet fund i den tidlige graviditet var dynamiske forandringer i moderens thyroidea funktionsparametre, som kan have betydning for, hvilket normalområde der anvendes til diagnostik af thyroidea sygdom hos gravide. Miljøfaktorer er associeret med forekomsten af autoimmun thyroidea sygdom og resultaterne af studier inkluderet i doktorafhandlingen indikerer, at moderens ryge- og alkoholvaner, kropsvægt, geografisk oprindelse og jodindtag influerer på forekomsten af thyroidea sygdom hos gravide. Forekomsten af thyroidea sygdom hos gravide blev undersøgt i den danske nationale fødselskohorte 'Bedre Sundhed i Generationer', som blev etableret i årene 1997 til 2003. På det tidspunkt var det fire procent af danske gravide, som havde en thyroidea sygdom, der var diagnosticeret og behandlet før, under eller i årene efter graviditeten. Ved analysering af de gravides thyroidea funktion i en blodprøve fra tidlig graviditet fandtes det imidlertid, at 12% havde en grad af abnorm thyroidea funktion, hvilket ofte var udiagnosticeret og ubehandlet. Thyroidea hormoner er vigtige udviklingsfaktorer og særligt involveret i reguleringen af den tidlige hjerneudvikling. Dette har ført til den hypotese, at thyroidea sygdom hos moderen under graviditet kan programmere fosteret til senere udvikling af sygdom. Eksperimentelle studier og observationer hos mennesker har påvist alvorlige fysiske og mentale handicaps hos børn født af mødre med svær, ubehandlet hypothyroidisme i graviditeten, men betydningen af mindre forstyrrelser i moderens thyroidea funktion under graviditet er fortsat uafklaret. Studier inkluderet i doktorafhandlingen beskriver en sammenhæng mellem svær, udiagnosticeret hypothyroidisme hos moderen i tidlig graviditet og lavere intelligens hos barnet i femårsalderen, men mindre abnormaliteter i moderens thyroidea funktion fandtes ikke at udgøre en entydig risiko. Behandling af thyroidea sygdom hos gravide er overvejende medicinsk, og hyperthyroidisme behandles med antithyroid medicin. Risikoen for alvorlige bivirkninger ved brug af antithyroid medicin under graviditet blev undersøgt i doktorafhandlingen. Det fandtes, at behandling med Thiamazol og Carbimazol i tidlig graviditet var associeret med alvorlige medfødte misdannelser hos børnene. Behandling med det andet præparat, Propylthiouracil, udgjorde også en risiko, men disse misdannelser var mindre alvorlige. Resultaterne bidrager til overvejelser omkring diagnostik og behandling af thyroidea sygdom hos gravide.

# ACKNOWLEDGEMENTS

The work included in the doctoral dissertation was carried out from 2012 to 2017 during my research fellowship in the Department of Endocrinology and medical specialist training in the Department of Clinical Biochemistry, Aalborg University Hospital. The opportunity to study thyroid disease in relation to pregnancy using the Danish nationwide registers emerged from initial contact in 2012 to Professor Jørn Olsen, Section of Epidemiology, Department of Public Health, Aarhus University, who kindly introduced me to the field of epidemiology and register-based research and provided data access. The initial work was carried out in collaboration with Professor Jørn Olsen and PhD Chunsen Wu who had excellent epidemiological knowledge and Professor Peter Laurberg, Department of Endocrinology, Aalborg University Hospital, who was an expert within clinical thyroidology. To extend our findings from the Danish nationwide register studies, we applied for access to biological specimens from pregnant women in the Danish National Birth Cohort for the measurement of maternal thyroid function in early pregnancy. I acknowledge the Steering Committee and the North Denmark Region Committee on Health Research Ethics for their interest and willingness to approve the study. I also thank staff in the Danish National Biobank, Siemens Healthcare Diagnostics, biomedical laboratory technologist Lene Lundø Møller, and Peter Hindersson, head of the Department of Clinical Biochemistry in the North Denmark Regional Hospital, who all provided invaluable assistance during the handling and analyzing of samples. I thank the data managers at Statistics Denmark and in Aarhus for their help with access and linkage to other data sources. I thank PhD Zeyan Liew and statistician Søren Lundbye-Christensen for inspiring talks during the work with the neurocognitive outcome data, and I thank Stefan Lönn and Ove Törring, Sweden, for great collaboration. I am grateful to my colleagues in the Department of Clinical Biochemistry, Aalborg University Hospital, who showed great interest in my research and willingness to provide time for the writing of the doctoral dissertation. I am thankful to my current and previous colleagues in the Department of Endocrinology, Aalborg University Hospital, especially Maggie Bloch, Ingelise Leegaard, Anne Krejbjerg, Louise Kærholm Schæbel, Inge Bülow Pedersen, Allan Carlé, Jesper Karmisholt and Peter Vestergaard. Special thanks to Professor Stig Andersen, Department of Geriatrics, Aalborg University Hospital. Most of all, I am deeply thankful to professor Peter Laurberg who introduced me to the field of iodine and thyroid research and was my supervisor during my PhD, and a close collaborator during the work within the field of thyroid and pregnancy. Peter Laurberg tragically died in an accident in June 2016. Enthusiasm, friendliness and invaluable knowledge were some of his characteristics, and he is greatly missed. His interest in our findings related to the field of thyroid and pregnancy was unique, and he was so excited to see the next results. Such memories have provided the courage to carry the work further as a young scientist. Last, but not least, I am thankful to my husband, family and friends.

# LIST OF ABBREVIATIONS

| | |
|---|---|
| **ATD** | Antithyroid drugs |
| **BMI** | Body mass index |
| **CMZ** | Carbimazole |
| **CRS** | Civil Registration System |
| **D3** | Type 3 iodothyronine deiodinase |
| **DNBC** | Danish National Birth Cohort |
| **DNHR** | Danish National Hospital Register |
| **DNPR** | Danish National Prescription Register |
| **DPCR** | Danish Psychiatric Central Register |
| **fT3** | Free triiodothyronine |
| **fT4** | Free thyroxine |
| **GD** | Graves' disease |
| **GP** | General practice |
| **hCG** | Human chorionic gonadotropin |
| **ICD** | International Classification of Disease |
| **IQ** | Intelligence quotient |
| **LDPS** | Lifestyle During Pregnancy Study |
| **L-T4** | Levothyroxine |
| **MBR** | Medical Birth Register |
| **MMI** | Methimazole |
| **PPT** | Postpartum thyroiditis |
| **PTU** | Propylthiouracil |
| **RCTs** | Randomized controlled trials |
| **SNPR** | Swedish National Patient Register |
| **SPDR** | Swedish Prescribed Drug Register |
| **T3** | Triiodothyronine |
| **T4** | Thyroxine |
| **TBG** | Thyroxine-binding globulin |
| **Tg-Ab** | Thyroglobulin antibodies |
| **TPO-Ab** | Thyroid peroxidase antibodies |
| **TPR** | Total Population Register |
| **TRAb** | TSH-receptor antibodies |
| **TSH** | Thyrotropin |
| **UK** | United Kingdom |
| **US** | United States |

# LIST OF PUBLICATIONS

LIST OF PUBLICATIONS INCLUDED IN THE DOCTORAL DISSERTATION

| | |
|---|---|
| **1.**<br><br>Ref. [1] | **Hyperthyroidism incidence fluctuates widely in and around pregnancy and is at variance with some other autoimmune diseases: a Danish population-based study**<br><br>Stine Linding Andersen, Jørn Olsen, Allan Carlé & Peter Laurberg.<br>*Journal of Clinical Endocrinology and Metabolism,100, 1164–1171, 2015.* |
| **2.**<br><br>Ref. [2] | **Hypothyroidism incidence in and around pregnancy: a Danish nationwide study**<br><br>Stine Linding Andersen, Allan Carlé, Jørn Olsen & Peter Laurberg<br>*European Journal of Endocrinology, 175, 387-393, 2016.* |
| **3.**<br><br>Ref. [3] | **Maternal thyroid disease in the Danish National Birth Cohort: prevalence and risk factors**<br><br>Stine Linding Andersen, Jørn Olsen, Peter Laurberg<br>*European Journal of Endocrinology, 174, 203-212, 2016.* |
| **4.**<br><br>Ref. [4] | **Dynamics and predictors of serum TSH and fT4 reference limits in early pregnancy: a study within the Danish National Birth Cohort**<br><br>Peter Laurberg*, Stine Linding Andersen*, Peter Hindersson, Ellen A. Nøhr & Jørn Olsen (*contributed equally to the work)<br>*Journal of Clinical Endocrinology and Metabolism, 101, 2484-2492, 2016.* |
| **5.**<br><br>Ref. [5] | **Early pregnancy thyroid function test abnormalities in biobank sera from women clinically diagnosed with thyroid dysfunction before or after pregnancy**<br><br>Stine Linding Andersen & Jørn Olsen<br>*Thyroid, 27, 451-459, 2017.* |
| **6.**<br><br>Ref. [6] | **Smoking reduces the risk of hypothyroidism and increases the risk of hyperthyroidism: evidence from 450,842 mothers giving birth in Denmark**<br><br>Stine Linding Andersen, Jørn Olsen, Chun Sen Wu & Peter Laurberg.<br>*Clinical Endocrinology, 80, 307-314, 2014.* |

| | |
|---|---|
| **7.**<br><br>Ref. [7] | **Maternal thyroid function in early pregnancy and child neurodevelopmental disorders: a Danish nationwide case-cohort study**<br><br>Stine Linding Andersen, Stig Andersen, Peter Vestergaard & Jørn Olsen<br>*Thyroid, 28, 537-546, 2018.* |
| **8.**<br><br>Ref. [8] | **Maternal thyroid function in early pregnancy and neuropsychological performance of the child at 5 years of age**<br><br>Stine Linding Andersen, Stig Andersen, Zeyan Liew, Peter Vestergaard & Jørn Olsen<br>*Journal of Clinical Endocrinology and Metabolism, 103 660-670, 2018.* |
| **9.**<br><br>Ref. [9] | **Birth defects after early pregnancy use of antithyroid drugs: a Danish nationwide study**<br><br>Stine Linding Andersen, Jørn Olsen, Chun Sen Wu & Peter Laurberg.<br>*Journal of Clinical Endocrinology and Metabolism, 98, 4373-4381, 2013.* |
| **10.**<br><br>Ref. [10] | **Severity of birth defects after Propylthiouracil exposure in early pregnancy**<br><br>Stine Linding Andersen, Jørn Olsen, Chun Sen Wu & Peter Laurberg.<br>*Thyroid, 24, 1533-1540, 2014.* |
| **11.**<br><br>Ref. [11] | **Antithyroid drug side effects in the population and in pregnancy**<br><br>Stine Linding Andersen, Jørn Olsen & Peter Laurberg<br>*Journal of Clinical Endocrinology and Metabolism, 101, 1606-1614, 2016.* |
| **12.**<br><br>Ref. [12] | **Birth defects after use of antithyroid drugs in early pregnancy: a Swedish nationwide study**<br><br>Stine Linding Andersen, Stefan Lönn, Peter Vestergaard & Ove Törring<br>*European Journal of Endocrinology, 177, 369-378, 2017.* |

None of the publications (paper 1-12 listed on page XI-XII and paper 13-18 listed on page XIII) have previously been included in a PhD dissertation and have not previously been submitted for evaluation of an academic degree or award at a Danish or foreign university.

LIST OF ADDITIONAL PUBLICATIONS NOT INCLUDED FOR SPECIFIC EVALUATION IN THE DOCTORAL DISSERTATION

| | |
|---|---|
| **13.**<br><br>Ref. [13] | **Spontaneous abortion, stillbirth and hyperthyroidism: a Danish population-based study**<br><br>Stine Linding Andersen, Jørn Olsen, Chun Sen Wu & Peter Laurberg.<br>*European Thyroid Journal, 3, 164-172, 2014.* |
| **14.**<br><br>Ref. [14] | **Hypothyroidism and pregnancy loss: comparison with hyperthyroidism and diabetes in a Danish population-based study**<br><br>Stine Linding Andersen, Jørn Olsen & Peter Laurberg<br>*Clinical Endocrinology, 85, 962-970, 2016.* |
| **15.**<br><br>Ref. [15] | **Low birth weight in children born to mothers with hyperthyroidism and high birth weight in hypothyroidism, whereas preterm birth is common in both conditions: a Danish National Hospital Register study**<br><br>Stine Linding Andersen, Jørn Olsen, Chun Sen Wu & Peter Laurberg.<br>*European Thyroid Journal, 2, 135-144, 2013.* |
| **16.**<br><br>Ref. [16] | **Maternal thyroid dysfunction and risk of seizure in the child: a Danish Nationwide Cohort Study**<br><br>Stine Linding Andersen, Peter Laurberg, Chun Sen Wu & Jørn Olsen.<br>*Journal of Pregnancy, 636705, 2013.* |
| **17.**<br><br>Ref. [17] | **Attention deficit hyperactivity disorder and autism spectrum disorder in children born to mothers with thyroid dysfunction: a Danish nationwide cohort study**<br><br>Stine Linding Andersen, Peter Laurberg, Chun Sen Wu & Jørn Olsen.<br>*BJOG, 121, 1365-1374, 2014.* |
| **18.**<br><br>Ref. [18] | **Psychiatric disease in late adolescence and young adulthood. Foetal programming by maternal hypothyroidism?**<br><br>Stine Linding Andersen, Jørn Olsen, Chun Sen Wu & Peter Laurberg.<br>*Clinical Endocrinology, 81, 126-131, 2014.* |

# TABLE OF CONTENTS

# CHAPTER 1. INTRODUCTION

Thyroid hormones have profound physiological effects, and play an important role in the regulation of metabolism, growth and development. In 1912, Gudernatsch described that the feeding of tadpoles with pieces of the thyroid gland triggered their transition into frogs [19]. This observation in amphibian metamorphosis has been substantially extended during the last century, and the role of thyroid hormones in human development, particularly early brain development, has been described. The crucial role of thyroid hormones in human brain development is evident from the severe and permanent brain damage observed in untreated congenital hypothyroidism [20]. Fortunately, prompt diagnosis and treatment can prevent such developmental brain damage, and neonatal screening has been implemented in many countries. Another area of concern is the developmental brain damage secondary to iodine deficiency with cretinism being the most severe clinical presentation [21]. Iodine is required for the synthesis of thyroid hormones and insufficient intake of iodine in pregnant women may cause lack of thyroid hormones in the mother and the fetus. The World Health Organization (WHO) [22] describes iodine deficiency as the single most common preventable cause of brain damage, and extensive programs of prevention have been implemented in large parts of the world. A third area of clinical and scientific awareness is maternal thyroid disease in pregnancy. The fetal thyroid gland is increasingly able to synthesize thyroid hormones from the second trimester of pregnancy, and thyroid hormones in the fetus are exclusively of maternal origin in the early pregnancy [23]. It is biologically plausible that maternal thyroid disease may adversely affect outcomes of pregnancy and fetal development, but many aspects remain unresolved, and the potential benefits and risks from routine testing of thyroid function in pregnant women are not completely clear [24].

## 1.1. MATERNAL THYROID DISEASE

Thyroid disease is one of the most common endocrine disorders in the population in general and in pregnant women. Characteristic for thyroid disease is the age dependency in the overall occurrence of disease and subtypes of disease [25,26]. The predominant types of thyroid disease in women of reproductive age include Graves' hyperthyroidism, autoimmune hypothyroidism and postpartum thyroiditis (PPT). On the other hand, benign goiter and nodules, and thyroid cancer are rarely observed in this age span. Another characteristic of thyroid disease is the multifactorial etiology of combined genetic and environmental factors [27]. The role of iodine is significant, and the U-shaped relationship between population iodine intake and the occurrence of thyroid disease is a key characteristic [28]. Thyroid disorders occur worldwide, but differences in population iodine intake within and between countries cause regional differences in the overall occurrence of thyroid disease and in the dominant subtypes of thyroid disease [28,29].

### 1.1.1. OCCURRENCE IN PREGNANCY

Pregnancy is associated with changes in maternal anatomy, physiology, metabolism and in the immune system [30]. The adaptations are primarily mediated via hormonal changes and ensure a condition that allow fetal development. The maternal immune system is suppressed during a pregnancy with a characteristic immune rebound after birth of the child. Physiological changes are seen in maternal thyroid function from the early pregnancy primarily due to the stimulation of the thyroid gland by the pregnancy related hormone, human chorionic gonadotropin (hCG), but changes in the metabolism of thyroid hormones and in thyroxine-binding globulin (TBG) also contribute [31]. These adaptations challenge the diagnosing of thyroid disease in pregnant women and the differentiation between physiological conditions related to the pregnant state and primary thyroid disease. Since thyroid diseases in women of reproductive age are primarily of autoimmune origin, the changes in the maternal immune system in and around pregnancy may influence the onset of thyroid disease. Thyroid autoantibodies show a concentration decline in pregnancy and an increase after birth, and the immune rebound may trigger the onset of thyroid disease [32]. Still, many aspects remain unresolved on the occurrence of maternal thyroid disease in and around pregnancy and on the interplay with the physiological changes related to the pregnant state. From a preventive perspective, it is important to identify risk factors for thyroid disease in pregnant women and to obtain data on the occurrence of unidentified thyroid disease, which is dependent on valid reference ranges for thyroid function in pregnancy.

### 1.1.2. OUTCOMES OF PREGNANCY

The interaction between the thyroid and pregnancy as well as outcomes of pregnancy after exposure to abnormal maternal thyroid function have been a matter of concern and focus of scientific work for decades. Early observations of cretinism were followed by a cluster of publications in the late 1990s that raised concern about the neurocognitive development in children born to mothers with abnormal thyroid function in pregnancy [33,34]. Much focus was on the adverse effects of low maternal thyroxine (T4) concentration in early pregnancy [23], and systematic screening for thyroid dysfunction in pregnant women was proposed [23,35]. Extensive research during the following decades have focused on outcomes of pregnancy in women with thyroid dysfunction and the role of thyroid autoimmunity. Still, many aspects remain unresolved on the role of maternal thyroid function and when and how to treat [36]. Furthermore, the clinical guidance is challenged by potential side effects to the drugs used for the treatment of thyroid disease in pregnancy. Levothyroxine (L-T4) used for the treatment of hypothyroidism is a replacement therapy, whereas antithyroid drugs (ATD) used for the treatment of hyperthyroidism are thionamides with blocking effects. For the use of ATD in general, severe adverse side effects such as agranulocytosis and liver failure are considered rare, but an additional concern to the use of ATD in pregnant women relates to potential teratogenic effects [37].

## 1.2. OBJECTIVES OF THE DOCTORAL DISSERTATION

The overall objective of the doctoral dissertation was to investigate the occurrence and outcomes of maternal thyroid disease in pregnancy. More specifically, the studies aimed to investigate i) the occurrence of maternal thyroid disease in and around pregnancy, ii) outcomes of child neurodevelopment and neuropsychological performance after exposure to maternal thyroid dysfunction in pregnancy, and iii) outcomes of severe side effects after exposure to drugs used for the treatment of maternal thyroid disease in pregnancy (Table 1-1). Data were retrieved from large, nationwide sources including Danish and Swedish nationwide registers and from the Danish National Birth Cohort (DNBC).

| | |
|---|---|
| i | **Occurrence of maternal thyroid disease in and around pregnancy** |
| | To study the occurrence and predictors of maternal thyroid disease in and around pregnancy using data from Danish nationwide registers and stored blood samples from the DNBC (paper 1-6). |
| ii | **Outcomes of neurodevelopment and neuropsychological performance in children born to mothers with thyroid dysfunction in early pregnancy** |
| | To study outcomes of neurodevelopmental disorders and neuropsychological performance at five years of age in children born to mothers with abnormal thyroid function in the early pregnancy using data from Danish nationwide registers and stored blood samples from the DNBC (paper 7-8). |
| iii | **Outcomes of severe side effects associated with maternal use of ATD in early pregnancy** |
| | To study the risk of birth defects after maternal use of ATD in early pregnancy and the risk of agranulocytosis and liver failure associated with the use of ATD in pregnancy and in the population using data from Danish and Swedish nationwide registers (paper 9-12) . |

*Table 1-1. Specified study objectives of the doctoral dissertation.*

The list of additional publications (paper 13-18, page XIII) includes the initial series of studies, which investigated the association between maternal thyroid disease and outcomes of pregnancy and child development using Danish nationwide registers. Characteristic for these studies was the indirect measure of exposure. A consistent finding was an association between maternal thyroid disease first diagnosed and treated in the years following the pregnancy and adverse outcomes of pregnancy and child development, which raised concern about undetected and untreated maternal thyroid disease in the pregnancy. The studies included in the doctoral dissertation (paper 1-12, page XI-XII) were subsequently performed and aimed to extend the initial hypothesis and to include more direct measures of exposure.

# CHAPTER 2. BACKGROUND

## 2.1. MATERNAL THYROID FUNCTION IN PREGNANCY

Physiological changes in pregnancy challenge the use of thyroid function parameters for the diagnosing and control of maternal thyroid disease. The changes occur from early pregnancy (Figure 2.1) and rising estrogen and hCG levels are described and considered as underlying mechanisms [38]. Estrogen increases in early pregnancy and stimulates the liver to an increased production of TBG [31], which leads to a rise in total thyroid hormone concentrations (Figure 2.1). hCG peaks in pregnancy week 9-10 [39], and stimulates the thyroid gland to an increased production of thyroid hormones with a concomitant lowering of thyrotropin (TSH) (Figure 2.1).

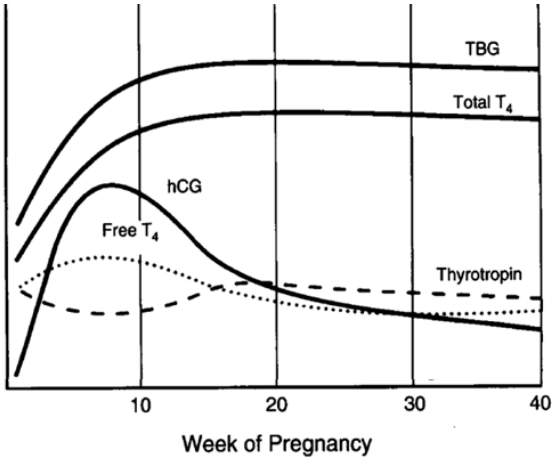


*Figure 2.1. Changes in maternal thyroid hormone parameters and hCG in pregnancy. Reproduced with permission from* [38], *copyright Massachusetts Medical Society.*

These physiological changes in early pregnancy neccessitate the use of pregnancy specific reference ranges for the diagnosing of thyroid disease in pregnant women, and clinical guidance recommends the use of trimester specific reference ranges [36,37]. In addition to estrogen and hCG, the type 3 iodothyronine deiodinase (D3) is present in the uteroplacental unit (Figure 2.2) from the early pregnancy [40] and involved in the pregnancy related changes in maternal thyroid function parameters. D3 inactivates thyroid hormones by catalyzing the conversion of T4 to revers triiodothyronine (T3) and T3 to diiodothyronine (T2) (Figure 2.2), which tends to increase TSH [31]. Accordingly, the balance between the various physiological mechanisms in early pregnancy affecting results of maternal thyroid function tests are complex and hitherto not completely clear.

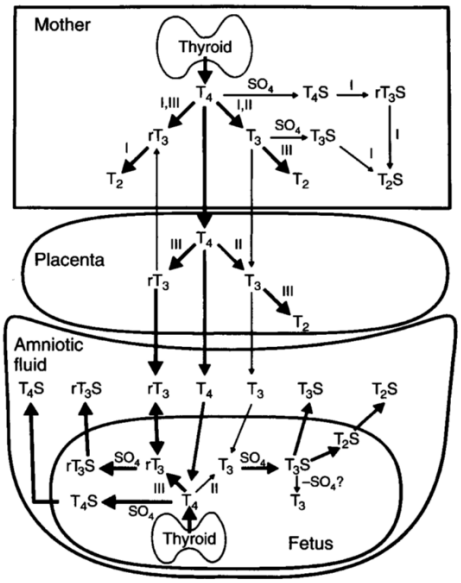


*Figure 2.2. Thyroid hormone metabolism in the mother, placenta and the fetus. I, II and III illustrate the type 1, type 2 and type 3 iodothyronine deiodinase. Reproduced with permission from* [38]*, copyright Massachusetts Medical Society.*

## 2.2. MATERNAL THYROID DISEASE IN PREGNANCY

Thyroid diseases are common endocrine disorders and part of the chronic disease burden in pregnant women [41]. Thyroid disease in a pregnant woman can be diagnosed prior to pregnancy, during or in the years following a pregnancy. The clinical challenge in the management of pregnant women with thyroid disease is to interpret thyroid function tests, and to decide who, when and how to treat. Hyperthyroidism is a condition with excess production of thyroid hormone by the thyroid gland, whereas the term thyrotoxicosis refers to any condition with excess of thyroid hormone [42]. Hyperthyroidism in women of reproductive age is most commonly due to Graves' disease (GD) with an autoimmune origin [26], and 95% of GD patients are positive for TSH-receptor antibodies (TRAb) [43]. Hyperthyroidism due to multinodular goiter or solitary nodules, which is seen in areas of iodine deficiency, is uncommon in women below the age of 40 years [26]. Gestational hyperthyroidism caused by high levels of hCG in early pregnancy is associated with hyperemesis gravidarum and may be difficult to distinguish from GD, but the presence of TRAb and the need for ATD favor a diagnosis of GD [44]. Other causes of hyperthyroidism and thyrotoxicosis are rare in pregnancy [26,44]. In the postpartum period, thyrotoxicosis may develop as part of PPT, which is a transient condition that should not be treated with ATD [37].

Hypothyroidism is a condition with lack of thyroid hormone [45]. Severe iodine deficiency can cause hypothyroidism, but in iodine sufficient areas and in areas with mild to moderate iodine deficiency, hypothyroidism is most commonly due to spontaneous hypothyroidism of autoimmune origin (also referred to as chronic autoimmune thyroiditis or Hashimoto's thyroiditis) [25,45]. This hypothyroidism is characterized by the presence of thyroid peroxidase antibodies (TPO-Ab) and/or thyroglobulin antibodies (Tg-Ab) in more than 99% of patients with overt disease [46]. These autoantibodies are present in 10-15% of the general population [47,48] and predispose to development of hypothyroidism [27]. Congenital and iatrogenic causes of hypothyroidism are rare in pregnancy [25]. In the postpartum period, hypothyroidism may develop as part of PPT, which is associated with TPO- and Tg-Ab and may present in various clinical types [49].
The various causes of thyroid disease and the autoimmune origin of thyroid function disorders imply a complex and multifactorial etiology with interaction between genetic and environmental factors. Population iodine intake is a strong determinant of thyroid disease occurrence [28] and lifestyle factors (e.g. smoking, alcohol intake) may show opposing effects on the development of different types of thyroid disease [50]. In pregnancy, focus is on maternal characteristics (e.g. age, parity, body mass index (BMI) and origin), and how these factors possibly influence the occurrence of maternal thyroid disease.

## 2.3. THYROID HORMONES AND BRAIN DEVELOPMENT

Thyroid hormones are important developmental factors, and maternal thyroid hormones regulate fetal brain development in the early pregnancy before the onset of fetal thyroid hormone production (Figure 2.3) [23]. Maternal thyroid hormones also play a role after the onset of thyroid hormone production in the fetal thyroid gland as indicated by the measurement of T4 in cord blood from newborns with a defect in thyroid hormone synthesis [51], and by the prevention of brain damage upon early treatment of athyrotic newborns with congenital hypothyroidism [21]. Consensus on the role of maternal thyroid hormones in fetal brain development has emerged over time [23]. An important role of maternal thyroid hormones was proposed in the 1960-1970s when clinical observations of neurological symptoms in endemic cretisnism were described [52]. Further, it was shown in a series of studies by Evelyn Man et al. that the neurocognitive development of children born to mothers with low serum butanol-extractable iodine concentration (as a measure of maternal T4) was normal when the mother had been adequately treated in the pregnancy [53]. However, the general consensus at this time was that placenta was impermeable for transport of thyroid hormones [54]. New findings during the 1980-1990s in experimental animals and in humans provided evidence of fetal thyroid hormone production [55], transfer of maternal thyroid hormones across the placenta [51,56] and the presence of thyroid hormone receptors in the fetal brain [57], but uncertainties on the role of maternal thyroid hormones prevailed [58].

In 1999, the hallmark study by Haddow et al. [33] was published, which described lower intelligence quotient (IQ) in children born to mothers with undiagnosed and untreated hypothyroidism in pregnancy. More evidence from experimental studies of human fetal brain tissue emerged concomittant [59] and experimental [60] as well as clinical investigations [34] drew attention to the role of maternal T4 [23]. However, uncertainties remain on the role of maternal hypothyroxinemia in pregnancy [61,62].

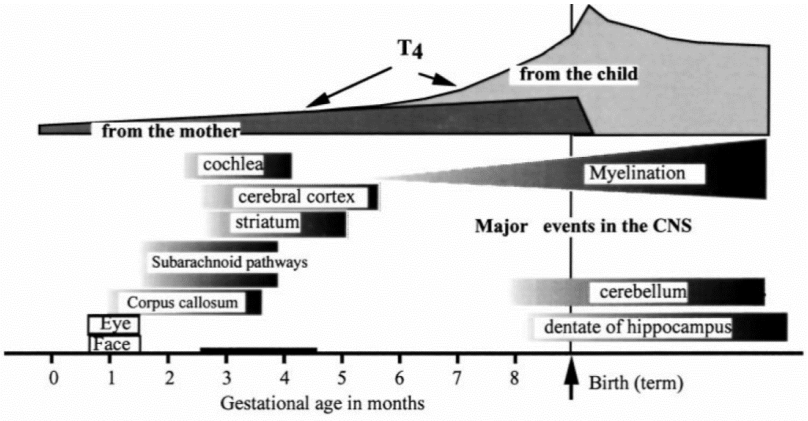


*Figure 2.3. Illustration of events during early brain development (lower part) and the sources of thyroid hormone (upper part). Reproduced with permission from* [23].

The transport of maternal T3 and T4 from the maternal to the fetal circulation depends on the expression of thyroid hormone transporters [63] and the local activity of D3 and the type 2 iodothyronine deiodinase (D2) [64] (Figure 2.2). The expression of D3 in placenta is higher that of D2, and is considered to present a barrier for the transport of T4 from the mother to the fetus [64]. Evidence on the role of thyroid hormones in the fetal brain primarily emerge from *in vivo* studies in rats and *in vitro* studies of cell cultures. Brain development is a complex and highly regulated process [65]. Passage of thyroid hormones to brain tissues requires the crossing of the blood-brain barrier via thyroid hormone transporters, and the main source of T3 is anticipated to derive from the conversion of T4 to T3 in the astrocytes [57]. T3 regulates gene expression via binding to nuclear receptors, which are present in the fetal brain at least from the 10th week of pregnancy [57], and genes involved in brain development that are transcriptionally regulated by T3 have been identified [66]. Many aspects are still to be resolved on the transfer of thyroid hormones across the placenta and the blood-brain barrier and on the role of thyroid hormones during different stages of brain development [65]. Observations from experimental studies on alterations associated with abnormal supply of thyroid hormones during early brain development include defects of proliferation, migration, differentiation, synaptogenesis and myelination [67] as well as abnormalities in the neurochemical enviroment [68].

More evidence in humans is needed to expand the hypothesis of fetal programming by maternal thyroid disease (Figure 2.4). The early observations of severe brain damage in neurological cretinism and impaired neurocognitive performance in children exposed to untreated severe maternal hypothyroidism [23] have encouraged a considerable number of observational association studies in humans. More recent studies included data on magnetic resonance imaging of the brain in children born to mothers with thyroid dysfunction [69-73], but many studies included indirect measures of exposure and outcome, which may challenge the interpretation and comparison of results [74]. Moreover, randomized controlled trials (RCTs) have not shown beneficial effects on child neurocognitive outcomes with the treatment of maternal subclinical hypothyroidism and hypothyroxinemia in pregnancy [75,76].

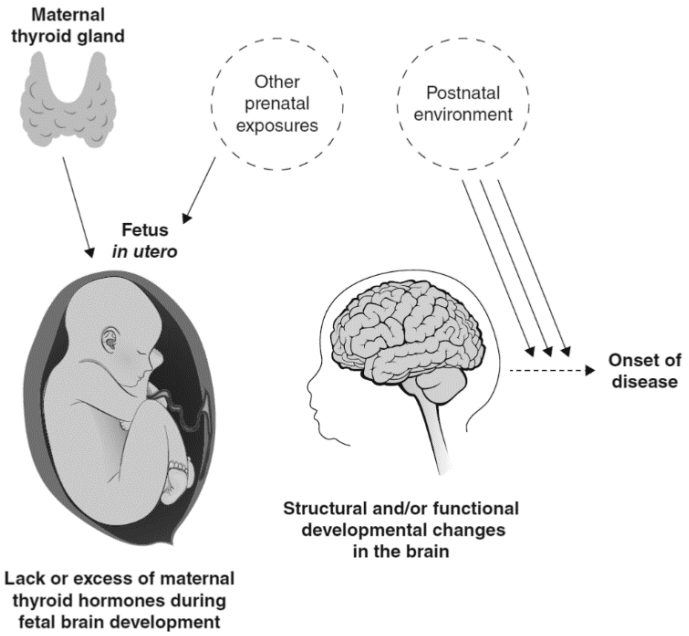


*Figure 2.4. The hypothesis of fetal programming by maternal thyroid disease. Reproduced with permission from* [74].

## 2.4. TREATMENT OF THYROID DISEASE IN PREGNANCY

The treatment of thyroid disease in a female patient who are or may in the future become pregnant constitutes a clinical challenge because the physiological changes in pregnancy influence the course of the disease, and because pregnancy introduces important considerations on the choice of treatment and concerns related not only to the pregnant woman, but also to the fetus and the neonate [77].

Overt hypothyroidism should be adequately treated in pregnant women, and evidence suggests beneficial effects of treating subgroups of pregnant women with subclinical hypothyroidism [36,78]. On the other hand, international clinical guidance does not recommend the treatment of maternal hypothyroxinemia [36,78]. The treatment of choice in pregnancy is L-T4, and the clinical challenge associated with this treatment is the increased requirements, which demand an early change in the dose of L-T4 [79]. However, studies from different countries have shown that hypothyroid women are not always adequately treated by the time they become pregnant [80-84].

The treatment of choice for Graves' hyperthyroidism in pregnant women is ATD, whereas ATD is not recommended for the treatment of gestational hyperthyroidism [36,37]. The clinical challenge associated with the use of ATD for the treatment of hyperthyroidism differs during the pregnancy. In early pregnancy, focus is on the risk of birth defects associated with the use of ATD [31,85]. Pregnancy weeks 6-10 represent the period of maximum sensitivity to teratogenic exposure (Figure 2.5), and revised guidelines of the American Thyroid Association address much concern about the early pregnancy and suggest the possibility of ATD withdrawal in appropriately selected patients [36,37]. In the second half of pregnancy and immediately after birth, focus is on thyroid function of the fetus and the neonate [31,85].

The available ATD include Methimazole (MMI) and its prodrug Carbimazole (CMZ) as well as Propylthiouracil [86]. ATD cross the placenta [87], the drugs are equally effective in the treatment of hyperthyroidism in pregnancy [88] and include the same risk of fetal hypothyroidism [89]. The choice of ATD for the treatment of hyperthyroidism is mainly based on the pharmacokinetic profile of the drug and the associated side effects.

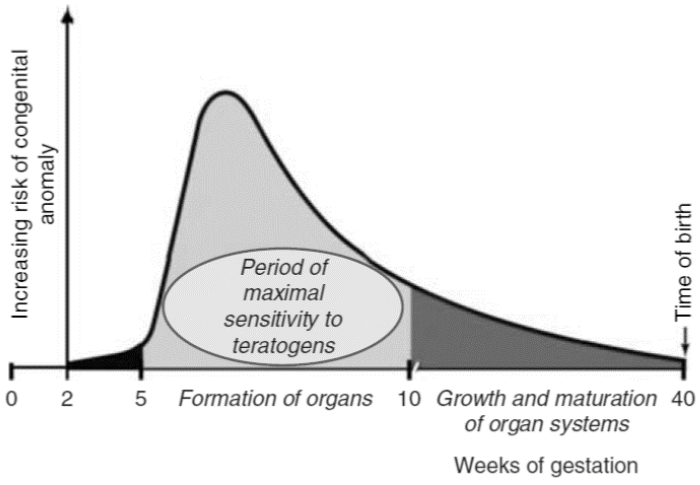


*Figure 2.5. Pregnancy period of maximum sensitivity to teratogenic exposure. Reproduced with permission from* [90].

In non-pregnant patients, the main treatment options for Graves' hyperthyroidism are ATD, radioiodine and thyroidectomy [37]. Radioiodine treatment is contraindicated in pregnancy, surgery in pregnancy is preferably avoided, and ATD is the treatment of choice in pregnant women. ATD is a commonly used treatment in many countries [91] and compared with definitive treatment options, it induces no damage to the thyroid gland. MMI/CMZ is the preferred ATD in non-pregnant individuals except for the treatment of thyroid storm [37]. MMI/CMZ can be administered once daily and is considered to have a lower risk of severe side effects [37]. Side effects to the use of ATD include minor cutaneous reactions and pruritus, which occur in 5-10% of patients [92]. On the other hand, major adverse reactions are rare and include agranulocytosis, liver failure and vasculitis [86]. Fulminant liver failure has been a major concern about PTU, which has favored the use of MMI particularly in children [93]. PTU was the third most common cause of drug-induced liver transplantation in the United States (US) when evaluated in 2009, and one third of the cases were children [94]. However, at this time it was stated that unanswerable questions remained on the preferential choice of ATD in women who are or may in the future become pregnant [94]. An additional concern about the use of ATD in pregnant women is the potential risk of adverse side effects in the fetus [31,85]. Until the 1970s, the major concern and focus of scientific research was the risk of fetal hypothyroidism associated with maternal use of ATD and the neurocognitive development of the child [95]. The first report on a possible teratogenic effect was published in 1972 and consisted of a short letter in which the authors described 11 mothers who gave birth to a child with a scalp defect, and two of the mothers had been treated with MMI in the pregnancy [96]. In the following decades more case reports and case series were published, which led to the proposal of a 'MMI/CMZ embryopathy' in the late 1990s including special facial features, aplasia cutis, choanal and esophageal atresia, omphalocele, and omphalomesenteric duct anomalies [97,98]. However, the risk was not quantified at this time [99], but not until 2011, when the first observational study including a non-exposed control group was published [100]. Evidence regarding PTU has been less comprehensive than for MMI/CMZ, and case series did not raise major concern [101]. The scientific evidence up to the year 2011 when the previous editions of international clinical guidance from different societies were drafted led to the recommendation that PTU should be preferred in early pregnancy and women on current MMI/CMZ should be shifted to PTU, when pregnancy was detected [102-104]. A major observational study from Japan in 2012 supported this recommendation [105]. However, subsequent observational studies in humans [9-12,90,106-108] as well as experimental studies [109-111] have added new evidence, which has challenged the choice of therapy in early pregnancy and has been considered in the revised clinical guidance of the American Thyroid Association drafted in 2016 [36,37,112].

# CHAPTER 3. METHODS

## 3.1. NATIONWIDE DATA SOURCES

Characteristic of the Nordic countries is the collection of population health data in nationwide registers [113] (Table 3.1 and 3.2). All citizens are assigned a unique personal identification number, which is used in all the nationwide registers and enables linkage between the different registers at an individual level. Data in such nationwide registers are secondary data meaning they have not been collected with a specific research purpose, but often for administrative purposes [114]. The use of secondary data for epidemiological research is advantageous in time, costs and size, and in the nationwide coverage, which in comparison to the collection of primary data reduces the risk of selection bias. However, the use of secondary data also poses challenges specifically related to data completeness, accuracy and registration periods, which should be considered in the design of a register-based study [114].

### 3.1.1. DANISH NATIONWIDE REGISTERS

The Danish personal identification number is a unique 10-digit number consisting of day of birth (six digits) and a four-digit serial number. The Danish Civil Registration System (CRS) was established on April 2, 1968 and includes data on date and place of birth, sex, place of residence, civil status, emigration and death [115]. Registration in CRS is required by law. Thus, data are virtually complete and the accuracy is high [116].

| Register | Abbrev | Year | Content |
|---|---|---|---|
| Danish Civil Registration System | CRS | 1968- | All citizens |
| Danish Medical Birth Register | MBR | 1973- | Live- and stillbirths |
| Danish Psychiatric Central Register | DPCR | 1969- | Hospital diagnoses |
| Danish National Hospital Register | DNHR | 1977- | Hospital diagnoses |
| Danish National Prescription Register | DNPR | 1995- | Prescription of drugs |

*Table 3.1. Danish nationwide registers used in the doctoral dissertation.*

The Danish Medical Birth Register (MBR) was established in 1968 and has been computerized since 1973 [117]. It includes maternal and child parameters for all live- and stillbirths in Denmark. Data registration is performed by midwifes after birth of the child, and electronic birth records have been used since 1995. Registration of some variables (e.g. birth weight and gestational age at birth) have changed over the time of the register [117] with a concomitant improvement in data accuracy [118,119], and variable definitions have been uniform since the transition to electronic recording in the mid-1990s. Gestational age at birth was previously based

on the first day of last menstrual period, but ultrasound estimation has been increasingly used over time [120]. All pregnant women in Denmark have been offered early pregnancy prenatal screening for chromosomal abnormalities including fetal ultrasound since 2004, and the rate of participation is high [121]. Estimation of gestational age by different methods did not show complete agreement [122], and whereas ultrasound is generally considered more precise, it has limitations [123]. Another variable of interest in reproductive epidemiology is maternal parity, which is self-reported by the pregnant woman in MBR, but showed a high agreement when compared with parity calculated from information in CRS [124].

Danish registers on hospital diagnoses of disease include the Danish Psychiatric Central Register (DPCR) and the Danish National Hospital Register (DNHR). The DPCR has systematically collected data on psychiatric hospital admissions since 1938, and data have been computerized since 1969 [125]. The DNHR includes data on all somatic hospital admissions since 1977, and the DPCR became an integrated part of DNHR in 1995 [126]. In both registers, only inpatient hospital visits were registered up to 1994, and outpatient hospital visits were included from 1995 and onwards. The coding of diagnoses is according to the International Classification of Disease (ICD), which was the eight edition (ICD-8) up to 1993, and ICD-10 from 1994 and onwards [125,126]. An important consideration to the use of these sources for research purposes is the completeness and accuracy of the registered data. Data validity has been continuously evaluated during the period of data registration and is encouraged to extend further [127,128]. A systematic review of validation studies in 2015 found a wide variation in the reported positive predictive values of different diagnoses, but different methodology in the validation studies may add to these findings [128]. For ICD-8, it was shown that the diagnostic validity decreased with the number of digits in the ICD code suggesting that the classification of disease categories is superior to the identification of specific diagnoses [127]. Moreover, the possibility to combine hospital diagnoses with information on surgical procedures and prescribed drugs in more recent years may compensate DNHR completeness for some diseases [128]. Patient visits in general practice (GP) and in specialty clinics are not included in the DPCR and the DNHR. However, information on patients diagnosed and treated outside a hospital can be assessed from other sources.

The Danish National Prescription Register **(**DNPR) covers all redeemed prescriptions of drugs in Danish pharmacies since 1995 including the type of drug according to the Anatomical Therapeutic Chemical (ATC) classification and the date of sale [129]. Data completeness and accuracy are considered high [129-131], but it is important to acknowledge that the DNPR does not include drugs sold without a prescription or dispensed at a hospital. Furthermore, non-compliance with the treatment is not captured, because the register does not include information on drugs that are prescribed by a doctor, but not redeemed by the patient, and information on actual use of the drug after the collection in a pharmacy is not available [129].

Along with the nationwide Danish registers listed in Table 3.1, Statistics Denmark provided nationwide information on income and education, which was available from 1980 and onwards.

### 3.1.2. SWEDISH NATIONWIDE REGISTERS

The Swedish unique personal identification number has consisted of 10 digits since 1976 and includes the six-digit date of birth [132]. The Total Population Register (TPR) was established in 1968 and includes data similar to the Danish CRS [133]. Data collection is regulated by law, and data quality is regarded as high [133].

| Register | Abbrev | Year | Content |
|---|---|---|---|
| Swedish Total Population Register | TPR | 1968- | All citizens |
| Swedish Medical Birth Register | MBR | 1973- | Live- and stillbirths |
| Swedish National Patient Register | SNPR | 1987- | Hospital diagnoses |
| Swedish Prescribed Drug Register | SPDR | 2005- | Prescription of drugs |

*Table 3.2. Swedish nationwide registers used in the doctoral dissertation.*

The Swedish MBR was established in 1973 and includes information on all live- and stillbirths in Sweden [134]. Like in Denmark, information on mother and child is registered by midwifes, but in Sweden information is not only collected at birth, but also during an early and late antenatal care visit in pregnancy [135]. The structure of the register has remained unchanged since 1973, but the content has been modified over time. This has been followed by a concomitant increase in data quality, but some variables are still subject to missing records (e.g. maternal drug use in pregnancy and infant diagnoses), where other data sources should be used [134]. Ultrasound for determination of gestational age has been offered to all pregnant women in Sweden since 1990, and the acceptance is high [135,136]. On the other hand, prenatal screening in early pregnancy with combined ultrasound and biochemistry has not been nationally implemented in Sweden, and the rate of participation is lower than in Denmark and varies considerably between counties [137,138].

The Swedish National Patient Register **(**SNPR) was launched in 1964, and complete national coverage was implemented from 1987 [139]. The register contains somatic and psychiatric inpatient hospital diagnoses and from 2001 outpatient visits in hospitals and in specialty clinics have been added, but data on patient visits in GP are not included in the register. Like in Denmark, the diagnoses are coded according to the ICD system, and many diagnoses have been validated [139]. Although the positive predictive value is varying, the overall validity is considered high and the use of the register for research purposes is encouraged [139].

Nationwide registration of redeemed prescriptions of drugs has been implemented in all Nordic countries (Denmark, Sweden, Norway, Finland and Iceland) and is a valuable source for epidemiological research [140]. The Swedish Prescribed Drug Register **(**SPDR) was established on July 1, 2005, a decade later than the DNPR in Denmark, and the structure, content and coverage of the SPDR is comparable to the DNPR [141,142].

### 3.1.3. DANISH NATIONAL BIRTH COHORT

The nationwide registers in the Nordic countries provide unique opportunities for studies within reproductive epidemiology because large cohorts of children can be identified with long-term follow-up. However, the use of secondary data often provide indirect measures of exposure and outcome in pregnancy, and additional data or more direct measures may be warranted for specific research purposes. In the Nordic and other European countries birth cohorts have been established, which have the advantage of including self-reported maternal information and biological specimens, but the collection of primary data is time consuming, associated with high costs, and includes a risk of selection bias [114].
The DNBC was established in 1996 to 2002 and aimed for nationwide inclusion of around 100,000 Danish pregnant women [143]. Inclusion was at the first antenatal pregnancy visit in GP, and all women who intended to carry their pregnancy to term and who spoke Danish well enough to participate in a telephone interview where eligible for the cohort [143]. Around 50% of pregnant women in Denmark received an invitation to the study in early pregnancy and 60% of the invited women participated with an overall participation rate of 30-35% [143,144]. Inclusion was nationwide as desired, but some discrepancies in socioeconomic factors have been described for non-participants [144,145]. After informed consent, participants completed a registration form with information on current medication, had a blood sample drawn in early pregnancy, and participated in a telephone interview twice during the pregnancy [143]. Furthermore, follow-up after birth of the child was part of the protocol, and seven years of follow-up was completed in 2010 with a response rate of 60-65% [146]. Notably, the time period of inclusion in the DNBC was within the periods of electronic data collection in Danish nationwide registers (Figure 3.1), which enabled linkage to other data sources for assessment of exposure and outcome.

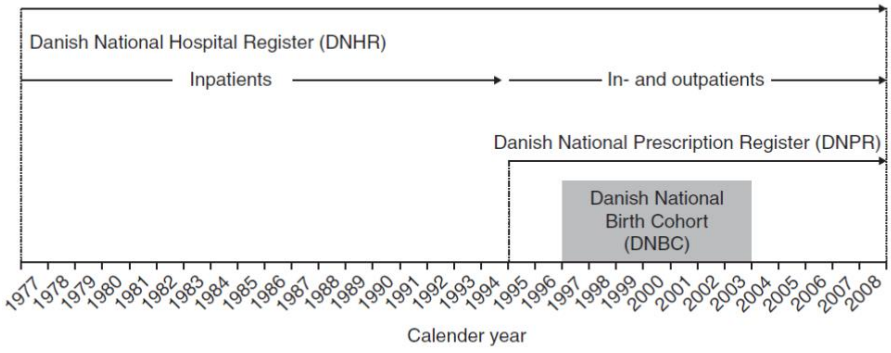


*Figure 3.1. The Danish National Birth Cohort in relation to the Danish nationwide registers. Reproduced with permission from* [3].

### 3.1.4. LIFESTYLE DURING PREGNANCY STUDY

The Lifestyle During Pregnancy Study (LDPS) is a prospective follow-up study within the DNBC, which was established to study the effects of maternal lifestyle during pregnancy on child neurodevelopment [147]. The primary exposure of interest in the LDPS was alcohol and participants were sampled from maternal alcohol intake and binge drinking episodes in pregnancy with oversampling of certain alcohol exposure categories. A total of 3,489 mother-child pairs were invited to participate when the child reached five years of age and 1,782 (51%) agreed to participate [148]. Exclusion criteria were multiple pregnancies, inability to speak Danish, and children with impaired hearing, vision or mental retardation. Neuropsychological testing by psychologists when the child was 60-64 months and testing of maternal intelligence took place from 2003-2008 in the four largest cities in Denmark. Outcome assessment included testing of child motor function by physiotherapists, and parent and teacher reports of child executive function and behavior [147]. The comprehensive outcome assessment is a unique feature of the LDPS especially for exposures associated with potential adverse neurodevelopmental of the child. However, an important note is the sampling procedure, which should be considered in the design of studies looking at alternative exposures than alcohol intake in pregnancy within this sub-cohort.

## 3.2. BIOCHEMICAL ANALYSES

The blood samples drawn at the antenatal visit in GP from pregnant women enrolled in the DNBC, 1996 to 2002, were venous blood samples collected in EDTA-coated tubes [143]. Samples were transported as whole blood by regular mail to the Danish National Biobank for processing and storage. Median time from sampling to arrival was 24 hours (range 4-48 hours). Upon arrival in the biobank, the samples were separated into plasma and stored in a -80 or -20 degrees Celsius freezer or in liquid nitrogen [143]. In 2015, samples drawn in early pregnancy were selected from a subgroup of women within the DNBC for measurement of maternal thyroid function (TSH and free thyroxine (fT4)). Upon request in 2018, the Danish National Biobank reported that these project samples had been stored at -20 degrees Celsius (and not at -80 degrees Celsius as initially specified [4,5,7,8]). A total volume of 100 µl plasma was obtained and kept frozen during transportation to the laboratory where thyroid function analyses were performed. In this laboratory, the samples were kept at -20 degrees until analyses. Considering the specific type of stored samples, a study [149] published in 2017 reviewed that the stored samples from the birth cohorts in Denmark (the DNBC) and in Norway (the Norwegian Mother and Child Cohort Study (MoBa)) are plasma samples. The term serum/sera is therefore incorrect [4,5,150]. Notably, TSH and fT4 were measured on a Dimension Vista (Siemens) immunoassay in DNBC samples and according to the manufacturer; serum and plasma can be used. Furthermore, a high agreement between plasma and serum measurements of thyroid function parameters in samples from pregnant women has been observed [149].

The handling of the samples from initial sampling in pregnancy to measurement of thyroid hormone parameters 13-19 years later is important to consider. One aspect is the pre-separation procedure, which includes a time delay during the transport of samples from GP to the Danish National Biobank. In samples from non-pregnant individuals, TSH and fT4 were stable in whole blood for up to 72 hours at room temperature [151]. Another aspect is the post-separation procedure including freeze and thaw cycles. In different studies [149,152], freezing and thawing did not affect TSH and free T4 in samples from pregnant women. Finally, the long-term storage for years may be a concern and has been a matter of debate [153,154]. However, in samples from non-pregnant [155] and pregnant individuals [154], thyroid function parameters were stable for more than 20 years of storage at -25 degrees Celsius.

## 3.3. DATA MANAGEMENT

All data analyses in the studies included in the doctoral dissertation (paper 1-12, page XI-XII) and in the additional list of studies (paper 13-18, page XIII) were performed by Stine Linding Andersen using the statistical software STATA version 11-14 (Stata Corp.). Data were made available in encrypted form and data linkage was performed: i) on the Statistics Denmark server for studies including Danish nationwide registers and data from the DNBC including biochemical measurements, ii) on the Aarhus University Citrix server for the study including data from the LDPS, and iii) in Department of Endocrinology, Aalborg University Hospital for the study using Swedish nationwide registers.

### 3.3.1. ETHICAL CONSIDERATIONS

All data analyses were approved by the Danish Data Protection Agency. Institutional review board permission is not required for studies that are exclusively based on data from nationwide registers in Denmark. The use of Swedish nationwide registers was approved by the regional ethical review board and the National Board of Health and Welfare in Sweden. The measurement of thyroid function in stored samples from pregnant women within the DNBC was approved by the DNBC Steering Committee and the North Denmark Region Committee on Health Research Ethics.

### 3.3.2. STATISTICAL ANALYSES

All the studies included in the doctoral dissertation were observational in design. Observational studies are prone to confounding [156] and it is essential to apply statistical methods that allow for consideration of potential confounding factors. Statistical methods included linear and logistic regression models (cohort studies), Cox proportional hazards model (cohort studies with varying follow-up), weighted Cox proportional hazards model (case-cohort studies with varying follow-up), and restricted cubic splines for evaluation of potential non-linear associations.

# CHAPTER 4. MATERNAL THYROID DISEASE IN PREGNANCY

This chapter includes a discussion of paper 1-6, which investigated the occurrence and predictors of maternal thyroid disease in pregnancy. The studies were all nationwide, but differed by the method used for assessment of maternal thyroid disease in the way that paper 1-2 and 6 included measures of diagnosis and treatment, paper 3 added measures of maternal self-report, and paper 4-5 included biochemical measurements of maternal thyroid function in early pregnancy.

## 4.1. DANISH NATIONWIDE REGISTER STUDIES

Paper 1 and 2 investigated the incidence of maternal hyper- and hypothyroidism in and around pregnancy. The large study population as part of the nationwide design made it possible to analyze and describe short time intervals before, during and after the pregnancy. Furthermore, the use of nationwide data sources makes loss to follow-up negligible, because mortality is low in this age group in Denmark, and reduces the risk of selection bias. This is in contrast to the inclusion of thyroid patients from hospital or specialist clinics with a risk of referral bias [157,158]. The use of prescription data for the identification of thyroid disease covered patients managed in GP alone, however, in studies of thyroid disease in pregnant women the risk of referral bias is considered low, because it is recommended that such patients are referred to an endocrinologist in Denmark for management of the disease during the pregnancy [157,158]. The specificity of the drugs used for the treatment of thyroid disease and the fact that thyroid medication is sold solely as prescription drugs in Denmark encourage the use of data from DNPR for studies within this field. Treatment with other thyroid hormone preparations than L-T4 are not included in the nationwide register, but these alternatives are not recommended for treatment of hypothyroidism in pregnant women and in general mostly used in more recent years [36]. Considering patient compliance and the lack of information on actual intake of the drug, a study within the DNBC evaluated compliance in pregnant women as the probability of self-reporting drug use in pregnancy after the purchase of a redeemed prescription [159]. Compliance was high for drugs used for the treatment of chronic diseases including thyroid disease, but low for local or short treatment drugs [159]. To increase the likelihood of actual treatment, women identified with thyroid disease in paper 1 and 2 redeemed minimum two prescriptions and data were linked with information on hospital diagnosis of disease [1,2]. The validity of the DNHR for the diagnosis of thyroid disease in and around pregnancy has not been evaluated specifically, however, for the use of ICD-8 and ICD-10 codes of hyper- and hypothyroidism in general, a Danish study reported that misclassification was less than two percent after the review of medical records from 900 patients [160].

The focus of paper 1 and 2 was the relationship between pregnancy and the onset of thyroid disease and other autoimmune diseases in women of reproductive age. Thus, the study population and inclusion criteria were selected for this purpose, and not to provide exact measures on the occurrence of the disorders in Denmark. Study assumption was the predominance of autoimmune hyper- and hyperthyroidism in this age group in Denmark [25,26], and inclusion criteria aimed to identify women with GD and persistent autoimmune hypothyroidism defined by treatment and duration of treatment as opposed to gestational hyperthyroidism and transient thyroid function abnormalities in PPT. In paper 1, women with hyperthyroidism were identified from minimum two redeemed prescriptions of ATD within one year over a period of more than one month, and in paper 2, women with hypothyroidism were identified from two or more redeemed prescriptions of L-T4 during a period of minimum two years. The inclusion period, 1999 to 2008, allowed for the identification of incident disease via linkage to previous prescriptions, hospital diagnoses or thyroid surgery and complete two year follow-up for all individuals. The primary analyses conditioned on the woman's first pregnancy in the inclusion period leading to the birth of a live-born child to exclude interference from subsequent and multiple pregnancies. Considerable variation in the incidence of maternal hyper- and hypothyroidism in and around pregnancy was observed (Figure 4.1) with some notable differences.

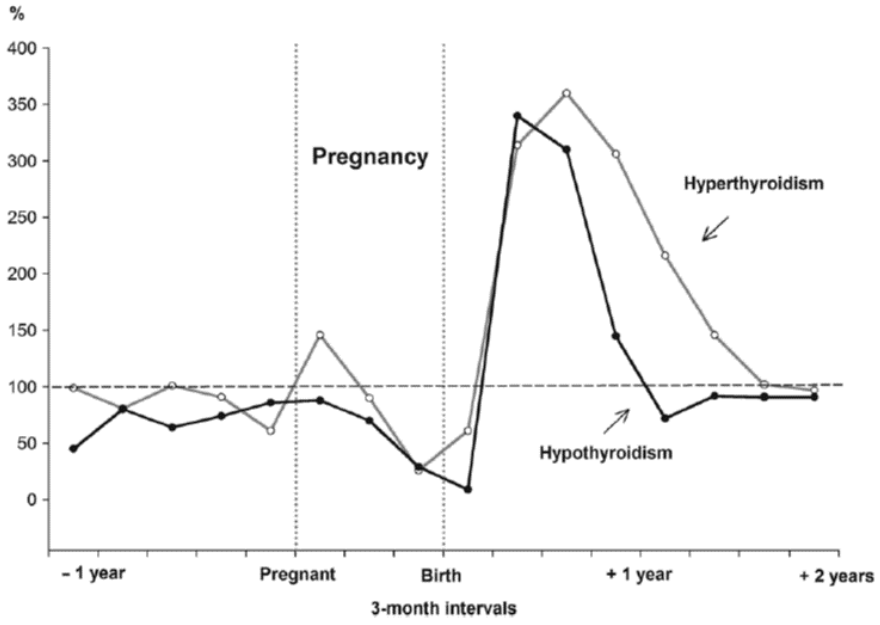


*Figure 4.1. Relative measures of incident maternal hyperthyroidism and hypothyroidism in and around pregnancy compared to the overall incidence in Danish women age 15-45 years. Reproduced with permission from* [2].

### 4.1.1. HYPER- AND HYPOTHYROIDISM DURING PREGNANCY

The incidence of hyper- and hypothyroidism decreased during the pregnancy (Figure 4.1), which is compatible with the general attenuation of the immune system in pregnancy and the decline in the levels of TPO- and Tg-Ab [161]. The level of TRAb usually declines as pregnancy progresses [162], which has been shown with different generations of assays [163-166], but stable or increased TRAb may also be seen [167]. The most intriguing finding was the disparity between hyperthyroidism and hypothyroidism in the early pregnancy (Figure 4.1), and the early pregnancy incidence peak of hyperthyroidism was not observed for other autoimmune diseases. Previous investigations have predominantly focused on the onset or worsening of GD in the postpartum period and this is the first study to provide population-based data on the onset during pregnancy. In a more recent study from Japan, different types of pregnancy related thyrotoxicosis were described in hospital outpatients [168]. Notably, onset of GD and gestational hyperthyroidism peaked around the same time (week 12-13), and all cases of GD were diagnosed in the first half of pregnancy [168]. In 1982, Amino et al. described the aggravation of Graves' hyperthyroidism in early pregnancy [169] and later proposed a causal role of hCG [170]. It seems biologically plausible that hCG may also trigger the onset of GD in susceptible individuals, but the underlying mechanisms and whether it is a direct thyroid stimulating hCG effect remain uncertain. Adding to this, it has been hypothesized that the hyperthyroid state itself may trigger disease activity in GD via effects on the immune system [171].

### 4.1.2. HYPER- AND HYPOTHYROIDISM POSTPARTUM

The incidence of hyper- and hypothyroidism increased postpartum (Figure 4.1), which is compatible with the general immune rebound [32,172] and the increase in the levels of TPO- and Tg-Ab [49,161,173,174] and in TRAb [163,164]. A notable finding was the timing and duration of the incidence peak postpartum, which differed between hyper- and hypothyroidism and other autoimmune diseases. The critical determinant in the interpretation of study results is, however, on the identification of women with GD and persistent autoimmune hypothyroidism as opposed to other types of postpartum thyroid dysfunction. In 1999, Amino et al. defined the postpartum autoimmune syndrome as any type of thyroid dysfunction newly occurring during the postpartum period in women with no previous thyroid dysfunction and proposed different subtypes (Figure 4.2) [32]. At present time, the distinction is typically between GD and thyroid dysfunction as part of PPT, where PPT is the occurrence of thyroid dysfunction, except hyperthyroidism caused by GD, in the first postpartum year in women who were euthyroid prior to the pregnancy [36]. The clinical course of PPT varies, which is reflected in considerable variation in the reported prevalence of the disorder [175] and as symptoms may be mild and undetected or untreated, the identification of cases from nationwide registers was methodologically challenged and consequently restricted to the identification of new onset Graves' hyperthyroidism and permanent hypothyroidism.

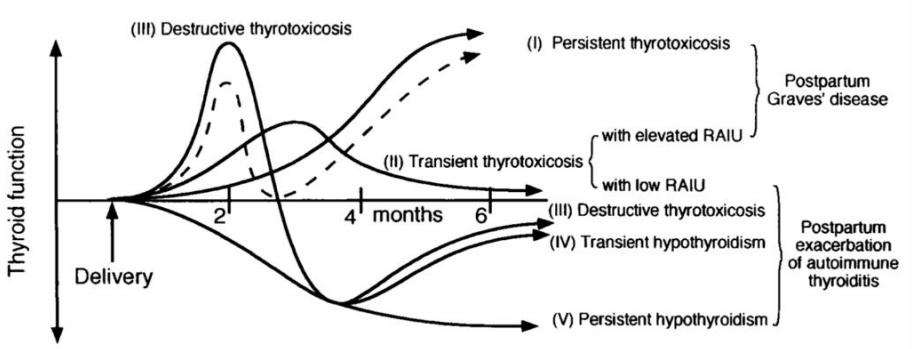


*Figure 4.2. Types of postpartum thyroid dysfunction. RAIU; radioiodine uptake. Reproduced with permission from [32].*

The finding that the postpartum period is a risk factor for onset of GD is in line with investigations from different countries [173,176-178]. Although the majority of studies agreed, a study from Italy concluded that their results did not support the postpartum period as a major risk factor for onset of GD [178]. Disparities in conclusions of previous retrospective studies may relate to the composition of the study population (e.g. age, parity) and the interpretation of results [173,176-178]. Paper 1 was a population-based study and the women with hyperthyroidism were selected from redeemed prescriptions of ATD. Furthermore, the timing of the incidence peak (Figure 4.1) is a plausible indicator that the cases identified did not suffer from thyrotoxicosis caused by PPT. In studies from Japan with measures of thyroid function and thyroid autoantibodies and determination of thyroid volume and blood flow by ultrasonography, onset of GD occurred 6-8 months postpartum, whereas the onset of thyrotoxicosis as part of PPT was within three months [168,179].

For the identification of cases of hypothyroidism in paper 2, the women were selected from continuous use of L-T4. Moreover, they had no prescriptions of ATD or hospital diagnosis of hyperthyroidism, or congenital or iatrogenic hypothyroidism. Hospital diagnoses in the DNHR were used to identify subtypes of hypothyroidism for the latter exclusion criterion although the validity of these specific diagnoses is unknown. The linkage to registrations of thyroid surgery is, however, expected to improve the diagnostic accuracy of iatrogenic hypothyroidism, but no information on radioiodine treatment was available. Although the hypothyroid phase of PPT may be more symptomatic than the hyperthyroid phase, symptoms may be mild, and the disease may not be clinically detected or may be managed in GP alone without initiation of L-T4 treatment [180]. These characteristics were evident from the lower frequency of transient hypothyroidism identified in the sub-analysis in paper 2, and limited the aim to the identification of persistent hypothyroidism with a need for prolonged L-T4 treatment and comparison with the incidence pattern of hyperthyroidism.

Results of paper 1 and 2 illustrate how pregnancy influence with the onset of different autoimmune diseases and the lack of a uniform pattern is noteworthy. Firstly, the early pregnancy incidence peak observed merely for hyperthyroidism may suggest that the underlying mechanism is not a general autoimmune phenomenon. Secondly, variability between autoimmune disorders is apparent from the female predominance of autoimmune thyroid diseases, which is similarly seen in rheumatoid arthritis, but not seen in inflammatory bowel disease [181]. Even considering the methodological challenges and a possible diagnostic delay, the observed disparity between hyper- and hypothyroidism in the way that persistent hypothyroidism peaked earlier than hyperthyroidism (Figure 4.1) is compatible with the pattern proposed by Amino et al. (Figure 4.2) [32]. They speculated if the immunological rebound after birth is initially a cellular response followed by a humoral response, which may coincide with the pathogenesis of autoimmune hypothyroidism and Graves' hyperthyroidism, respectively [32,182]. Underlying mechanisms for the development of autoimmune thyroid dysfunction postpartum remain uncertain, but the specific role of thyroid autoantibodies, alterations in immune cells during pregnancy, genetic susceptibility and fetal microchimerism are areas of investigation [174,183-187], and the clinical perspective is on the possibility of disease prediction [188,189].

## 4.2. DANISH NATIONAL BIRTH COHORT

Paper 3-5 investigated the occurrence of maternal thyroid disease among pregnant women enrolled in the DNBC and as part of this objective; the aim was to establish reference ranges for maternal thyroid function tests in early pregnancy and to evaluate the occurrence of maternal thyroid function abnormalities in the early pregnancy.

### 4.2.1. MATERNAL THYROID DISEASE

Paper 3 was the initial publication within the DNBC, which described the occurrence of maternal thyroid disease before, during and up to five years after the pregnancy, when evaluated from maternal self-report at the first telephone interview in early pregnancy and via linkage to nationwide registers. Exposure information in the DNBC was collected via computer-assisted telephone interview (median week 17) [190]. In addition to this, the women filled out a registration form at the time of recruitment in early pregnancy (median week 8) with information on current medication [190]. The women included in paper 3 and the subsequent publications within the DNBC all gave birth to a singleton live-born child and the woman's first live birth in the study period was chosen. This conditioning was made, because complete interview data were not available for women terminating with pregnancy loss and because the subsequent studies in the protocol included follow-up of the children. In the register-based studies on pregnancy complications (paper 13 and 14), maternal hyper- and hypothyroidism were associated with pregnancy loss, which should be considered in relation to measures of disease occurrence.

For assessment of self-reported thyroid disease among women in the DNBC, information from the first pregnancy interview and from the registration form was included. This methodology was chosen to ensure complete data for all individuals, because some loss to follow-up was observed between subsequent interviews (five percent between first and second pregnancy interview) [190]. The content and phrasing of the interviews were made by project coordinators in consultant with external experts (midwifes, general practitioners, obstetricians and pediatricians). All interviewers were women of reproductive age and employed by a commercial call center [191]. Training courses were conducted for the interviewers and pilot testing of the interviews in pregnancy was satisfactory [143]. The questions related to the occurrence of thyroid disease included an overall question of previous thyroid disease, and whether it was diagnosed by a doctor and still present. In addition to this, the woman was asked about subtypes of thyroid disease with the options of answering: hyperthyroidism, GD, toxic adenoma, hypothyroidism, Hashimoto's, iatrogenic, others, and unknown). It appeared from the results that many women answered the option 'others' on the subtype of thyroid disease, which was filled out in free text by the interviewer. Thus, individual review of the records was performed as part of the data analyses in paper 3, which revealed that a proportion of the answers were unlikely to be thyroid disease (e.g. polycystic ovarian syndrome, psoriasis). Whether this disparity can be attributed to the pregnant woman and/or the interviewer remains uncertain. Variation in health beliefs and personal habits have not been found to influence the questions on smoking and alcohol intake in the DNBC [191]. Still, the findings questioned the use of self-reported data for assessment of thyroid disease, which is compatible with other reports [192,193]. Consequently, self-reported data were combined with information on hospital diagnoses, surgery and prescriptions of drugs via linkage to nationwide registers, which has also been the suggestion for assessment of other endocrine diseases in pregnancy [194].

Overall, four percent of the 77,445 Danish pregnant women studied in paper 3 were found to have thyroid disease before, during or after the pregnancy. The study further aimed to classify the women with known or new onset thyroid disease in or after the pregnancy and to classify the major subtypes of thyroid disease. As described in detail in the method section of paper 3, such classification had to rely on predefined criteria, when indirect measures from nationwide registers were used. Nevertheless, the findings that onset of disease in pregnancy was rare and that 0.2% of the women were treated with ATD and 0.5% with L-T4 in early pregnancy are comparable with general figures [44,195]. Hyperthyroidism was more frequent before and during the pregnancy, whereas hyperthyroidism and hypothyroidism were equally frequent when diagnosed in the years following the pregnancy. This probably relates to a change in age and population characteristics (e.g. iodine status). Certain population characteristics that affect the occurrence of thyroid disease in general and in pregnancy will be addressed in section 4.3 'Predictors of maternal thyroid disease'.

### 4.2.2. REFERENCE RANGES

Paper 4 was the first publication in the DNBC that included measurements of maternal thyroid function in stored samples from the early pregnancy. The aim was to establish method- and pregnancy week specific reference ranges for TSH and fT4. The inclusion of pregnant women in the DNBC was before the current organization of prenatal screening for chromosomal anomalies in Denmark [121]. Thus, the first antenatal visit in GP was on average earlier than current practice, which facilitated a considerable number of samples from the early pregnancy weeks. An important methodological note is on the determination of pregnancy week of blood sampling. Pregnancy weeks are counted from the first day of the last menstrual period [196], and fertilization on average takes place two weeks later, but the terminology used for the counting of pregnancy weeks may differ (Figure 4.3). Obstetricians often use the terminology full weeks plus days by which week 0 (+…) is the first week of pregnancy. The terminology 1st, 2nd, 3rd week was used in paper 4 and week of blood sampling ranged from the 5th (4+0 to 4+6) to the 19th week (18+0 to 18+6).

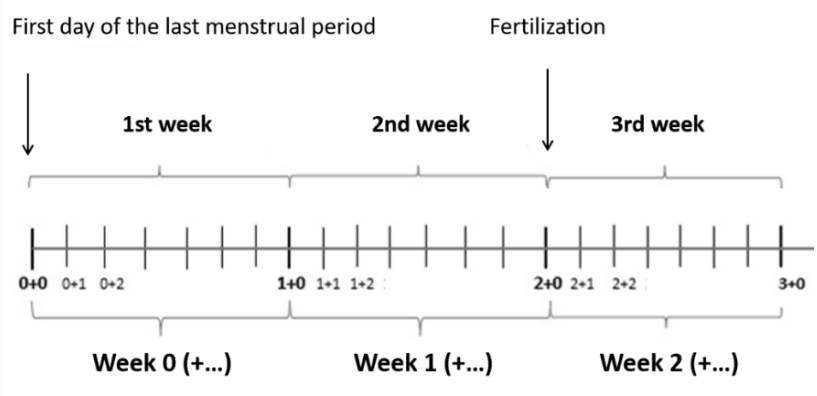


*Figure 4.3. Illustration of the terminology of pregnancy weeks.*

The approved protocol allowed for 100 µl of biological specimen from each pregnant woman, which restricted the number of biochemical analyses to TSH and fT4 and excluded the possibility to measure TPO- and Tg-Ab. The log-linear relationship between TSH and fT4 favors a TSH screening strategy during stable conditions [197], but the choice of measurement of peripheral thyroid hormone concentrations differ (total and/or free thyroid hormone concentrations). When evaluated in 2016, nearly 90% of clinical laboratories in Denmark offered free thyroid hormone measures (fT4 and/or free triiodothyronine (fT3)) [198], but in the North Denmark Region, total thyroid hormone measurements are exclusively performed by the clinical laboratory and recommended in clinical practice. Results may be supported by the measurement of TBG and total T4 corrected according to TBG levels is reported upon request.

The main reason for the widespread use of free thyroid hormone measurements has been that any misinterpretation of total thyroid hormone concentrations due to changes in binding proteins may be avoided [199]. Direct methods for the measurement of free thyroid hormones include an initial separation of free and protein bound hormone by equilibrium dialysis or ultrafiltration and subsequent measurement of the free thyroid hormone concentration by a radioimmunoassay or mass spectrometry [200,201]. On the other hand, current methods used in clinical laboratories for measurement of fT4/fT3 are automatic, competitive immunoassay based on an indirect measure with no initial separation. Furthermore, the specific methodology may differ between assays (one-step, two-step, labeled antibody) [201]. The concentration of free thyroid hormones changes during a pregnancy when measured with both direct and indirect methods (lower levels in second and third trimester) [202-204]. However, the indirect methods are to a variable degree affected by the physiological changes in binding proteins in pregnant women and may also show diverse results in critical ill patients [204-206]. These characteristics necessitate the use of method- and pregnancy week specific reference ranges. Otherwise, the risk of misclassification is considerable [204,207], which has raised concern about the use of free thyroid hormone measurements in pregnant women [204]. The choice of supplementary measurement to TSH in paper 4 was fT4. The automatic immunoassay that was used did not provide the possibility to measure total thyroid hormone concentrations, measurement of TBG was not possible, and free thyroid hormone measurements are often requested in a scientific context. T3 is less protein-bound than T4, which makes measurements of fT3 even more uncertain [206].

TPO- and/or Tg-Ab are markers of autoimmune hypothyroidism and associated with higher TSH in a population [47]. Recommendations on the establishment of reference ranges for thyroid function tests in pregnancy suggest the exclusion of TPO-Ab positive women from the reference cohort [36]. In paper 4, exclusion criteria were established in an attempt to define a thyroid healthy reference cohort despite the lack of thyroid autoantibody measurements. Participants were a 12% random sample of pregnant women in DNBC who gave birth to a singleton live-born child and had a blood sample drawn in early pregnancy. It was possible to exclude women with previous, current or later diagnosed thyroid disease or other autoimmune diseases as well as individuals with prescriptions of thyroid interfering medication via linkage to Danish nationwide registers. Furthermore, women with TSH at or above 4.5 mIU/l or free T4 at or above 21 pmol/l were excluded from the reference cohort. How to deal with outliers in a reference cohort is controversial, and no universal consensus exist [208]. Although criticism may be raised on the method of outlier detection in paper 4 [209], the approach was in line with a previous report from a national birth cohort in Finland [210], and excluded participants in paper 4 had TSH ranging up to 80 mIU/l and fT4 up to 42 pmol/l, which suggests the presence of undiagnosed thyroid disease [211]. Moreover, as reported in paper 4, results did not considerably change if these individuals were not excluded from the reference cohort. Still, other methods of outlier detection could have been applied e.g., Tukey's fences or Dixon's test, but all methods include pros and cons [208].

The method- and pregnancy week specific reference ranges for TSH and fT4 in paper 4 were determined by non-parametric methods and reported as 2.5 and 97.5 percentiles with 95% confidence interval (Figure 4.4.) The outermost early and late pregnancy weeks were collapsed, however, all intervals except weeks 15-19 included more than 120 individuals, which is the minimum recommended number [208].

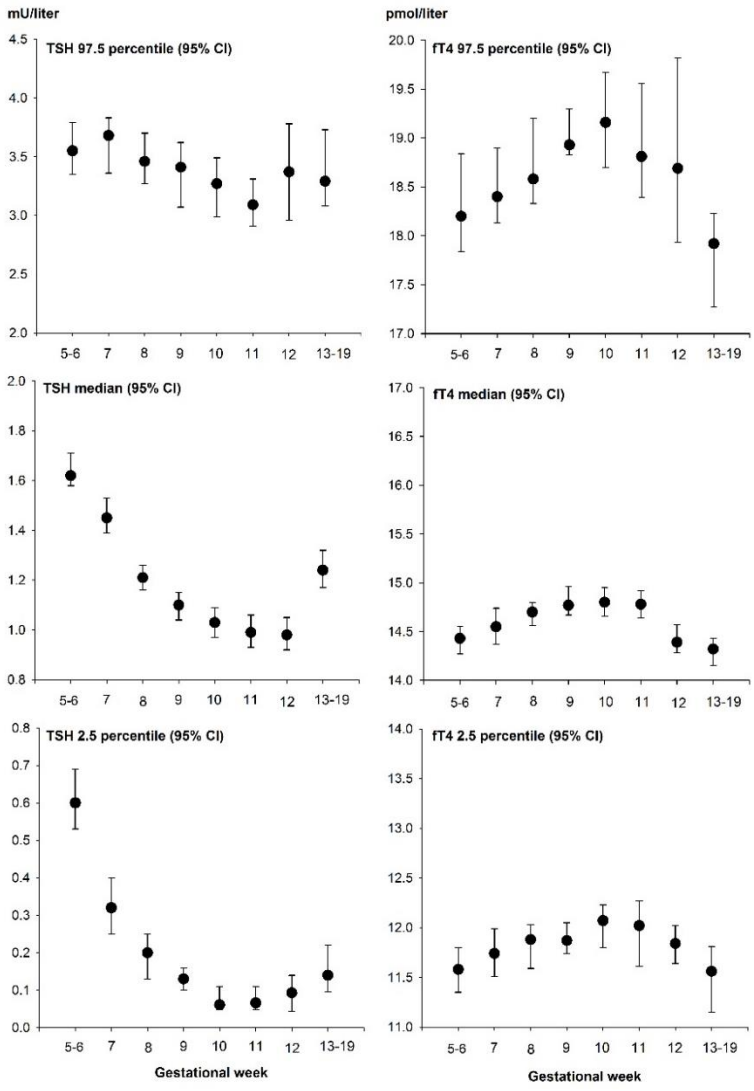


*Figure 4.4. Reference ranges for maternal TSH and free T4 (fT4) in the early pregnancy. Reproduced with permission from* [4].

The reference range for TSH showed considerable variation during the first trimester (Figure 4.4). TSH levels were low in pregnancy week 9-12, and a decreasing trend in the lower reference limit prior to the 9th week was observed. An indication of higher TSH levels in pregnancy week 5-6 has also been observed in other reports (Table 4.1) from different countries with varying iodine intake [29,212,213].

| Pregnancy week | 2.5 percentile | Median | 97.5 percentile |
|---|---|---|---|
| Dashe et al., 2005, US, Immulite 2000 (Siemens) [214] | | | |
| 6 | 0.23 | 1.36 | 4.94 |
| 7 | 0.14 | 1.21 | 5.09 |
| 8 | 0.09 | 1.01 | 4.93 |
| 9 | 0.03 | 0.84 | 4.04 |
| Stricker et al., 2007, Switzerland, Architect i2000 (Abbott) [215] | | | |
| ≤ 6 | 0.45 | 1.24 | 3.23 |
| 7-12 | 0.07 | 0.95 | 2.82 |
| Mannistö et al., 2011, Finland, Architect i2000 (Abbott) [210] | | | |
| 2-6 | 0.26 | 1.26 | 3.87 |
| 7 | 0.14 | 1.15 | 3.09 |
| 8 | 0.12 | 1.10 | 3.04 |
| 9 | 0.07 | 1.07 | 3.20 |
| Shen et al., 2014, China, Architect i2000 (Abbott) [216] | | | |
| ≤ 6 | 0.37 | 1.41 | 3.98 |
| 7-12 | 0.07 | 1.12 | 3.38 |
| Li et al., 2014, China, Cobas e601 (Roche) [217] | | | |
| 4 | 0.65 | 2.24 | 6.28 |
| 5 | 0.76 | 2.20 | 6.70 |
| 6 | 0.52 | 2.08 | 4.84 |
| 7 | 0.20 | 1.57 | 4.42 |
| 8 | 0.11 | 1.37 | 4.81 |
| 9 | 0.04 | 1.25 | 4.22 |
| Liu et al., 2017, China, Modular Analytics E 170 (Roche) [218] | | | |
| 5-8 | 0.47 | 2.18 | 5.00 |
| 9-12 | 0.09 | 1.47 | 4.52 |
| Liu et al., 2017, China, Architect i2000 (Abbott) [218] | | | |
| 5-8 | 0.23 | 1.80 | 4.46 |
| 9-12 | 0.03 | 1.05 | 3.83 |
| Liu et al., 2017, China, UniCel DxI 800 (Beckman Coulter) [218] | | | |
| 5-8 | 0.03 | 1.69 | 4.16 |
| 9-12 | 0.05 | 1.24 | 3.55 |

*Table 4.1. Reports of week specific reference ranges for TSH (mIU/l) in the first trimester of pregnancy. All studies besides the study by Dashe et al. excluded women who were positive for TPO- and/or Tg-Ab from the reference cohort.*

One possible mechanism of the dynamics in TSH in the early pregnancy that were observed in paper 4 and in other reports is the balance between D3 and hCG [31]. The peak in hCG concentration is overlapping with the low TSH period observed in week 9-12 (Figure 4.4), and the higher TSH levels in week 5-6 may relate to a dominant D3 activity in these early weeks of pregnancy. D3 expression in the uteroplacental unit is high from the early pregnancy [24,40,219,220]. Furthermore, the replication of the findings in other studies from different countries with different assays of TSH and exclusion of TPO- and/or Tg-Ab positive women may support the hypothesis. FT4 showed much less, but opposite, variation to that of TSH (Figure 4.4). Thus, the use of a uniform method specific reference range for fT4 in first trimester may be reasonable, but results advocate that TSH needs to be considered in shorter intervals during the first trimester of pregnancy. An important consideration in relation to reference ranges is on the clinical applicability. Uncertainty prevails on the exact week of pregnancy in early pregnancy before ultrasound determination and the use of week specific reference range may be too ambitious. An alternative practice compatible with the findings in paper 4 and other reports [217] is to consider three periods in the first trimester; an early high TSH period (week 5-6), a dynamic TSH period (week 7-8) and a low TSH period (week 9-12), but further studies in different populations are warranted to evaluate this approach. Another clinical discussion concerning early pregnancy reference ranges is on the upper limit of TSH. Previous recommendations of the American Thyroid Association, Endocrine Society, and the European Thyroid Association suggested an upper limit of 2.5 mIU/l [78,103,104]. However, revised guidelines of the American Thyroid Association in 2017 [36] recommends that the upper limit is increased to 4.0 mIU/l. Results of paper 4 support that a uniform upper limit of 2.5 mIU/l in the first trimester of pregnancy is too low and raise concern about the diagnosing of thyroid disease in the early pregnancy weeks if the dynamics of TSH are not considered.

### 4.2.3. THYROID FUNCTION ABNORMALITIES

Paper 5 aimed to describe the frequency of maternal thyroid function abnormalities in early pregnancy using the week specific reference ranges established in paper 4. A feasibility of measurements in stored biobank samples was the possibility to detect unidentified disease. Participants in paper 5 included the 12% random sample of pregnant women in the DNBC from paper 4 (n=7,624) and in addition to this, all women identified with thyroid disease via linkage to nationwide registers in paper 3 were included to evaluate the frequency of thyroid function abnormalities in women with known thyroid disease (n=1,241), and in women who were diagnosed with thyroid disease after the blood sampling in pregnancy or in the years following pregnancy (n=1,204). Thyroid dysfunction was defined by TSH and/or fT4 outside the method- and pregnancy week specific reference ranges and classified into six subtypes of thyroid function abnormalities (Table 4.2). The overall frequency of thyroid dysfunction among all women in the random sample was 12.5% (Table 4.2), which would simulate a general routine testing of thyroid function in early pregnancy.

| | All in random sample | | Known thyroid disease | | Later thyroid diagnosis | |
|---|---|---|---|---|---|---|
| | n = 7,624 | | n = 1,241 | | n = 1,204 | |
| | n | % | n | % | n | % |
| **Thyroid dysfunction** | 951 | 12.5 | 432 | 34.8 | 441 | 36.6 |
| Hyperthyroidism | 271 | 3.6 | 137 | 11.0 | 115 | 9.5 |
| Overt | 118 | 1.6 | 75 | 6.0 | 70 | 5.8 |
| Subclinical | 153 | 2.0 | 62 | 5.0 | 45 | 3.7 |
| Hypothyroidism | 357 | 4.7 | 214 | 17.2 | 274 | 22.8 |
| Overt | 55 | 0.7 | 42 | 3.4 | 120 | 10.0 |
| Subclinical | 302 | 4.0 | 172 | 13.8 | 154 | 12.8 |
| Isolated abnormal fT4 | 323 | 4.2 | 81 | 6.6 | 52 | 4.3 |
| Hypothyroxinemia | 174 | 2.3 | 33 | 2.7 | 30 | 2.5 |
| Hyperthyroxinemia | 149 | 1.9 | 48 | 3.9 | 22 | 1.8 |

*Table 4.2. Frequency of early pregnancy thyroid function abnormalities in a random sample and in women with known or later diagnosed thyroid disease [5].*

The subtypes of maternal thyroid dysfunction in the random sample varied (Table 4.2). More evidence on the prevalence and impact of isolated changes in maternal fT4 is needed [61,62] and these abnormalities were consequently also identified and described. The comparison of figures on maternal thyroid dysfunction in pregnancy between studies is challenged by several factors. Firstly, population characteristic may influence the occurrence and secondly, the timing in pregnancy as well as the information source and criteria used for the identification of maternal thyroid dysfunction differ between studies. In line with the majority of other reports, overt hypothyroidism was observed in less than one percent of the pregnant women in paper 5 [24,76]. Overt hyperthyroidism was more frequently observed (Table 4.2), even after the exclusion of women with known thyroid disease, which has similarly been observed in other European birth cohorts [221-223]. The regional differences in iodine intake in Denmark at the time of inclusion and the implementation of a mandatory iodine fortification in the year 2000 [224] need to be addressed and will be discussed in section 4.3 ‘Predictors of maternal thyroid disease’.

An intriguing finding in paper 5 (Table 4.2) was the high frequency of thyroid function abnormalities in women with known or later diagnosed disease. Inadequate treatment of hypothyroidism is commonly observed in the population in general with up to 40% of patients being either over- or undertreated [225-227]. For the treatment of hypothyroidism in pregnant women, studies from Sweden [80,81], the United Kingdom (UK) [83], Scotland [82] and Denmark [84] have indicated a similar trend, when evaluating the frequency of abnormal high TSH in pregnant women on current L-T4 treatment according to uniform upper limits in pregnancy. Other studies have indicated a low adherence with maternal L-T4 treatment in pregnancy [228-230]. The majority of TSH measurements in these studies were performed in the first trimester

of pregnancy, and 50-60% of the women were inadequately treated, when a TSH upper limit of 2.5 mIU/l was applied, and 30-40% when the upper limit was around 4.0 mIU/l [80-84]. In paper 5, pregnancy week specific TSH reference ranges were used, and the measurement of TSH was in stored biobank samples. Hypothyroidism was identified in 40% of the 180 pregnant women with known hypothyroidism in current treatment with L-T4. TSH ranged from 3.5-30 mIU/l in this group of women, and four of the women were newly diagnosed in the pregnancy. The frequency of hypothyroidism was similar among the 58 women with previous hyperthyroidism who received current replacement therapy with L-T4, and the frequency of hyperthyroidism was 30% in the 84 women who received current ATD for the treatment hyperthyroidism. These figures raise concern about insufficient treatment of maternal thyroid disease in the early pregnancy, but uncertainties to the evaluation from a single measurement should be acknowledged. However, in the UK study, 67% of the women with TSH above 2.5 mIU/l in first trimester also had a TSH above the 3.0 mIU/l limit in the second and/or third trimester [83]. In the general population, concern has been raised about overtreatment of thyroid disease in relation to long-term outcomes [231]. In paper 5, seven percent of L-T4 treated women with known hypothyroidism were identified with biochemical hyperthyroidism. This is in line with the UK study in which 6.5% had TSH < 0.2 mIU/l in the first trimester [83]. The UK study raised concern about inadequate treatment of maternal hypothyroidism as the risk of miscarriage was significantly increased in women with a TSH above 4.5 mIU/l [83]. The reason for the inclusion of women with later diagnosed thyroid disease in paper 5 was the findings in earlier register studies (paper 13-18) of adverse pregnancy and child outcomes in women who had no diagnosis of thyroid disease at the time of pregnancy, but were first diagnosed in the years following the pregnancy. These findings generated the hypothesis of undetected disease in the pregnancy, which the actual measurement of thyroid function in paper 5 corroborated. Whether such unidentified maternal thyroid function abnormalities associate with adverse child outcomes was addressed in paper 7 and 8.

## 4.3. PREDICTORS OF MATERNAL THYROID DISEASE

Genetic and environmental factors have been associated with the development of autoimmune thyroid disease in the population in general (Table 4.3). If the same associations are seen in pregnant women, it may imply the inclusion of patient characteristics in the strategy for detection and management of thyroid disease. Accordingly, international clinical guidelines recommend the measurement of TSH in pregnant women considered of high risk for thyroid disease [36]. Some of the high-risk criteria are maternal age (> 30 years), multiple prior pregnancies (≥ 2), morbid obesity (BMI ≥ 40 kg/m$^2$), and women living in an area of known moderate to severe iodine deficiency. Paper 6 specifically addressed the association between maternal smoking in pregnancy and the development of hyperthyroidism and hypothyroidism, whereas paper 1-4 investigated alternative predictors of maternal thyroid disease.

| | Graves' disease | Autoimmune hypothyroidism | References |
|---|---|---|---|
| Increasing age | ↑ | ↑ | [25,26] |
| Increasing parity | ↑ | ↑ | [232,233] |
| Increasing iodine intake | ↑ | ↑ | [25,26,234,235] |
| Smoking | ↑ | ↓ | [236-238] |
| Alcohol intake | ↓ | ↓ | [239,240] |

*Table 4.3. Predictors of autoimmune thyroid disease observed in the general Danish population (risk factor (↑), protective factor (↓)).*

### 4.3.1. MATERNAL AGE AND PARITY

A Danish study with detailed classification of newly diagnosed hyperthyroid patients described that the incidence rate of GD shows a slight increase from 20 to 40 years and then levels out [26], whereas for autoimmune hypothyroidism the incidence rate is stable low up to the age of 40 years and then increases [25]. Considering the role of maternal age for the development of autoimmune thyroid disease in pregnancy, age above 30 years was a risk factor for hyper- and hypothyroidism in paper 3, but maternal age did not predict TSH and fT4 early pregnancy reference limits in paper 4. The disparity may be explained by the fact that thyroid disease was identified from diagnosis and treatment up to five years after the pregnancy in paper 3 signifying follow-up to an older age. Other studies of pregnant women did not observe that maternal age above 30 years was a risk factor for hyperthyroidism or hypothyroidism in pregnancy [241-243], but one study demonstrated that maternal TSH in pregnancy increased by 0.03 mIU/l for every increasing year of maternal age [244].

Previous live births and induced abortions were found to be a risk factor for onset of autoimmune hypothyroidism and GD after the postpartum period in Danish case-control studies [232,233], but an association with parity has not been consistently shown in other reports [244-251]. Considering the role of maternal parity for the development of autoimmune thyroid disease in pregnancy, multiparous women did not have a higher risk of being diagnosed with hyperthyroidism or hypothyroidism up to five years after the pregnancy in paper 3. On the other hand, multiparous women had lower reference limits for TSH in early pregnancy in paper 4. Another study similarly observed that maternal TSH decreased in pregnancy by increasing maternal parity independently of age [244]. Considering the onset of maternal thyroid disease postpartum, the risk of onset of hypothyroidism in the postpartum period was higher in multiparous women than after first time pregnancies in paper 2. A possible mechanism is that repeated pregnancy exposure accelerates the development of disease in susceptible individuals [232,233]. However, the risk of postpartum onset of hyperthyroidism in paper 1 was higher in nulliparous women, which may suggest that a single pregnancy period is sufficient for the development of GD in susceptible individuals.

### 4.3.2. MATERNAL ORIGIN AND BODY MASS INDEX

The role of ethnicity in the development of autoimmune thyroid disease has not been examined in the Danish population specifically, but a study of US military personnel showed that GD was more common and autoimmune hypothyroidism less common in black and Asians than in whites [252]. The role of ethnicity in relation to maternal thyroid function in pregnancy is debatable, and an increasing number of studies have suggested that ethnic differences should be considered [253-256]. The measure of ethnicity from Danish nationwide register data was a dichotomous variable on place of birth in or outside of Denmark. In line with a study from the US [244], the reference limits in paper 4 were not influenced by maternal ethnicity, but the number of pregnant women born outside of Denmark in the DNBC was low [143]. Ethnicity was a risk factor for maternal hyper- and hypothyroidism assessed from hospital diagnoses and redeemed prescriptions of drugs in paper 3 and this finding was in line with the previous observations using data from the DNHR in paper 15. However, diagnostic awareness in the clinical management of pregnant women born outside of Denmark may contribute partly to this association.

The association between BMI and autoimmune thyroid disease specifically has not been described in the Danish population, but increasing BMI has been associated with higher TSH and lower T4 [257], consistent with other reports [258]. Considering the role of body weight for the development of autoimmune thyroid disease in pregnancy, TSH reference limits in early pregnancy were higher and fT4 lower in individuals with BMI at or above 30 $kg/m^2$ in paper 4, and increasing BMI was a risk factor for maternal hypothyroidism, but not for hyperthyroidism, in paper 3. These findings are in line with other reports of pregnant women [259-261], and much focus is on the association between maternal body weight and hypothyroxinemia [262,263].

### 4.3.3. MATERNAL IODINE INTAKE

Denmark was previously iodine deficient with regional differences, and a mandatory iodine fortification of salt was introduced in the year 2000 [264]. Before iodine fortification, hypothyroidism was more frequent in East Denmark with mild iodine deficiency and dominated by autoimmune hypothyroidism [25]. Hyperthyroidism was most frequent in West Denmark with moderate iodine deficiency, particularly in individuals above 40 years of age, and dominated by multinodular toxic goiter [26]. Pregnant women suffered from iodine deficiency with signs of thyroidal stress [265,266]. As expected, mandatory iodine fortification has increased iodine intake in the Danish population [267]. This was associated with an increase in the incidence of overt hypothyroidism, particularly in West Denmark and in young and middle-aged individuals [234,268]. The incidence of hyperthyroidism has shown a transient increase after iodine fortification, and unexpectedly, the highest increase has been observed in individuals younger than 40 years [235,269], where GD is the dominant cause [26]. Further, the presence of TPO- and Tg-Ab has increased [48]. Studies of Danish pregnant women before iodine fortification elucidated the beneficial effects

of iodine supplementation on maternal thyroid function [270,271]. Evaluation of iodine intake in Danish pregnant women in East and West Denmark more than 10 years after the introduction of mandatory iodine fortification showed that 85% of the women used iodine supplement in pregnancy, but iodine intake was insufficient both in iodine supplement users and non-users, when evaluated by measurement of urinary iodine concentration [272,273]. Adding to this, iodine intake in the Danish population has decreased in recent years [274] and considerations to increase the amount of iodine added to salt are ongoing. The introduction of mandatory iodine fortification of salt in Denmark was during the period of study inclusion in the DNBC, and the regional differences in iodine intake as well as the changes in the occurrence of thyroid disease in a population related to increased iodine intake need to be addressed. In paper 4, the reference limits for TSH in early pregnancy were lower in West Denmark compared with East Denmark compatible with regional differences in TSH in the Danish population before iodine fortification [275]. Figure 4.5 illustrates the occurrence of thyroid function abnormalities in early pregnancy stratified by region and year of blood sampling in paper 5, and in a study of pregnant women in East Denmark in 2008 [223]. Non-significant trends in the occurrence of hyperthyroidism were observed, whereas hypothyroidism was more common in East compared with West Denmark before iodine fortification, and the occurrence of hypothyroidism significantly increased in West Denmark after iodine fortification (Figure 4.5). Uncertainties to the crude stratified analyses and the influence of other factors should be acknowledged, but the findings in Danish pregnant women seem compatible with observations in the general Danish population before and after iodine fortification and call for further reports and monitoring of the changes over time.

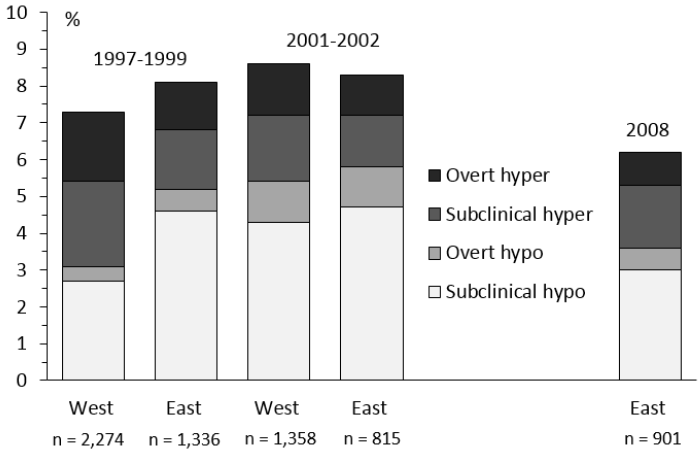


*Figure 4.5. Frequency of hyperthyroidism and hypothyroidism in a random sample of Danish pregnant women in the DNBC with no known thyroid disease stratified by region and year of blood sampling (1997-1999 and 2001-2002), and in a study of Danish pregnant women in East Denmark, 2008 [223]. Mandatory iodine fortification was introduced in the year 2000.*

### 4.3.4. MATERNAL SMOKING AND ALCOHOL INTAKE

Maternal smoking and alcohol habits are not part of the high-risk criteria for assessment of maternal thyroid function in pregnancy in international guidelines [36], but considerable awareness persists on these potential modifiable factors in reproductive epidemiology. The lack of information on individual smoking habits is often a limitation in studies of the general population using nationwide registers, but information on maternal smoking in pregnancy has been registered in the DNHR since 1996. The registration is performed by midwifes at birth of the child and includes information on current smoking or smoking cessation in the pregnancy. In paper 6, women who gave birth to a live-born child in Denmark from 1996-2008 were included, and the observed frequency of maternal smoking in pregnancy considerably decreased during the study period in line with other reports [276]. The study only included women who reported current smoking in the pregnancy, and the role of smoking cessation will need to be addressed in a future study. Furthermore, women who stopped smoking prior to pregnancy could not be identified, and information on smoking habits after the pregnancy was only available for a sensitivity analyses in women with consecutive pregnancies. Information on maternal hyper- and hypothyroidism was obtained from the combined use of DNHR and DNPR for identification of diagnoses and treatment. Even if some misclassification of exposure and outcome is likely to be present, consistent associations between maternal smoking and the development of maternal thyroid dysfunction before or after the pregnancy were observed, and any misclassification is expected to be non-differential with bias towards the null [156]. Smoking increased the risk of hyperthyroidism and decreased the risk of hypothyroidism, and these findings were subsequently corroborated in paper 3 with self-reported information on maternal smoking habits in early pregnancy. In paper 4, measurements of maternal thyroid function revealed no difference in the reference limits for TSH according to maternal smoking, but fT4 limits were lower in smokers. The association between smoking and the thyroid has been studied for decades, and the summarizing of current evidence in 1994 and in 2013 have shown the developments within the field [277,278]. It has long been evident that smoking is a risk factor for GD [277], and particularly Graves' ophthalmopathy, but the findings that smoking reduces the risk of autoimmune hypothyroidism and the occurrence of TPO- and Tg-Ab have come about in more recent years [278]. A Danish study [238] showed in 2012 that smoking cessation was associated with an increased risk of developing autoimmune hypothyroidism in the years immediately after cessation. Considering changes in thyroid function parameters associated with current smoking, studies performed within the general population have shown that TSH typically decreases and fT4 and fT3 increase, which is considered to be induced by activation of the sympathetic nervous system [278]. The lower fT4 reference limits in pregnant women who were current smokers observed in paper 4 are in contrast to this general notion. Other studies of pregnant women have similarly shown such decrease [244,279], whereas others showed no difference in fT4 between smokers and non-smokers [280,281]. The possible mechanisms of alterations in thyroid function parameters in smoking pregnant women are not clear, and the findings must be addressed in further studies.

Information on maternal alcohol intake in pregnancy is not available in Danish nationwide registers, but self-reported information on pre-pregnancy alcohol intake in early pregnancy was available in the DNBC. In paper 3, maternal alcohol intake reduced the risk of hyperthyroidism, but no associations with hypothyroidism was observed, and maternal alcohol intake was not a significant predictor of TSH and fT4 reference limits in paper 4. In the population in general, Danish case-control studies have shown that alcohol reduced the risk of both GD [240] and autoimmune hypothyroidism [239], but the underlying mechanisms are unknown.

### 4.3.5. CLINICAL AND SCIENTIFIC IMPLICATIONS

The combined summary of the findings in paper 1-6 illustrates similarities with other studies in pregnant women and in the population in general on possible predictors of autoimmune thyroid disease, but it also highlights that numerous aspects are still to be elucidated. One aspect is the underlying mechanisms for the associations observed. Autoimmune thyroid disease is considered to develop upon interaction between genetic and environmental factors, and the relative contribution of these factors has been considered and discussed [27,50,282,283]. One model of causation is the sufficient cause model introduced by Rothman in 1976 [284]. In this model, a variable number of sufficient causes may explain the development of disease and each sufficient cause is constituted by a number of component causes. All component causes must be present in a sufficient cause for disease to develop, and a component cause may be present in more than one sufficient cause. The model adds a theoretical framework, but also serves to illustrate that the role of different component causes does not add up to 100%, and that there is no upper limit of the proportion due to interaction between component causes. Thus, a disease can be entirely dependent on the presence of genes, but environmental factors may still be component causes. Considerable contributions have been made regarding genetic and environmental factors involved in the development of autoimmune thyroid disease, and still more potential component causes have been described (e.g. selenium, vitamin D, stress, infections) [27]. How different component causes interact in the development of autoimmune thyroid disease (gene-environment interactions) and why both opposing and similar effects are observed in GD and autoimmune hypothyroidism and other autoimmune diseases remain uncertain. Another aspect is the clinical implication of identifying causal components. Considering genetic factors, much focus is on the identification of polymorphisms, which may predict relapse of GD after ATD treatment [285] or treatment response in autoimmune hypothyroidism [286]. For the evaluation of environmental factors, studies from the Netherlands have suggested clinical prediction models for female relatives of patients with autoimmune thyroid disease [248] and for low maternal thyroid function in pregnancy [287], but the validity of such models is hitherto unknown. Finally, the scientific implication of identifying genetic and environmental factors should be considered. Since many of the environmental factors associated with autoimmune thyroid disease may also be associated with adverse outcomes of pregnancy and child development, the role of confounding is important to consider in observational outcome studies [74].

# CHAPTER 5. FETAL PROGRAMMING BY MATERNAL THYROID DISEASE

This chapter includes a discussion of paper 7 and 8, which investigated the hypothesis of fetal programming by maternal thyroid disease on child neurodevelopment and neurocognitive performance. The studies were performed within the DNBC and used the same methodological approach for assessment of maternal thyroid function, but differed by the type of neurodevelopmental and neurocognitive outcome in the child.

## 5.1. NEURODEVELOPMENTAL DISORDERS

Neurodevelopmental disorders develop secondary to disturbed brain development. Depending on the timing and magnitude of disruption, symptoms may range from specific disabilities to global impairments [288]. The etiology of the disorders is only partly understood and a variety of exposures have been proposed. Paper 7 was a case-cohort study within the DNBC, which aimed to examine the association between maternal thyroid function and child neurodevelopmental disorders. In addition to the random sample of pregnant women in paper 4 and women with known or later diagnosed thyroid disease in paper 5, the selection was made from registrations of neurodevelopmental disorders in the child. Thus, all women in the DNBC whose child had a registration of a neurodevelopmental disorder during follow-up in Danish nationwide registers up to December 31, 2010, were selected for measurement of maternal thyroid function in the stored blood sample from early pregnancy. The types of neurodevelopmental disorders were selected from the findings in the earlier register-based cohort studies (paper 16 and 17) in which a large cohort of children live-born in Denmark were followed for a diagnosis of seizures, autism spectrums disorders (ASD) and attention deficit hyperactivity disorder (ADHD) (Figure 5.1), and the association with maternal thyroid disease was investigated.

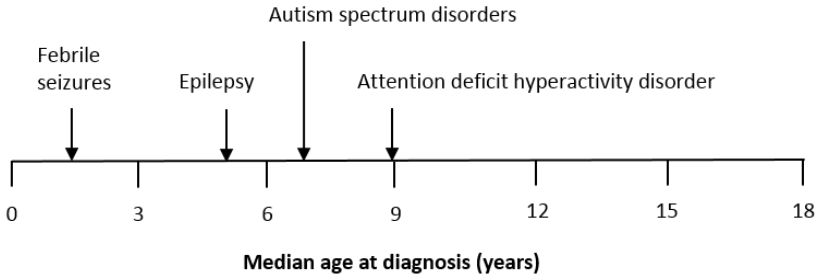


*Figure 5.1. Median age of the child at diagnosis of neurodevelopmental disorders in Danish nationwide register studies. Reproduced with permission from [289].*

In the register studies (paper 16 and 17), maternal hyperthyroidism was a risk factor for epilepsy and ADHD in the child, and maternal hypothyroidism was a risk factor for febrile seizure, epilepsy and ASD in the child. Associations were mainly observed when maternal thyroid dysfunction was first time diagnosed and treated after the pregnancy. It was speculated if these women had undiagnosed thyroid dysfunction in the pregnancy, which was untreated, and could disturb fetal brain development and program the child to later onset of neurodevelopmental disorders. Register-based studies are feasible due to the large study population and nationwide inclusion, but the design primarily aim to generate hypotheses and the findings called for alternate studies with actual measurement of maternal thyroid function in the pregnancy.
A case-cohort design is characterized by the collection of exposure data in a subset of the full cohort [290]. It includes all cases and a random sub-cohort, which favors that the same sub-cohort can be used for investigation of different outcomes. This is in contrast to the matching performed in a nested case-control design. Analyses in the case-cohort study were performed using a weighted Cox proportional hazards model to account for the overlap between cases and the random sub-cohort [291]. In paper 7, the overall frequency of abnormal maternal thyroid function in the early pregnancy was higher among cases of ASD. On the other hand, when looking at subtypes of maternal thyroid function abnormalities, the findings were diverse and challenged by a small number of cases exposed to overt maternal thyroid dysfunction.

### 5.1.1. SEIZURE DISORDERS

Seizures result from abnormal discharge of neurons in the brain [292]. Epilepsy is considered to have a neurodevelopmental origin, and malformations of cortical development have been described [293]. Neurodevelopmental vulnerability has also been suggested in part for febrile seizures [294]. An association between maternal hypothyroidism in pregnancy and seizure in the rat offspring has been described [295,296], and hyperthyroidism increased seizure susceptibility in non-pregnant rats [297,298]. The definition of outcomes differed in the Danish studies in the way that the inclusion criterion in the register-based study (paper 16) was a hospital diagnosis, whereas febrile seizures were defined by minimum two diagnoses and epilepsy by a diagnosis of disease and minimum two redeemed prescriptions of antiepileptic drugs in the case-cohort study (paper 7). A diagnosis of epilepsy and febrile seizure in the DNHR has been validated and was considered high [299,300]. In the case-cohort study, no association with febrile seizure was observed. For epilepsy, associations with hyper- and hypothyroidism were observed in sub-analyses depending on time of onset of epilepsy in the child and maternal TSH. In these analyses, overt maternal hyperthyroidism was a risk factor for onset of epilepsy in the child before one year of age, but the small number of exposed cases limited the possibility to draw definite conclusions. No associations with maternal TSH or fT4 as continuous variables were observed, but results indicated that maternal TSH was more often above 10 mIU/l in cases of epilepsy exposed to maternal overt hypothyroidism. However, the range of maternal TSH among non-cases was wide suggesting a low predictive value.

### 5.1.2. AUTISM SPECTRUM DISORDERS

ASD are pervasive developmental disorders with impairments in social, behavioral and communicative skills, and a neurodevelopmental origin has been proposed [301]. ASD consist of a group of developmental disorders with a continuum of symptoms [301]. Hospital diagnoses of the disease were used for identification of ASD in the register-based study (paper 17) and in the case-cohort study (paper 7), and the validity of a diagnosis of childhood ASD is considered high [302]. The diagnostic validity is expected to decrease as the number of digits in the diagnostic code increases, and analyses on sub-diagnoses (e.g. infantile autism and Asperger syndrome) were not performed. Clinical studies on the association between maternal thyroid disease and ASD have shown associations with maternal iodine deficiency, hypothyroxinemia, and thyroid autoimmunity [303-305], but experimental hypothyroidism in pregnant rats did not support an association [306]. Maternal hypothyroidism was a risk factor for ASD in the Danish register study. Such association was similarly observed in the case-cohort study, in which also hypothyroxinemia was a risk factor for ASD in girls. Specific developmental disorders (SDD) are classified together with ASD in ICD-10 and include difficulties in language and speech and in school skills such as reading, calculating and writing. Children diagnosed with SDD during follow-up were sampled in the case-cohort study in paper 7, but the validity of these diagnoses is not known, and no association with maternal thyroid dysfunction was observed.

### 5.1.3. ATTENTION DEFICIT HYPERACTIVITY DISORDER

ADHD is a neurobehavioral disorder characterized by difficulties in concentration, attention and behavior most commonly observed in boys [307]. It is considered a neurodevelopmental disorder and abnormalities in brain structure and function have been described [308]. Clinical studies on the association between maternal thyroid disease and ADHD in the child have been heterogeneous in the definition of exposure and outcome, and results have not shown a consistent trend [289]. The Danish studies were the only studies that assessed outcomes of ADHD from diagnosis and treatment. The validity of diagnosis of ADHD has recently been evaluated and was considered high [309]. In the register study (paper 17), ADHD was defined by hospital diagnosis or at least two redeemed prescriptions of the drugs used for the treatment of the disorder. In the case-cohort study (paper 7), all cases were identified from redeemed prescriptions of drugs, which may introduce disparities in the clinical characteristics. An association between maternal hyperthyroidism and ADHD was observed in the Danish register study, which is compatible with a high frequency of ADHD observed in individuals with resistance to thyroid hormone [310] and with the hyperactivity behavior of mice with D3 deficiency [311]. However, such association was not corroborated in paper 7 and in other reports from birth cohorts in the Netherlands and in Finland [312-315]. Associations with lack of maternal thyroid hormones and thyroid autoantibodies have been described, and maternal hypothyroxinemia was a risk factor for ADHD in girls, but not in boys, in paper 7.

### 5.1.4. CLINICAL AND SCIENTIFIC IMPLICATIONS

The case-cohort design in paper 7 included the possibility to study the impact of *in utero* exposure to undetected maternal dysfunction. Assessment was based on a single measurement in early pregnancy, but the finding in paper 5 that up to 50% of the women who were diagnosed and treated for thyroid disease after the pregnancy had thyroid function abnormalities already in the early pregnancy suggests that the abnormalities were likely to persist. Results of paper 7 were diverse, but some of the observed associations support the hypothesis of fetal programming by maternal thyroid disease and relates to previous findings. The most prominent association was observed for the outcome of ASD in the child. In line with the Danish register study (paper 17) and other reports [303,304], an association with indicators of lack of maternal thyroid hormones was observed. Maternal thyroid autoantibodies have been proposed to play a role in the development of ASD in the child [316,317]. This aspect could not be directly addressed in the Danish studies, but the lack of an association with paternal hypothyroidism and the specific association with maternal hypothyroidism diagnosed after, but not before, the pregnancy in the register study (paper 17) argue against a dominant role of thyroid autoimmunity. This is in contrast to a nested case-control study within a national birth cohort in Finland, in which maternal TPO-Ab was a risk factor for ASD independently of maternal thyroid function [305]. Another debatable aspect is on the role of *in utero* exposure to thyroid disrupting chemicals and development of ASD [318-320]. The association between maternal hyperthyroidism and ASD observed in paper 7 needs to be addressed in further reports. On the other hand, the association between maternal hyperthyroidism and hypothyroidism and epilepsy in the child is compatible with the findings in the Danish register study (paper 16) and with experimental findings [295-298], but more studies in humans are warranted. Considering ADHD, the literature is conflicting [289], and the impact of maternal hypothyroxinemia is disputable [61,62]. The fact that for example maternal body weight associates with isolated changes in maternal fT4 [262,263] and divergent observations of possible interaction with child's sex in neurodevelopmental outcome studies [314,315] call for more evidence to understand and quantify the scientific and clinical impact. An important focus of future studies is on the role of endocrine disrupting chemicals and how such *in utero* exposure interact with maternal thyroid function and fetal brain development [321-324]. Noteworthy, interaction with child's sex has been reported in studies on the association between fetal exposure to endocrine disruptors and brain development, and the underlying mechanisms are uncertain [323,324].

The hypothesis of fetal programming by maternal thyroid disease via disturbed fetal brain development is biologically plausible from experimental evidence [325,326]. On the other hand, results of observational studies in humans provide less consistent evidence to support the hypothesis [74,289,327,328]. Methodological aspects of the observational design are deemed of importance in the interpretation of results [74]. The internal validity of individual studies is threatened by selection and information bias as well as confounding. A strength of the Danish studies was the nationwide

design, which reduces the risk of selection bias. This is in contrast to the selection of patients from a single hospital department. Such selection includes a risk of referral bias since severity of the disease and age of the patient may decide whether a thyroid patient is managed in hospital [157,158]. Information bias due to misclassification of exposure and outcome will be present in register-based studies even though many diseases have shown acceptable diagnostic accuracy and the combination with drug prescription data is a possibility [128]. Such misclassification is likely to be non-differential in designs with collection of exposure information independently from outcome assessment, but differential recall bias may be a concern in case-control studies [156]. Observational studies are prone to confounding and the many environmental factors associated with maternal thyroid disease and possibly with child neurodevelopmental disorders challenge the design. A strength of the Danish nationwide registers is the information on maternal smoking and other maternal comorbidities. Maternal and paternal psychiatric diseases were observed to coincide with thyroid disease in paper 18 and in other Danish reports [329-331]. Thyroid disorders also associate with socioeconomic factors [332-334] and more detailed data on such maternal characteristics may be of interest. Moreover, the extent to which maternal and child characteristics serve as confounders or intermediates on the causal pathway and the role of interaction are uncertain. Considering external validity, comparison of studies is hampered by the diversity in the definition of exposure and outcome as well as population characteristics [289]. For exposure assessment, no uniform definition of maternal hypothyroxinemia exists, and it varies considerably for outcomes of neurodevelopmental disorders in the child, whether information relies on parent- or teacher report, psychological testing, or diagnosis and treatment of the disease. Furthermore, the age of the child at assessment of disease varies between studies [289]. Another concern about neurodevelopmental disorders is the diagnostic accuracy. Some neurodevelopmental disorders, particularly ADHD, have shown a considerable diagnostic increase in many countries in recent years [335]. This trend may question the severity of disease and the clinical indication for treatment and may favor studies with alternative outcomes of brain development.

## 5.2. NEUROPSYCHOLOGICAL PERFORMANCE

Data on outcomes of child neuropsychological performance were obtained from the LDPS and described in paper 8. All pregnant women in the DNBC, who participated in the LDPS together with their five-year-old child, were sampled for the measurement of thyroid function in the early pregnancy blood sample. An important methodological note is that the primary aim of the LDPS was to investigate the impact of maternal alcohol intake. This was reflected in the LDPS sampling procedure [147], and necessitated the weighting by the sampling fraction in all analyses. Thus, unweighted means and medians reported in paper 8 cannot be directly compared between groups, but crude and adjusted estimates of the mean difference and exponentiated β were weighted by the sampling fraction for comparison.

### 5.2.1. INTELLIGENCE

IQ is a commonly used method for assessment of intelligence and an IQ test score is calculated based on a norm group with a mean score of 100 and a standard deviation of 15. IQ norms for Danish children were not available at the time of the LDPS and Swedish norms were used, which implies that the distribution differs from a theoretical mean of 100 [336]. The Wechsler intelligence scale includes measures of full, performance and verbal IQ. Performance IQ includes among others tests of geometric design and object assembly, whereas verbal IQ addresses arithmetic skills and vocabulary. Studies of focal brain damage have provided clues on the link between measures of IQ and areas in the brain [337], but aspects of interpretation and development of verbal and performance IQ in children and the discrepancy between the two measures at an individual level seem unanswered [338].

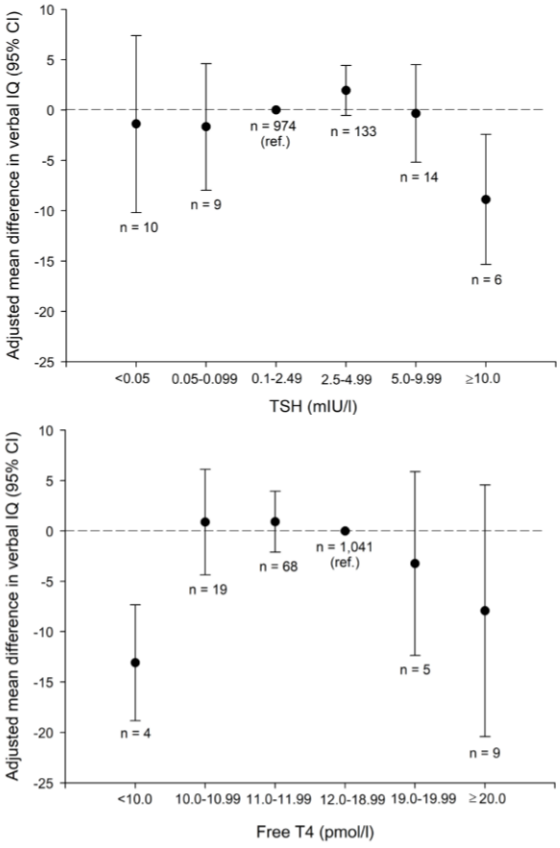


*Figure 5.2. Adjusted mean difference in child verbal IQ by maternal TSH and free T4 in early pregnancy. Reproduced with permission from [8].*

An intriguing finding in paper 8 was the association between maternal TSH and fT4 and verbal IQ in the child (Figure 5.2). A similar non-significant trend was observed for performance and full IQ. The association was assessed using categories of maternal TSH and fT4 (Figure 5.2), which were decided from clinical guidance. Since evaluation of categorical exposure may be sensitive to the choice of cut-points, non-linear associations were also modelled using restricted cubic splines [339]. This model corroborated a significant association with maternal TSH (supplemental Figure 1 in paper 8). Restricted cubic splines provide a flexible model of non-linear associations in which separate curves are fitted for different segments and combined to a smooth fitted curve [339]. However, the method is sensitive to the choice and location of dots. Notably, the association between TSH and child verbal IQ was stable for a change in the number and location of dots. Albeit the number of children in the most outer categories of TSH and fT4 was limited, results suggested that marked maternal hypothyroidism with TSH at or above 10 mIU/l, but not smaller deviations or low TSH, were associated with child IQ (Figure 5.2). The choice of independent variables in multivariate analyses is a matter of discussion [340]. A systematic approach using the change in estimate method via backward elimination was applied in paper 8 [340], but results did not change from a fully adjusted model. Maternal IQ showed considerable impact in the analyses, and it is noteworthy that previous investigations on neurocognitive outcomes in children born to mothers with thyroid disease did not include measures of maternal intelligence.

### 5.2.2. OTHER NEUROPSYCHOLOGICAL OUTCOMES

The neuropsychological assessment in the LDPS also included testing of child motor function and parent- and teacher reports of problem behavior and executive function. No associations with maternal TSH or fT4 as categorical or continuous variables were observed for these outcomes. An intriguing finding was the association between maternal thyroid dysfunction and teacher, but not parent, reports of difficulties in the Behavior Rating Inventory of Executive Function and the Strengths and Difficulties Questionnaire. Similarly to the observation of impairment in child motor function, these associations were dominated by exposure to maternal hypothyroxinemia.

### 5.2.3. CLINICAL AND SCIENTIFIC IMPLICATIONS

Clinical guidance indisputably states that overt hypothyroidism in pregnant women should be treated considering the adverse effects on pregnancy and child outcomes [36]. The study by Haddow et al. in 1999 [33] and the follow-up study by Klein et al. [341] showed from measurement of maternal TSH in pregnancy week 17 and assessment of IQ in the child at age 7-9 years that untreated maternal TSH (range ~5 to 90 mIU/l) was associated with lower child IQ and that there was an inverse correlation between the severity of maternal hypothyroidism and child IQ. On the other hand, child IQ was normal when maternal hypothyroidism was treated in the pregnancy, in line with other reports [342-345].

Results of paper 8 corroborate an association between markedly high maternal TSH and child IQ and depict no association with smaller aberrations in TSH. Contrary to overt hypothyroidism, the evidence regarding maternal subclinical hypothyroidism and hypothyroxinemia is less consistent [24], and clinical recommendations consequently less strong [36,78]. Observational studies have largely investigated the impact of smaller aberrations in maternal thyroid function on child development in the first three years of life, and the study by Pop et al. in 1999 showed that maternal fT4 below the 10th percentile in pregnancy week 12 was a risk factor for impaired psychomotor development of the child at 10 months of age [34]. Many of the subsequent studies have likewise suggested an association [346-353], but reports have been heterogeneous regarding population and design [328,354]. In line with results of paper 8 on the outcomes of child behavior, associations between maternal hypothyroxinemia and child outcomes at 5-8 years of age are diversely reported in observational studies [70,355-358]. The role of confounding and interaction by maternal and child characteristics seem unresolved. During the last decade, two RCTs have been published on the effects of L-T4 treatment for maternal subclinical hypothyroidism and hypothyroxinemia in pregnancy on child IQ [75,76]. Both trials showed no effect of treatment on child neurocognitive outcomes, but differed by the timing of initiation and dose of L-T4 treatment (week 12-13, 150 µg L-T4 in the Controlled Antenatal Thyroid Screening (CATS) study [75]; week 16-17, 100 or 50 µg L-T4 in the study by Casey et al. [76]) and by the age of the child at IQ assessment (three years in the CATS study [75]; five years in the study by Casey et al.[76]). It has been discussed if the outcomes of the trials may be explained by some of these characteristics [359]. A follow-up on the CATS study (CATS-II) with outcome assessment at nine years of age and the inclusion of children born to mothers with normal thyroid function addressed some of these concerns [359,360]. Results of CATS-II similarly showed no beneficial effects of treatment and IQ was similar in children born to mothers with abnormal thyroid function and in the normal thyroid function group. In contrast to the study by Casey et al. [76], the CATS study [75] included women with combined high TSH and low fT4, but results were similar in this subgroup. A study within the Generation R birth cohort in the Netherlands showed that both maternal low and high fT4 in pregnancy week 13 was associated with low child IQ at six years of age and adverse magnetic resonance imaging measures of brain morphology, which raised concern about maternal overtreatment [73]. However, in the CATS study no indication of lower IQ in children born to mothers with high fT4 during treatment was observed [359]. In paper 8, very few women reported current treatment with L-T4 and the potential role of treatment could not be addressed. Future studies on the role of maternal subclinical hypothyroidism and hypothyroxinemia in relation to child neurocognitive development are warranted, and possibly with intervention at an early stage of pregnancy. However, many other aspects remain uncertain in the assessment of the risk. One is on the definition of exposure and outcome [361,362]. Others include the role of thyroid autoimmunity, iodine intake, lifestyle factors and co-morbidities as well as interaction with the postnatal environment during child development [61,74,363-365].

# CHAPTER 6. TREATMENT OF MATERNAL THYROID DISEASE IN PREGNANCY

This chapter includes a discussion of paper 9-12, which investigated outcomes of pregnancy associated with the medical treatment of maternal thyroid disease. More specifically, the studies addressed the risk of severe side effects to the use of ATD. The studies were all nationwide, but differed by the source population in the way that paper 9-11 were performed in the Danish and paper 12 in the Swedish population.

## 6.1. SEVERE SIDE EFFECTS TO ANTITHYROID DRUGS

Severe side effects to the use of ATD are considered rare, but clinical detection is of major importance as the conditions potentially may be life threatening. The primary aim of the studies included in the doctoral dissertation was to evaluate the association between the use of ATD in early pregnancy and birth defects in the child, but to set the risk into perspective, investigations on ATD associated agranulocytosis and liver failure in pregnant women were additionally performed.

### 6.1.1. BIRTH DEFECTS

Birth defects (also referred to as congenital malformations or embryopathies) are structural abnormalities that develop secondary to *in utero* exposure, and a teratogen is an exposure, which may adversely disturb the development of the fetus [366]. Fetal development occurs in various stages and as organs are differently affected by teratogenic exposures, the window and type of exposure are determinants of the outcome [367]. The embryonic period up to pregnancy week 10 with formation of organs is considered more sensitive to teratogenic exposure than the later fetal period with maturation and growth [366]. The definition of exposure in paper 9-12 was from redeemed prescriptions of ATD, which are indirect measures of exposure. However, several points support the use of this method for exposure assessment. ATD is used solely for the treatment of hyperthyroidism, the coverage and validity of the nationwide prescription registers are high, and the compliance with redeemed drugs for the treatment of chronic disorders is high in pregnant women [159]. A challenge with such indirect method of exposure assessment is on the time window of redeemed prescriptions to include actual drug intake in the early pregnancy [368]. In paper 9 and 12, ATD exposure in early pregnancy was *a priori* defined as registrations of redeemed prescriptions of ATD in the period from six months before pregnancy start up to an including pregnancy week 10. This decision was based on clinical knowledge about typical dose, packet size and duration between prescriptions. Furthermore, individuals who redeemed both MMI/CMZ and PTU were individually reviewed for assessment of the time of shifting between drugs.

Outcomes of birth defects in paper 9-12 were defined from hospital diagnoses. The diagnostic validity has been evaluated in Denmark and was considered acceptable for epidemiological research [369]. The prevalence of birth defects depends on the types of malformations studied and the timing of assessment. Birth defects are by definition present at birth, but if symptoms are absent or vague, the malformation may first be clinically detected later in life [367]. In paper 9 and 11-12, diagnoses of birth defects registered before the child was two years of age were included in the assessment. This decision was made to include minor malformations and to obtain similar follow-up for all children according to birth year and duration of the registers. Others sources of birth defect registrations e.g. the European Surveillance of Congenital Anomalies (EUROCAT) apply a one-year follow-up for assessment and excludes a subset of minor malformations [370], but methodological pros and cons are debatable [371]. The prevalence of birth defects in non-exposed children in the Danish population (paper 9) was six percent at two years of age and three percent when assessed at three months of age (upper part of Figure 6.1) compatible with general figures [366]. The prevalence of birth defects in the Swedish population (paper 12) was higher (eight percent at two years of age), which presumably is explained by the inclusion of outpatient visits to specialist clinics in the SNPR. Notably, the findings in paper 9 and 12 were similar in sensitivity analyses, which restricted the window of exposure or age of the child at outcome assessment. As illustrated in the lower part of Figure 6.1, a higher frequency of birth defects after MMI and PTU exposure compared with non-exposed children was observed in the Danish study (paper 9) at all time points of outcome assessment up to the age of two years [112].

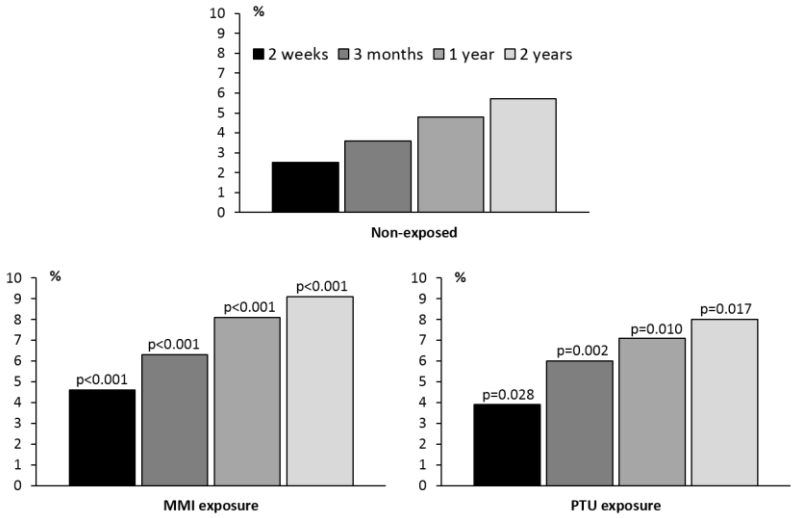


*Figure 6.1. Prevalence of birth defects stratified by age of the child at follow-up. The p-values are results of the comparison with non-exposed. Reproduced with permission from* [112].

An intriguing finding in the Danish study (paper 9) was the risk of birth defects associated with both MMI/CMZ and PTU. However, the types of malformations associated with each drug differed markedly, and the face and neck malformations observed after PTU exposure were not associated with the use of MMI/CMZ (Figure 6.2). The findings for MMI/CMZ were in line with another large study from Japan [105] and a particular high risk of the cluster of severe birth defects previously described in the embryopathy was observed. Further, results of paper 9 suggested that also ventricular septal defect is part of the embryopathy, which previous observations had likewise indicated [372]. On the other hand, the finding that PTU was associated with an increased risk of birth defects was new and in contrast to the findings from Japan [105], and challenged current clinical guidance on the choice of ATD in early pregnancy [373].

The aim of paper 10 was to evaluate the severity of the birth defects in the face and neck region and the urinary system associated with maternal use of PTU. In the design of the study, the decision was made to extend follow-up for a diagnosis of birth defects in the registers beyond the age of two years to a median age of eight years to possibly include less severe and later diagnosed defects. This methodological approach identified additional two cases of PTU associated birth defects in the face and neck region and one case of urinary system malformations, and the overall figures were similar when the risk was assessed with extended follow-up. Severity of the malformations was evaluated via linkage to nationwide registers. Malformations of the face and neck region were predominantly preauricular and branchial sinus, fistula or cysts, none of the children were diagnosed at birth and the majority underwent surgery for the disorder. Malformations of the urinary system were predominantly obstructive malformations, all cases were boys and often diagnosed at birth. Surgery had been performed in less than half of the cases and two of these children also had genital malformations. It is to be expected from the identification of cases via hospital diagnoses that only cases referred to hospital for surgery or other hospital related management are identified. Thus, the review of individual cases highlights that the birth defects associated with PTU often required surgery, but does not cover how the risk of PTU associated birth defects would quantify if asymptomatic cases or cases treated outside hospital were to be identified. Further, paper 10 described individual characteristics of the cases, but did not provide data on similar characteristics among non-exposed cases. Such comparison would be warranted to further quantify the severity and to evaluate if PTU associated birth defects show specific characteristics. However, the descriptive data in paper 10 indicated that the malformations associated with PTU were less severe than those observed after MMI, in which the number of hospital contacts might be much larger and the outcome even fatal [90]. The data on birth characteristics (gestational age and birth weight) and maternal characteristics were included in paper 10 to support causality in the associations observed. Many risk factors have been examined and associated with birth defects, some of which may also associate with the exposure [366,367]. Thus, the role of intermediate and confounding factors is a potential concern.

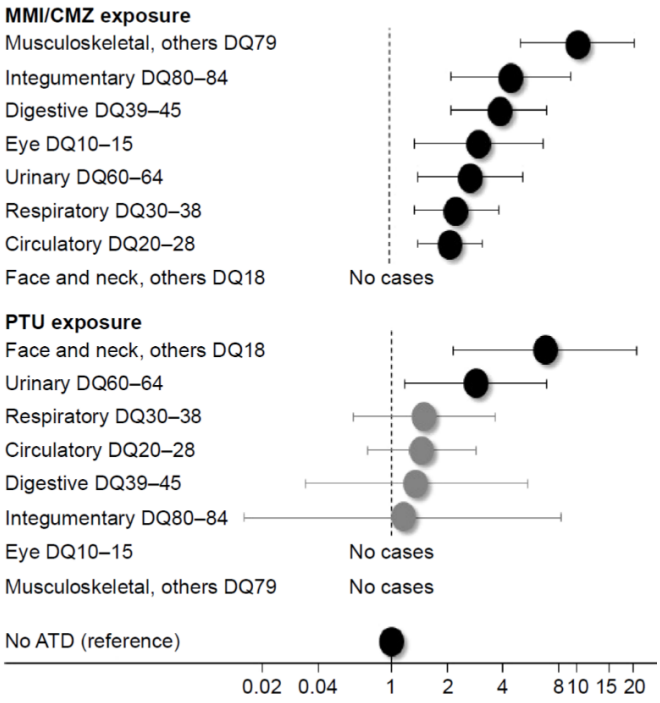


*Figure 6.2. Odds ratios with 95% confidence interval for subgroups of birth defects after exposure to MMI/CMZ or PTU in early pregnancy. Reproduced with permission from* [9,85].

Results of paper 9 and 12 were consistent in multivariate analyses adjusting for potential confounders and in sensitivity analyses considering potential intermediates, but unknown, unmeasured or residual confounding may persist. Paper 10 was descriptive and did not include data on maternal characteristics among non-exposed cases, but the review of exposed cases did not raise concern about a specific competing cause. The Danish study (paper 9) also identified a group of 159 children exposed to both MMI/CMZ and PTU in the early pregnancy because maternal treatment was changed after pregnancy had been confirmed and the majority of these women shifted from MMI/CMZ to PTU in line with the clinical guidance in 2011-2012 [102,104]. Unexpectedly, the prevalence of birth defects in this group was high. Individual review of exposed cases according to the timing of the shift in treatment in early pregnancy indicated that MMI associated birth defects were often observed when the shifting had occurred relatively late in early pregnancy [90]. Such detailed evaluation must be interpreted with caution due to the small number of exposed cases in this group and the potential uncertainty about the exact timing of exposure in pregnancy. However, the indication of a relatively late shift was compatible with a review of all MMI/CMZ associated cases of birth defects described in the literature after maternal shift in therapy in early pregnancy [90].

One of the Bradford Hill criteria of causality is consistency [374] and to extend the findings from the Danish study (paper 9 and 10), a similar design was adopted and used in the Swedish population (paper 12). This possibility emerged from similarities between nationwide registers in the Nordic countries, but a notable disparity in the frequency of exposure was observed. Thus, the use of ATD in early pregnancy was less frequent in the Swedish population compared with the Danish population, which seemed to be in line with general figures on redeemed prescriptions of ATD in the countries. Historically, iodine fortification was implemented earlier in Sweden and iodine intake levels are higher than in Denmark [213]. Population differences in health behavior e.g. smoking habits may further add to the disparity [375]. Finally, the clinical management of thyroid disease in women of reproductive age may differ concerning the preferential choice of definitive therapy before pregnancy. Overall, no association between the use of ATD in early pregnancy and birth defects was observed in paper 12, but for subtypes of birth defects a differential pattern for MMI and PTU was seen. A systematic approach was used according to the evaluation of subtypes of birth defects similar to the Danish study. For subgroups of malformations, a higher frequency of heart septal defects was seen after MMI exposure and a higher frequency of ear and urinary system malformations was observed after PTU. Considering severe malformations described as part of the MMI embryopathy, one case of choanal atresia was observed after exposure to both MMI and PTU. The most intriguing finding relates to the similarity in the malformations of the face and neck region and the urinary system observed after PTU exposure, but further large studies are needed to corroborate the findings and support consistency. The number of exposed pregnancies in the Swedish study and in other previous studies should be considered in the interpretation of the results [376]. A similarity between the studies [12,100,106,107,377,378] that found no association was the small number of MMI exposed pregnancies (less than 200) as opposed to more than 1000 exposed pregnancies in the studies from Denmark (paper 9) and Japan [105]. Recently, another large and nationwide study [108] was published (Table 6.1). The study was based on data from the Korean National Health Insurance database and the design of the study was very similar to that of the Danish study (Table 6.1). In the Korean study [108], both MMI and PTU were associated with an increased risk of birth defects, and a particular high risk was observed with MMI alone and with the shifting from PTU to MMI in early pregnancy. For subgroups of malformations, PTU was significantly associated with defects of genital organs and the musculoskeletal system. On the other hand, MMI was associated with defects of the nervous, circulatory and digestive system and a specific high risk of birth defects described in the MMI embryopathy. When summarizing the findings (Table 6.1) the large studies showed consistent results for the association with MMI/CMZ. On the other hand, results regarding PTU were more diverse. The study from Japan [105] found no association, and the studies from Denmark (paper 9) and Korea [108] found that PTU was associated with an increased risk of birth defects, but the subtypes of malformations differed. On the other hand, the birth defects associated with PTU in the Swedish study (paper 12) showed similarities with the Danish study.

| | Japan [105] | Denmark [9] | Korea [108] |
|---|---|---|---|
| **Publication year** | 2012 | 2013 | 2018 |
| **Population** | Hospital setting | Entire population | Entire population |
| Children (n) | 4,513 | 817,093 | 2,210,253 |
| **Exposure** | | | |
| Data source | Medical records | Prescribed drugs | Prescribed drugs |
| Dose of the drug | Yes | No | Yes |
| Thyroid function | Yes | No | No |
| MMI exposed (n) | 1,226 | 1,097 | 1,120 |
| PTU exposed (n) | 1,392 | 564 | 9,930 |
| MMI and PTU (n) | none | 159 | 1,841 |
| **Outcome** | | | |
| Data source | Maternal report | ICD-10 diagnosis | ICD-10 diagnosis |
| Age at assessment | At birth | 2 years | 1 year |
| Prevalence (%) | 2.1 | 5.7 | 5.9 |

*Table 6.1. Characteristics of the large cohort studies that reported an association between the use of ATD in early pregnancy and birth defects [112].*

Other studies on the association between PTU and birth defects include experimental studies in *Xenopus* embryos, mice and rats [109-111], human case reports [101], reports from malformations registries [378-380], and observational cohort studies with non-exposed control groups [100,106,107]. Results have not consistently raised concern about the use of PTU and birth defects though teratogenic effects have been observed in experimental studies, human case reports and in reports from malformations registries. Characteristic for the observational studies that found no association was the relatively small exposure groups [100,106,107], but one study from 2013 included 915 PTU exposed pregnancies [377]. This US study was based on health insurance data including prescribed drugs and ICD-9 diagnoses of birth defects, which were assessed up to the age of one year. The prevalence of birth defects in non-exposed children was 5.9% as opposed to 7.2% in PTU exposed children. The overall association did not reach statistical significance, but analyses of subgroups of birth defects may indicate a risk of urinary system malformations associated with PTU [377]. In addition to considerations on the number of exposed pregnancies, the method used for assessment of outcomes of birth defects in the child should be considered in the interpretation of results [376]. Notably, the overall prevalence of birth defects was reported to be less than one percent in a negative outcome study based on health insurance data from Taiwan [100]. In the study from Japan [105], birth defects were diagnosed by an obstetrician at birth and the findings were reported by the mother at the first hospital visit after birth. On the other hand, studies that indicated an association with PTU included a longer follow-up and hospital diagnoses of disease [9,12,108,377]. Especially for less severe defects such differences in outcome assessment are likely to influence the findings.

Another principle of causation in the view of Bradford Hill is the observation of a biological gradient [374]. Paper 9-12 did not include information on maternal dose of ATD in early pregnancy, because information was not available in the nationwide registers. However, other large studies included such information (Table 6.1). In the study from Japan [105], the dose of ATD was available in medical records and no dependency on the dose was observed in multivariate analyses. In the study from Korea [108], duration and cumulative dose of ATD in first trimester were reported, and results indicated that a high cumulative dose of MMI was a risk factor for birth defects compared with a low dose. Another important consideration is on the possible impact of maternal thyroid function *per se* [381]. In the study from Japan [105], information on maternal thyroid function parameters in early pregnancy was available from medical records (Table 6.1), and no dependency on the thyroid function was observed in multivariate analyses. It should be stressed that the Japanese study included patients with GD managed in a hospital who became pregnant, which includes a risk of selection bias. Further studies on the differential role of ATD dose and maternal thyroid function are warranted.

### 6.1.2. AGRANULOCYTOSIS AND LIVER FAILURE

The clinical recommendation on the choice of ATD in early pregnancy has in recent years been challenged by evidence suggesting a risk of birth defects associated with both types of available ATD and other determinant may be incorporated in clinical decisions. The aim of paper 11 was to evaluate the frequency of agranulocytosis and liver failure as opposed to birth defects associated the use of ATD in the population in general and in pregnancy. A methodological challenge in the design phase was the assessment of general population data. The population emerged from all children live-born in Denmark, 1973 to 2008, and their parents and consequently a disparity in the age distribution compared with the entire Danish population was observed. However, estimates were standardized to the age distribution of the Danish population on January 1, 2003, which was in the middle of the registration period for DNPR. Another methodological challenge was the criteria for identification of ATD associated events from the use of secondary data. Consequently, criteria were *a priori* considered and individual review of all registrations from possible cases was performed. The figures on agranulocytosis and liver failure observed in the general population and the characteristic of the timing of development support the identification of ATD associated cases. In paper 11, the frequency of agranulocytosis associated with MMI was 0.11% and 0.27% with PTU, the majority of cases developed within three months after initiation of treatment and around 25% of the cases developed during treatment for hyperthyroidism relapse. Previous studies have been retrospective evaluations from the review of medical records, and have reported a frequency of ATD associated agranulocytosis from 0.1-0.5% [382-386]. Further, many studies have observed a similar time dependency in the development of agranulocytosis after initiation of treatment [382,384-387], and a similar frequency of cases developing during a second or later course of treatment [386,387] .

Considering ATD induced liver failure, prevalence data are varying, which presumably reflects the heterogeneous clinical presentation ranging to fulminant liver failure with a need of liver transplantation [388]. In paper 11, the frequency of liver failure associated with MMI was 0.03% and 0.05% for PTU, and the side effect typically developed within three months in line with observations from China [389]. It has been proposed that MMI associated liver failure is cholestatic, while PTU induces hepatocellular damage [390]. Specification of the type of liver failure was not possible in paper 11, but studies from Asia have indicated that the types of liver failure associated with each drug may be different and overlapping [389,391].
For the use of ATD in pregnancy, data on the frequency of agranulocytosis and liver failure are limited [106,377,392]. An intriguing finding in relation to the use of ATD in pregnancy in paper 11 was the lower frequency of ATD associated agranulocytosis and liver failure as compared with birth defects which suggests that birth defects is the predominant side effect to the use of ATD in pregnant women.

### 6.1.3. CLINICAL AND SCIENTIFIC IMPLICATIONS

The clinical guidance for the treatment of hyperthyroidism in pregnancy drafted in 2011-2012 suggested that PTU was the preferred treatment in early pregnancy and that women treated with MMI should shift to PTU, when pregnancy was confirmed [102-104]. Potential teratogenic effects of PTU and the finding that such timing of a shift in therapy may be too late questioned current guidance [90]. The lack of other safe and readily available treatments have drawn attention in revised clinical guidance to the possibility of ATD withdrawal in appropriately selected patients [36,37]. If a pregnancy is planned, a change in therapy to PTU can be initiated before pregnancy, but if pregnancy is not planned, focus is on early detection of pregnancy to consider a change in therapy before pregnancy week six [36,37]. Clinical guidance suggest that a woman treated with ATD should immediately contact the responsible physician when pregnancy is detected. If the risk of relapse is considered low, ATD can be withdrawn followed by weekly testing of thyroid function, otherwise PTU should be used. The risk of relapse must be considered from severity of the disease at diagnosis and current stage, duration and dose of ATD, results of thyroid function tests and TRAb. Notably, the guidance is classified as weak recommendation with a need for more evidence [36,37]. Data on the risk of relapse emerge from studies of non-pregnant individuals where a high dose of ATD and short duration of treatment, high TRAb, low TSH, and signs of Graves' ophthalmopathy are some of the factors associated with a high risk [393-397]. However, the risk in pregnant women specifically is not known and neither is the time course of a relapse in early pregnancy. A key concern is the risk associated with maternal hyperthyroidism *per se*. Fetal loss has been associated with maternal hyperthyroidism [13,398], whereas a large study from the US showed no risk of adverse pregnancy outcomes with maternal subclinical hyperthyroidism [399]. A pertinent question and aim of future studies is on predictors and course of relapse in pregnancy and on other possible treatment options (e.g. iodine, cholestyramine and perchlorate) [400-405].

# CHAPTER 7. PERSPECTIVES

The management of thyroid disease in fertile women includes the possibility of a current or future pregnancy. The age dependency in the occurrence of thyroid disease, the physiological changes in pregnancy and concerns about the fetus influence the clinical management and introduce further aspects. The nationwide investigations included in the doctoral dissertation addressed the diagnosis, occurrence and predictors of maternal thyroid disease, and child outcomes associated with maternal thyroid dysfunction and with the treatment. More specifically, results illustrated the interplay between pregnancy and the onset of maternal hyper- and hypothyroidism and showed that predictors of autoimmune thyroid disease considered in general similarly influence the occurrence of thyroid disease in pregnant women. Results challenge the use of a uniform reference range for TSH in the first trimester of pregnancy and demonstrated a high frequency of undetected thyroid function abnormalities in Danish pregnant women. Results of outcome analyses add evidence to the ongoing debate on the benefits and risks of routine testing for thyroid dysfunction in pregnant women and add to the discussion on the impact of subtypes of maternal thyroid function abnormalities. Finally, potential harm related to the medical treatment was addressed and the findings that all current available ATD may be teratogenic challenge the clinical management.

According to the World Health Organization (WHO) [406], a screening program should *respond to a recognized need and include defined objectives and a defined target population. There should be scientific evidence of screening effectiveness and the program should integrate education, testing, clinical services and program management as well as quality assurance with mechanisms to minimize potential risks of screening. The program should ensure informed choice, confidentiality and respect for autonomy, and promote equity and access to screening for the entire target population. Program evaluation should be planned from the start, and the overall benefits of screening should outweigh the harm.* This definition of a screening program includes that the method of detection and the definition and occurrence of a disease are clearly defined and known. The benefits of early detection by screening should be evident, and the indication for treatment should be defined. Further, the method of detection and the treatment should not include substantial adverse risks and cost-effectiveness should be addressed.

The debate on screening for thyroid disease in pregnant women has been ongoing for years and no definite answer has been reached [23,24,35,407-414]. An evaluation of European clinical practice in 2010 showed considerable variation; 42% screened all pregnant women and 43% performed targeted screening of high-risk groups [415]. For comparison, the screening for gestational diabetes has been considered and implemented in many countries, but the current strategy varies considerably [416].

## 7.1. OCCURRENCE IN PREGNANCY

The drawing of a blood sample for the measurement of thyroid function is simple, but the choice and evaluation of thyroid function test for the diagnosing of disease and the definition of normal range in pregnant women are disputable [417]. Clinical guidance includes suggestions of a uniform TSH reference range in the first trimester of pregnancy if local reference ranges are not available, and the upper limit was recently changed from 2.5 to 4.0 mIU/l [36]. Results of paper 4 support that an upper limit of 2.5 mIU/l is too low and the dynamic changes observed for the lower limit of TSH further indicate that a uniform reference range may be too simple. The lack of thyroid autoantibody measurements in paper 4 and the time period of study inclusion encourage further studies. Preliminary results of week specific reference ranges for TSH and fT4 measured with an alternative immunoassay in a large, recent cohort of Danish, TPO- and Tg-Ab negative, pregnant women corroborate a similar trend [418], but the clinical applicability of the dynamic changes remain uncertain. Another important aspect is on the choice of thyroid function parameters in pregnant women. Initial emphasis on the use of free thyroid hormone measures to overcome the changes in total thyroid hormone concentration caused by alterations in TBG has been followed by concern about the indirect method of assessment via immunoassays [198,204,207]. More studies on the use of alternative parameters in pregnant women (total thyroid hormones corrected by TBG and free thyroid hormone estimates), improvements of automatic immunoassays and alternative methods such as mass spectrometry are needed [419]. Another area of interest considering the evaluation of maternal thyroid function in pregnancy is on biological variation. Studies in non-pregnant [420] and pregnant [421] Danish individuals have shown that the intra-individual variation of TSH, T3 and T4 is smaller than the inter-individual variation suggesting that the longitudinal course of thyroid function parameters in each individual is valuable as opposed to population reference ranges. The role of individual characteristics and environmental factors that may influence the reference ranges and the occurrence of autoimmune thyroid disease is another area of importance. Different predictors were assessed in paper 1-4 and 6, but the link between epidemiological associations and clinical practice is not clear and further studies are needed to evaluate the clinical applicability of disease predictors [287]. Many alternative predictors may exist and the predominant factors may change over time and between populations. Recent emphasis is on endocrine disruptors [422] and selenium [423], and population iodine intake is continuously important to monitor [28]. Adding to this, many aspects remain uncertain on the underlying mechanisms for the development of autoimmune thyroid disease in general and in pregnancy. Results of paper 1-2 illustrate the dynamic incidence of thyroid disease in and around pregnancy, but more evidence is needed on the onset of GD in and around pregnancy including the prediction of remission and relapse. Studies with actual measurement of TRAb in and after pregnancy are warranted and future aspects include the use of TRAb bioassays for the measurement in pregnant women and the role of stimulating and blocking antibodies [187,424-426].

## 7.2. OUTCOMES OF PREGNANCY

Screening programs aim to identify undiagnosed disease. Results of paper 5 raised concern about a high frequency of undetected thyroid function abnormalities in pregnant women, but it is essential whether the recognition and treatment of such abnormalities ameliorate adverse effects. Paper 7-8 and 16-18 addressed outcomes of child neurodevelopment and neurocognitive performance, but thyroid hormones also have important effects in the utero-placental unit, and outcomes of pregnancy loss and complications addressed in paper 13-15 add to the discussion [427].

Considering hypothyroidism, overt disease should be treated in pregnant women, but severe maternal hypothyroidism does not preclude normal outcomes of pregnancy [428,429]. For subclinical hypothyroidism, the recommendations of treatment are primarily based on studies investigating pregnancy complications e.g. pregnancy loss and preterm delivery where associations have been observed, particularly in TPO-Ab positive women [36,78]. RCTs have indicated beneficial effects of treatment in TPO-Ab positive women who were euthyroid or had subclinical hypothyroidism [430-432], and in TPO-Ab negative women with TSH above 4.0 mIU/l [433]. Thus, recommendations on treatment consider TPO-Ab status and TSH [36,78], but it should be emphasized that studies are heterogeneous, limited by small sample sizes and the impact on child neurocognitive development is not clear [36,78,434-436]. For isolated changes in maternal fT4, a limited number of studies addressed the risk of pregnancy complications [437,438]. More studies evaluated and observed associations with child neurocognitive development [36,78], but RCTs did not show beneficial effects of treatment [75,76]. Results of paper 7 and 8 substantiate the role of overt maternal thyroid disease in pregnancy. On the other hand, the diverse findings in relation to other types of maternal thyroid dysfunction and the specific association between markedly high maternal TSH and child IQ argue against detrimental adverse effects of smaller aberrations in maternal thyroid function. The impact of smaller deviations in maternal thyroid function on outcomes of pregnancy complications is similarly not clear [439,440]. An important consideration is on the distinction between association and causation. Many factors associate with maternal thyroid disease, pregnancy complications and child development, and observational studies are prone to confounding [24,74,435]. The diversity in results of outcomes studies on the association with maternal subclinical thyroid disease and isolated changes in fT4 may question the underlying mechanisms for the associations observed and the role of other maternal and child characteristics [441-443]. Adding to this, the mechanisms of thyroid hormone transfer across the utero-placental unit are still poorly understood with new methods to come, and the optimal marker of impaired fetal development and effects of treatment remain unclarified [435,444]. Another unresolved area is on the role of maternal thyroid function *per se*, thyroid autoantibodies, and the interplay with hCG [445,446]. Finally, the timing of exposure and outcome assessment is likely to be important and large studies with early detection and treatment of maternal thyroid dysfunction are warranted.

The hypothesis of fetal programming is that lack or excess of maternal thyroid hormones *in utero* may lead to structural or functional abnormalities in the fetal brain, which later predispose the child to development of disease [74]. Brain plasticity is the phenomenon that the human brain may adapt its structure and function according to the postnatal environment [447]. Thus, it is a possibility that alterations induced during fetal life may to some extent recover after birth. It has been proposed if maternal thyroid dysfunction in pregnancy can alter the immune system in the offspring [448,449] and epigenetic aspects of fetal programming remain unresolved [450,451]. Studies with alternative outcomes of child development, for example educational performance, and long-term follow-up would be of interest [452-455]. Another interesting aspect of fetal programming hypotheses is on the role of exposure in pregnancy as opposed to prepregnancy exposure of the oocyte [456,457].

Considering hyperthyroidism, the literature regarding pregnancy outcomes and subsequent child development is less comprehensive, but overt hyperthyroidism should be adequately treated to prevent maternal and fetal complications [36,37]. Results of paper 8-12 addressed concerns about the use of ATD in early pregnancy. L-T4 mimics endogenous thyroid hormone and teratogenic risks associated with this drug were not considered. Block replacement therapy with ATD and L-T4 should not be used in pregnancy except in the case of isolated fetal hyperthyroidism in a woman who still produces TRAb after previous ablative therapy [31]. A few studies have addressed the risk of birth defects associated with the use of L-T4 with inconsistent results [458-462]. Results of paper 8-12 and other reports particularly add concern about the use of MMI/CMZ in early pregnancy [463]. However, further studies are needed to substantiate the optimal management of hyperthyroidism in pregnancy and to evaluate the proposal of early ATD withdrawal in selected individuals [36,37]. Uncertainty is on the optimal method of evaluating disease activity and the need for treatment. Another concern about the focusing on pregnancy detection and side effects is on the psychological well-being of the female patient who is or may in the future become pregnant [464]. Patient knowledge is considerable and information about potential risks associated with the treatment seems imperative. An important focus of future studies is to address the role of maternal hyperthyroidism *per se* and to evaluate the severity and types of birth defects associated with MMI/CMZ and PTU. Long-term follow-up and data on prenatal diagnosis of birth defects would be of interest [465,466]. The focus in paper 8-12 was on the early pregnancy, which is considered most sensitive to teratogenic exposure [90]. However, evaluation of the risk of birth defects associated with later use of ATD in pregnancy would add further evidence to the choice of treatment. The finding that ATD associated liver failure and agranulocytosis in pregnant women were rare may favor continuous use of PTU after the first trimester of pregnancy, but further studies are warranted and assessment of the risk of other types of severe side effects (e.g. vasculitis) [467] would add to the discussion. Finally, genetic predictors of side effects to the use of ATD in pregnancy may be a future perspective [468,469] and optimally new treatments with less severe side effects should be developed.

# CHAPTER 8. CONCLUSION

Thyroid disease affects pregnant women worldwide. The clinical and scientific focus are considerable [470,471], and strategies for optimal management and possibly prevention will have significant impact. Diagnostic methods and treatments have been known for decades, but like other endocrine disorders many challenges are still ahead [472,473]. Evaluation of effects of treatment rely on accurate identification of exposure and outcome. Over the years, the clinical and scientific focus within thyroidology has moved from overt disease to the investigation of smaller aberrations in thyroid function. It is important to clarify the role of such thyroid function abnormalities in non-pregnant and pregnant individuals, but lack of consensus on the definition of smaller aberrations challenges the comparison of studies and interpretation of results. The autoimmune origin of thyroid diseases is another challenge related to population differences in genetic and environmental factors. Overt hyperthyroidism and hypothyroidism should be treated in pregnant women. An important focus is on the need for a change in therapy by the time pregnancy is detected, which is also the case for other endocrine, autoimmune and neurological diseases [474-476]. Clear strategies for preconception counselling and management during the pregnancy within and across countries are warranted [477,478], but population differences in iodine intake and other determinants should be integrated and considered. Adding to this approach, precision medicine has been proposed as a novel future perspective in endocrinology and in thyroidology specifically [472,473]. Routine testing for overt thyroid disease in pregnancy may well be justified from scientific evidence [24], and the conduction of RCTs to evaluate the effect of treatment for overt thyroid disease in pregnant women is considered unethical [36]. However, uncertainties still prevail on the diagnosis of abnormal thyroid function in pregnancy and the definition of normal thyroid function [479]. Further aspects to consider is the cost-effectiveness of a screening program and the benefits of universal screening as opposed to selective screening of high-risk individuals [241,480-485]. Lessons learned from other screening programs in pregnant women emphasize the need for consensus on the optimal strategy prior to clinical implementation [416]. Current guidance contains a suggestion of risk factors for the selective measurement of TSH in pregnant women including symptoms or signs of thyroid disease [36], but the value of individual symptoms is disputed [486]. In contrast to overt thyroid disease, the indication for treatment and routine detection of smaller aberrations in maternal thyroid functions is less clear and a screening program to identify such abnormalities in pregnant women is currently not justified [24,36]. More and large studies are needed to evaluate the impact of smaller aberrations in maternal thyroid function on pregnancy complications and child development and to investigate the interplay with various maternal and child characteristics and thyroid autoimmunity. Comprehensive knowledge on the optimal definition and timing of exposure and outcome assessment is warranted in the design of large RCTs.

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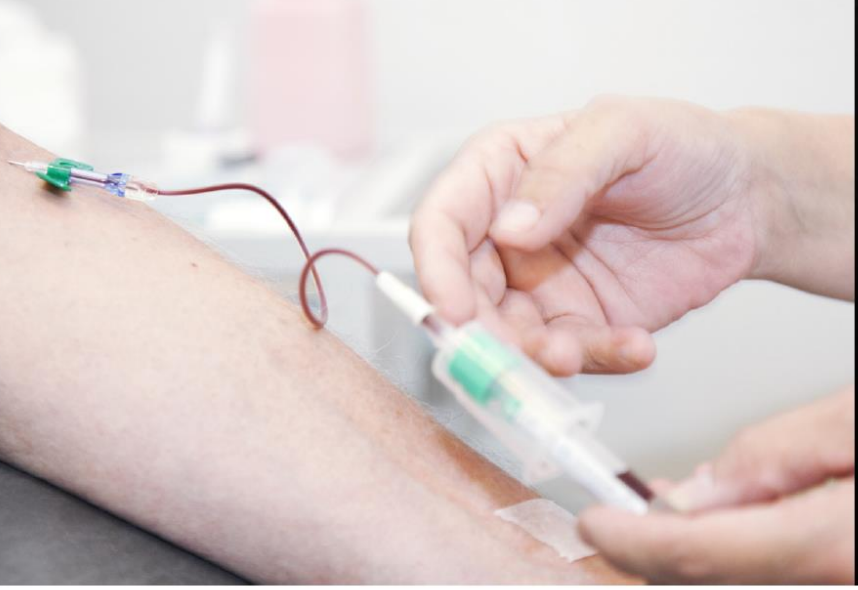

## SUMMARY

Thyroid disease in women of reproductive age is mainly of autoimmune origin. Pregnancy initiates a number of physiological changes in the maternal immune system and thyroid function, which influence the occurrence of thyroid disease in and around pregnancy and challenge the interpretation of thyroid function tests. The doctoral dissertation includes a series of nationwide investigations on the occurrence of maternal thyroid disease in pregnancy and address environmental factors associated with the development of autoimmune thyroid disease. Thyroid hormones are considered crucial developmental factors involved in the regulation of early brain development, and a hypothesis of fetal programming by maternal thyroid disease has been proposed. The doctoral thesis includes investigations within the Danish National Birth Cohort on neurodevelopmental and neuropsychological outcomes in children born to mothers with abnormal thyroid function in the early pregnancy. The treatment of choice for thyroid dysfunction in pregnant women is medical treatment. Another outcome investigated in the doctoral dissertation is the risk of severe side effects to the use of antithyroid drugs for the treatment of hyperthyroidism in pregnancy. The findings challenge the clinical guidance for the management of thyroid disease in pregnant women and the choice of treatment.