Fetal Programming in Rheumatoid Arthritis

Florentien D.O. de Steenwinkel

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Cover: This illustration is created by Leonie Boelens, a dear friend of the author. It visualizes the difference in growth and development of children.

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Spreuken 8: 11
Wijsheid is beter dan koralen
al wat men zal kunnen begeren
kan haar niet evenaren

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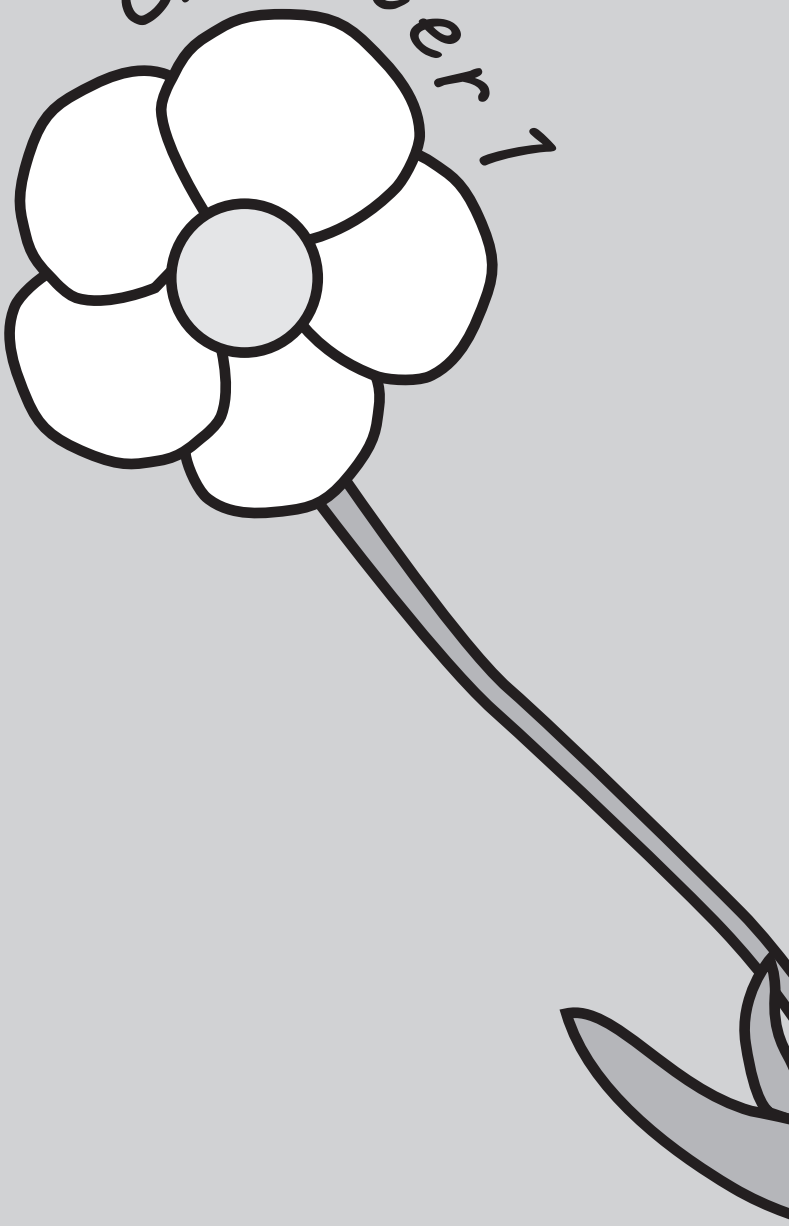
Chapter 1




General Introduction

1.1 Rheumatoid arthritis (RA)

Rheumatoid arthritis (RA) is a chronic, systemic, inflammatory, autoimmune disease mainly affecting synovial tissues, which can lead to severe morbidity and progressive joint destruction resulting in deformations and disability. Other important outcomes include extra-articular features and comorbidities, like vasculitis, cardiac disease and infections[1]. RA affects approximately 1% of the adult population and each year, 5 to 50 per 100.000 persons develop this condition[2]. Women are affected two to three times more often than men. The age of onset in women is usually between 40 and 50 years, but it often affects women of childbearing age[3].

1.1.1 Etiology of RA

RA is a multifactorial disease in which genetic and environmental factors interact in the etiology. First-degree relatives prevalence rate is 3–5%, which means that the risk of developing RA is just a few percent higher when parents or siblings are affected.

The risk of developing RA is for approximately 50% attributable to genetic factors[4]. Of these genetic factors the strongest associations are found with certain Human Leukocyte Antigen (HLA)-alleles. These HLA-alleles are located on chromosome 6 and are involved in antigen presentation and are therefore key molecules in the human immune system.

The presence of RA associated autoantibodies, like rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA) indicates a more severe type of RA, characterised by higher disease activity, more joint destruction and extra-articular manifestations[5]. The risk of developing RA is three times more common in smokers than non-smokers[6]. Studies have also shown that the risk of developing ACPA-positive RA is associated with a strong gene-environment interaction between smoking and a specific HLA allele. The risk of developing RA depends on the magnitude of smoking and genotype[7]. Besides smoking, other environmental factors are thought to be associated with the development of RA, including infections, the use of oral contraceptives and parity[8,9].

In addition to autoantibodies, the release of cytokines, especially pro-inflammatory cytokines like tumor necrosis factor-α (TNFα), interleukin-6 (IL-6) and interleukin-1 (IL-1) are thought to play an important role in the pathogenesis of RA. Several cells of the immune system, including T-cells, B-cells, dendritic cells and macrophages, produce and interact with these cytokines[10]. This all leads to an amplification of the immune response, resulting in synovial inflammation and finally joint destruction[11].

1.1.2 Treatment of RA

Fortunately, pharmacological management in RA has greatly improved these last decades. There are several treatment options, depending on the severity of the disease. This improvement of RA medication has created a strong reduction of inflammation and pain, inhibiting joint destruction and preserving functionality in the majority of RA patients.

Disease-modifying antirheumatic drugs (DMARDs) are the most important treatments in RA therapy. The most widely used DMARD is methotrexate, which is the corner stone of modern RA-treatment. Other DMARDs commonly used are sulfasalazine and hydroxychloroquine.

DMARDs may be combined with glucocorticoids like prednisone or dexamethasone. Glucocorticoids have anti-inflammatory and immunosuppressant assets. They have been shown to influence the course of RA and can therefore be considered DMARDs as well. They can be given orally, intramuscularly, or intra-articularly.

Since the late 1990s, the so-called biologicals have provided clinically important improvement in patients not responding to traditional DMARDs. Biologics are mainly, though not exclusively, therapeutic antibodies targeting key molecules of the immune system like, pro-inflammatory cytokines, including TNF and IL-6[12-14].

1.2 Pregnancy and RA

While pregnant, the RA disease activity may improve, but contrary to what most people think, it does not happen in all women. In literature, the reported improvement of RA during pregnancy has declined from 90% to 53%[15,16]. An explanation for this difference might be that past studies have been performed retrospectively, without objective information on the disease activity, and without a validated scoring system.

The most recent study of De Man *et al.* did use a prospective cohort, and a well-validated scoring system for RA disease activity. She found that around 25% of all pregnant women have a remission in the third trimester of the pregnancy, despite the fact that medication use was reduced during pregnancy[15]. In general, RA disease activity might decrease during pregnancy but will increase postpartum. Almost 50% of the women will have a moderate response during pregnancy and more than 35% has a moderate flare postpartum. The postpartum flare may be underestimated, because medication was often re-started soon after delivery. Medication during pregnancy is mostly restricted to prednisone, sulfasalazine and to less extent hydroxychloroquine. Until recent, the use of biologicals was inhibited during pregnancy. Nowadays TNF-blockers have been categorized as “group B” by the Food and Drug Administration (FDA). Although only a few reports of congenital malformations have been reported, no firm conclusions can be drawn about the safety of anti-TNF therapy during pregnancy[17]. Nevertheless, TNF-blockers are nowadays more often prescribed during pregnancy to control active RA. Even if adequate medication is given during pregnancy, becoming pregnant can be difficult while suffering from RA. Fertility can be reduced and the risk of early miscarriage is higher[18,19].

1.2.1 RA and pregnancy outcome

Pregnancy complications, like gestational hypertension, preeclampsia, but also caesarean sections are slightly increased in pregnant women with RA compared to the

general population. Furthermore pregnancy outcome complications, like low birth weight or prematurity are more common in RA[20-23].

Elevated RA disease activity during pregnancy is associated with a lower birth weight of the child independently of many covariates, like medication use, parity, smoking, gender of the child, gestational age, maternal age or educational level[20].

Prednisone use during pregnancy shortens the gestational age in women with RA. De Man *et al*. showed that women with prednisone use deliver (on average) one week earlier and had also more often pre-term pregnancies (<37 weeks of gestational age), compared with women who did not use prednisone[20]. Both results concerning prednisone use will indirectly lower the birth weight of the child.

1.3 Early life determinants associated with adult diseases

Several hypotheses are settled on the idea that certain environmental determinants during the fetal and postnatal period are important risk factors for developing adult diseases. In the following paragraphs, all known associations of these environmental determinants will be described during 3 different periods in early life. Beginning with the fetal period (I), followed by the postnatal period during the first year (II), and the prepubertal age from 5-10 years (III) (Figure 1.1).

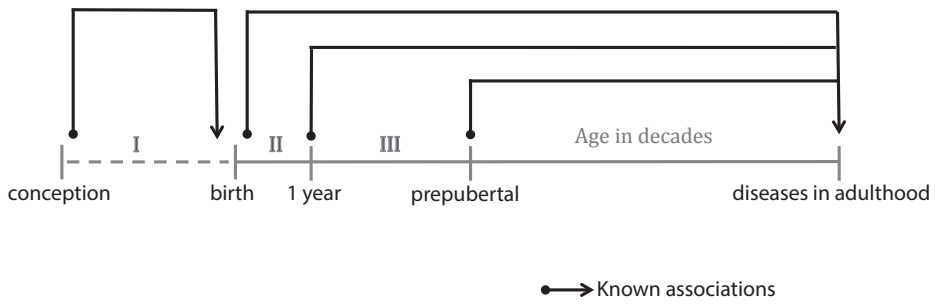


Figure 1.1- Known associations between determinants during the fetal period (I), during the first year of life (II), or during prepubertal age (III), and the development of diseases in adulthood.

1.3.1 Fetal determinants of diseases in adulthood

Several environmental determinants are associated with fetal growth deprivation, which is often, but not always, manifested in lower birth weight. Besides maternal malnutition there are many other maternal environmental determinants, which are associated with low birth weight, like smoking, maternal weight, height and body mass index (BMI), gestational weight gain and maternal hypertension[24-27]. Birth weight is expressed in birth weight standard deviation scores (birth weight SDS), corrected for

gestational age and gender[28]. Lower birth weight SDS, independent of the reason, is associated with diseases in adulthood, like hypertension and cardiovascular disease[29-31].

Barker *et al*. were the first to create a hypothesis based on the inverse association found between birth weight and several diseases in adulthood[32]. They hypothesized that low intrauterine supply of nutrients, could lead to fetal re-programming[33]. Obviously, the fetus will benefit from these adaptions, because it will survive despite the malnutrition. However when the child enters the nutrient-enriched, and in some cases nutrient-over enriched world, this re-programming is not necessary anymore and can lead to hypertension and coronary heart diseases in adulthood. Importantly, fetal malnutrition should not solely be based on the size of the baby at birth. A study done on the effect of Dutch famine during World War II showed that maternal malnutrition during gestation permanently affected adult health without affecting the birth weight. The authors did imply that the adaptations made by the fetus to continue to grow may nevertheless have adverse consequences for health in later life like coronary heart disease[34].

Concerning RA, several maternal RA determinants could potentially lead to low birth weight. Rise of RA disease activity often leads to the rise of pro-inflammatory cytokines, which might negatively influence the fetal growth as seen in mice-studies[35-37]. This could be an explanation for the low birth weight found when pregnancy is accompanied by elevated RA disease activity. Glucocorticoids might also create an adverse effect on the fetal programming resulting in impairment of fetal growth[38].

1.3.2 First year determinants of diseases in adulthood

About a decade ago, Singhal and Lucas suggested that not the low birth weight, but importantly the acceleration in weight during childhood is associated with increased risk for adult diseases later in life[39]. They assumed that a child is genetically determined to grow to its growth potential. When a child is born below his or hers genetic growth potential, it can experience postnatal catch-up growth and the tempo of this catch-up is essential[40].

Leunissen *et al.* specified the hypothesis of Singhal and Lucas by postulating that it is not just postnatal growth acceleration, but more importantly it is the fat accumulation during childhood, which increases the risk of diseases in adulthood[41]. They showed that fat accumulation during childhood was related to insulin sensitivity, higher levels of total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL), and blood pressure in early adulthood[42-44]. Recent studies of Kerkhof *et al*. specified that is more the catch-up growth in weight during the first 3 months of life, which is related to unfavorable cardiovascular and metabolic profiles later in life[40,41]. Catch-up growth is defined as a weight gain of more than 0.67 standard SDS during the first year, and is called rapid, when the weight gain in first 3 months is more than 0.5 SDS[40,41,45].

Concerning RA, elevated RA disease activity and RA medication use are related, directly or indirectly, with lower birth weight of the child[20]. Catch-up growth is therefore unavoidable, but it is unknown in which time and tempo these children experience catch-up growth in the first year of life.

1.3.3 Prepubertal determinants of diseases in adulthood

High amount of total fat mass during childhood tends to track into adulthood, meaning that children with high body mass index (BMI) will become adults with higher BMI, thereby increasing the risk for cardiovascular disease (CVD) or Type II Diabetes Mellitus (T2DM) in adulthood[46,47]. Abdominal distribution of body fat is associated with insulin resistance and dyslipidemia[48].

A cluster of cardiovascular risk factors that have been shown to predict the development of CVD and T2DM in adulthood is called the metabolic syndrome. The International Diabetes Federation has defined this metabolic syndrome for children[49]. Insulin resistance is a precursor of the metabolic syndrome and is evaluated using the Homeostasis Model of Assessment Insulin Resistance (HOMA-IR) and the level of serum adiponectin in the child.

Animal studies showed a reduced, negative feedback of the hypothalamic-pituitary-adrenal (HPA) axis when exposed to synthetic glucocorticoids *in utero*, which led to elevated blood pressure and glucocorticoid levels in adult offspring[50]. Studies postulate that synthetic glucocorticoids can influence the activity of the fetal HPA axis especially in the early stage of pregnancy, when the placenta has not been fully developed[51,52]. Furthermore, in adults increased total cortisol exposure is associated with athoresclerosis of the carotid arteries[53].

The risk for adult disease can be evaluated by assessing the body composition of the prepubertal child, the presence of metabolic syndrome (MetS) including insulin sensitivity or by the presence of elevated daily cortisol levels in the children. Body composition is assessed by anthropometric measurement including weight, height, skin folds, waist and hip circumferences. The total amount and distribution of the fat mass can be assed using a DXA-scan.

Concerning RA, previous studies have suggested that in utero exposure to glucocorticoids can lead to persistently increased glucocorticoid effect throughout adulthood leading to diseases in adulthood like CVD and T2DM[51]. This fetal exposure to prednisone could influence the fetal programming of the unborn child. This could be by ‘re- programming’ the fetal HPA-axis. It is unknown if this ‘re- programming’ happens in pregnant women with RA and if the consequences are still present in their offspring at prepubertal age. Due to the fact that elevated RA disease activity can create fetal growth restriction it could also create similar consequences.

1.4 Aims of the study

In the FEPRA-study (FEtal Programming in Rheumatoid Arthritis) we explored the association between RA related variables during pregnancy, found in the PARA-study (Pregnancy-induced Amelioration of Rheumatoid Arthritis), and the growth and development of the offspring until prepubertal age. All subjects of the FEPRA-study are children born to women who participated in the PARA-study. The designs of the two studies are described in Appendix A.

The main aim of the FEPRA-study was to assess if RA variables during pregnancy, like RA disease activity, medication use and the presence of RA autoantibodies, are associated with early determinants of diseases in adulthood. The early life determinants of diseases in adulthood can be measured at birth (I), during the first year of life (II), or at prepubertal age with a range from 5 until 10 years old (III) (Figure 1.2). At these time points different outcomes were measured, but all outcomes added to the main aim, trying to assess risk factors for future diseases in adulthood and then relating these risk factors to maternal RA disease activity and medication use during pregnancy.

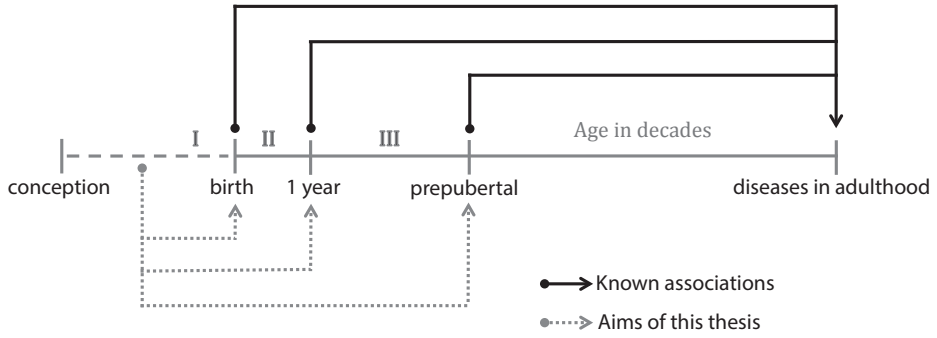


Figure 1.2 -This thesis aims to describe associations between maternal RA determinants throughout pregnancy, and determinants of adult disease at birth (I), during the first year of life (II), or at prepubertal age (III).

I) At birth

- Do RA variables during pregnancy, negatively influence the birth weight SDS of the child?

To investigate whether the RA associated cytokines IL-10, IL-6 and TNFα, in pregnant women with RA, influence the fetal growth resulting in lower birth weight SDS of the child.

II) During the first year of life
- *Do RA variables during pregnancy, negatively influence the tempo first year growth?* To examine the influence of RA disease activity, medication use and the presence of RA associated autoantibodies during pregnancy, on the growth tempo of the child in the first year of life.

III) At prepubertal life
- *Do RA variables during pregnancy, influence the body composition of the prepubertal offspring?* To investigate if RA associated variables during pregnancy, like RA disease activity and medication use, increase the prevalence of early risk factors of diseases in adulthood at the prepubertal age. The early risk factors were assessed based on the body composition of the child, including the total amount and distribution of the fat mass in the body. It was also assessed on the prevalence of metabolic syndrome (MetS).

- *Do RA variables during pregnancy, influence the insulin resistance of the prepubertal offspring?* Insulin resistance was assed using the HOMA-IR. Furthermore, early risk factors were assessed based on lipid profiles, including the level of serum adiponectin of the offspring.

- *Do RA variables during pregnancy, influence bone mineral density of the prepubertal offspring?* To investigate if the bone mineral density of the children differs from the normal population, because both elevated disease activity and prednisone use are involved in developing low bone mineral density in patients[54,55].

- *Do RA variables during pregnancy, influence the cortisol levels of the prepubertal offspring?* Finally, to explore whether maternal prednisone use during pregnancy had an influence on the fetal hypothalamic–pituitary–adrenal (HPA) axis by assessing the daytime cortisol levels of the prepubertal offspring.

1.5 Design of the studies

1.5.1 PARA-study

The PARA-study (Pregnancy-induced Amelioration of Rheumatoid Arthritis) is the world's largest prospective, nationwide cohort study on pregnancy and RA, which started in 2002 and ended in 2010[15]. The main purpose of this study was to observe the mutual interaction between RA disease activity and pregnancy, including pregnancy outcome. In addition, it was set up to examine pathogenic mechanisms underlying this mutual interaction.

In the PARA-study women were recruited by their rheumatologist and were eligible for inclusion if they had a pregnancy wish or when they were already pregnant. All women met the American College of Rheumatology 1987 revised criteria for RA[56,57].

Patients were visited at their home address at preconception, 3 times during pregnancy and 3 times postpartum. During every visit, a physical examination was performed, medication use was collected and RA disease activity calculated using the DAS28CRP, a well-validated tool for RA disease activity during pregnancy[20]. This tool calculates the RA disease activity score by examining 28 joints using 2 variables per joint: number of swollen joints and number of tender joints, and measuring the serum C-reactive protein (CRP) level[62].

Birth weight was expressed in birth weight standard deviation scores (birth weight SDS), corrected for gestational age and using a gender specific formula[28].

Medication use during pregnancy was restricted to prednisone, sulfasalazine and hydroxychloroquine. The following table is and indication of the medication used in the PARA study[15].

Table 1.1 – Indication of the medication used in the PARA-study (de Man *et al*.[15]).

	ever used n=84	pre-pregnancy n=41	1st trim n=84	2nd trim n=84	3rd trim n=84
	n(%)	**n(%)**	**n(%)**	**n(%)**	**n(%)**
prednisone (oral)	33 (39)	16 (39)	30 (36)	30 (36)	29 (35)
sulfasalazine	65 (77)	17 (41)	26 (31)	28 (33)	27 (32)
hydroxychloroquine	31 (37)	2 (5)	2 (2)	3 (4)	2 (2)
methotrexate	46 (55)	0 (0)	0 (0)	0 (0)	0 (0)
leflunomide	4 (5)	0 (0)	0 (0)	0 (0)	0 (0)
biologicals	10 (12)	0 (0)	0 (0)	0 (0)	0 (0)
no medication	n/a	7 (17)	22 (26)	29 (35)	29 (35)

Abbreviations: trim: trimester

*This table is kindly provided by dr Y.A. de Man and has been published before[15]

The medical treatment during pregnancy was adapted in some women due to the fact that RA disease activity declined during pregnancy. The median oral dose of prednisone in the first trimester was 6.00 mg/day with an interquartile range (IQR) of 1.0 to 10.0 mg/day. In the third trimester the median dose was 5.00 mg/day (IQR = 4.4-10.0 mg/day). Other medication included sulfasalazine, sometimes in combination with prednisone. The median (IQR) sulfasalazine dose was 2000 (500-4000) mg/day throughout the entire pregnancy.

One of the main conclusions from the PARA-study was that elevated RA disease activity during pregnancy is associated with a lower birth weight and that prednisone shortens the gestational age[20]. These conclusions served as the basis of the FEPRA-study, which resulted in this doctoral dissertation.

1.5.2 FEPRA-study

After participating in the PARA-study, all mothers were contacted by mail and phone to participate in the FEPRA-study (FEtal Programming in Rheumatoid Arthritis). Growth charts of the children were collected from birth onwards. Information was obtained out of the growth booklet (Dutch: groeiboekje) or records of the infant welfare centre (Dutch; consultatiebureau).

When the child reached the age of 5 years, both parents and the child were invited to visit the Sophia Children's Hospital in Rotterdam. Less than one week before the visit salivary sampling was completed at home. Sampling was done using polyesther swabs during four time-points: at awakening, 30 minutes after awakening, afternoon, before going to bed. The parents recorded the sampling times immediately after completing saliva sampling. At the hospital the swabs were centrifuged and stored at -20°C until measured. Salivary sampling is a non-invasive technique in domestic setting, without the need of repeated blood samples. Saliva cortisol levels are highly correlated with serum levels and there advantage is that the cortisol level is not contaminated with stress resulting from needle or hospital[59-61]. In the present thesis, saliva cortisol samples were used to assess the hypothalamic-pituitary-adrenal axis activity.

During the visit at the hospital, several characteristics of child and parents were determined like, weight, height, head circumference and blood pressure. The anthropometric data of the child were extended with sitting height and circumference of arm, waist and hip and also included skin folds (triceps, biceps, subscapular and suprailiac) measured with a Holtain caliper. All values were transformed to standard deviation scores (SDS) for age and gender according to Dutch reference values[64,65], using the Growth Analyser (version4.0; Growth analyser BV, Rotterdam, the Netherlands, www.growthanalyser.org).

Fasting blood samples of the child were taken to measure glucose fasting levels, insulin fasting levels, high-density lipoprotein fasting levels, triglycerides fasting levels, total cholesterol and adiponectin concentrations.

All children underwent a Dual-Energy X-ray Absorptiometry (DXA)-scan to assess bone mineral density and body composition[58]. The radiation dose during a DXA scan is approximately 1/10 of a chest X-ray[58].

1.6 Outline of this thesis

This thesis gives a detailed description of RA associated variables during pregnancy, namely prednisone use and RA disease activity, and the consequences of these variables on offspring. **Chapter 1** gives an introduction in the topics described in this thesis. **Chapter 2** defines the association between maternal serum cytokine levels in the pregnant women with RA, with birth weight SDS of the child. **Chapter 3** illustrates the postnatal growth pattern in the first 2 years of the children, born from mother with RA. In **Chapter 4** the associations between RA medication plus RA disease activity and the

bone mineral density of the child is described. **Chapter 5** investigates whether prednisone or elevated RA disease activity in pregnant women with RA influence the body composition of their offspring, or increase the presence of metabolic syndrome. **Chapter 6** focuses on signs of insulin resistance at the children focusing on 3 alternative outcomes to evaluate the insulin resistance: body fat distribution, Homeostasis Model of Assessment Insulin Resistance (HOMA-IR), and level of serum adiponectin. The objective of **Chapter 7** was to investigate whether prednisone-exposure in utero influences the cortisol levels of prepubertal children. In **Chapter 8** we discuss the significance and clinical implications of the results found. It debates the conclusion in the light of current literature, and gives recommendations for future research. In **Chapter 9**, a summary of the dissertation is given in English and in **Chapter 10** a summary of the dissertation is given in Dutch. The addendum of this thesis contains a list of abbreviations and a few words about the author. It further includes the PhD portfolio, the list of co-authors and affiliations and the acknowledgments.

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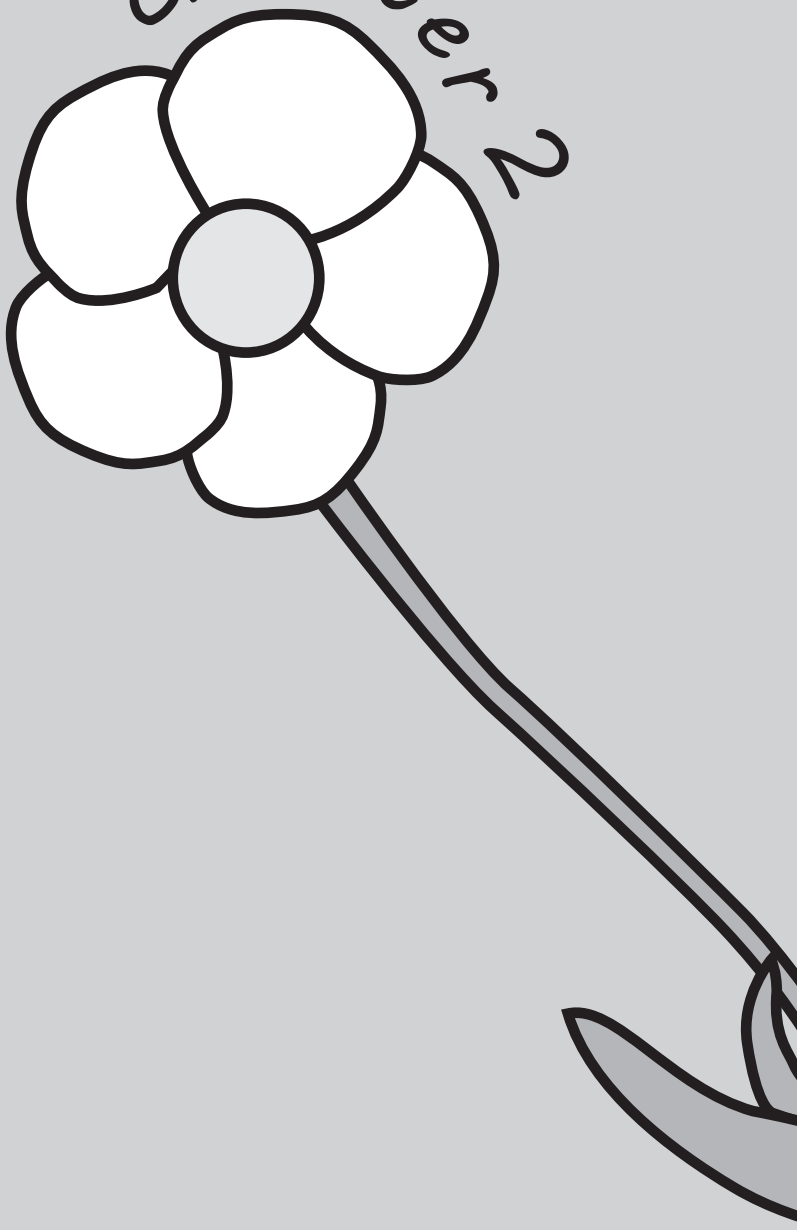
Chapter 2



Circulating maternal cytokines influence the fetal growth in pregnant women with rheumatoid arthritis

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ABSTRACT

Background
High rheumatoid arthritis (RA) disease activity during pregnancy is associated with a lower birth weight. Active RA is characterised by high circulating levels of cytokines, which can mediate placental growth and remodelling.

Objectives
To assess the influence of maternal serum cytokine levels on birth weight in RA pregnancy.

Methods
This study is embedded in the PARA Study, a prospective study on RA and pregnancy. In the present study, 161 pregnant women with RA and 32 healthy pregnant women were studied. The main outcome measures were birth weight SD score (birth weight SDS) in relation to maternal serum levels of interleukin-10 (IL- 10), interleukin-6 (IL-6) and tumour necrosis factor-α (TNFα) at three different time points: preconception and during the first and third trimester. Single-nucleotide polymorphisms (SNPs) in the corresponding cytokine genes were also studied.

Results
During the first trimester, IL-10 was detectable in 16% of patients with RA, IL-6 in 71%, and TNFα in all patients with RA. Mean birth weight SDS of children born to mothers with RA was higher when IL-10 level was high compared with low (difference=0.75; $p=0.04$), and lower when IL-6 was high compared with low (difference=0.50; $p<0.01$) in the first trimester. No correlation was seen at the other time points studied or with TNFα. Cytokine levels were not related to their corresponding SNPs.

Conclusions
Maternal IL-10 and IL-6 levels are associated with fetal growth in RA. In the first trimester, high IL-10 levels are associated with higher birth weight SDS, and high IL-6 levels are associated with lower birth weight SDS, even after correction for disease activity.

INTRODUCTION

In normal pregnancies, cytokine levels are not always detectable, but active rheumatoid arthritis (RA) is characterised by high serum levels of cytokines. RA is a chronic, systemic autoimmune disease in which cytokines play a crucial role in the disease activity[1].

High maternal cytokines can initiate and intensify the cascade of inflammatory cytokine production during normal pregnancy, resulting in maldevelopment of the placenta leading to spontaneous abortion, intrauterine growth restriction (IUGR) or preterm delivery[2]. Aberrant maternal cytokine levels have been reported in several perinatal complications, such as pre-eclampsia[3], pre-term delivery[4] and threatened miscarriages[5].

It has been shown that high RA disease activity is associated with a lower birth weight[6]. High disease activity can be related to high cytokine levels, but it is not known if high cytokine levels influence birth weight. A lower birth weight, even within the normal range, might be a risk factor for perinatal complications and is associated with developmental delay, cardiovascular disease, hypertension, non-insulin-dependent diabetes mellitus and neuropsychiatric disorder in adulthood[7-9].

In the present study, three different points in time were chosen to evaluate cytokine levels: pre-conception, first and third trimester. If high circulating cytokines influence placentation and even implantation of the fetus, as mice studies have emphasised[10], the focus should be on the beginning of the pregnancy. The third trimester was chosen because of the rapid growth and maturation of the fetus in that period. Interleukin-10 (IL-10), interleukin-6 (IL-6) and tumour necrosis factor-α (TNFα) were studied, because they are implicated in the pathogenesis of RA as well as in pregnancy outcome [1,5,11-13].

Certain functional variations due to single- nucleotide polymorphisms (SNPs) in the genes of IL-10, IL-6 and TNFα have been shown to influence cytokine levels, increase the risk of developing RA, and be associated with preterm birth and increased risk of pre-eclampsia[14]. In our study, these specific SNPs were taken into account.

We hypothesised that high maternal serum levels of cytokines influence fetal growth during pregnancy in women with RA. Describing these effects should lead to a better understanding of the function of cytokines during pregnancy and their influence on pregnancy outcome.

SUBJECTS & METHODS

Study population

This study is embedded in the PARA-study (Pregnancy-induced Amelioration of Rheumatoid Arthritis), a prospective, nationwide cohort study in pregnant women with RA[15]. For present analyses, information was obtained at preconception (range of 3 month-1 year before conception) and during first trimester (range 8-12 weeks of gestation) and the third trimester (range 28-32 weeks of gestation). Preconception was

defined as actively trying to become pregnant after stopping all teratogenic RA medication for at least 3 months. Women were recruited by their rheumatologist and were eligible for inclusion if they wished to become pregnant or when they were already pregnant, but no further than the second trimester. All women met the American College of Rheumatology 1987 revised criteria for RA[16]. Only singleton pregnancies of Caucasian women, who delivered a child without congenital deformities, were included[17]. A total of 162 participants were enrolled, of whom 73 were seen at preconception, 139 were visited in the first trimester, and data for 158 were available for the third trimester. One patient was excluded from the study because of having breast cancer during her pregnancy. Therefore, 161 Caucasian women with RA were eligible for the study.

Since serum cytokine levels may depend on the detection method, a comparison group was included to observe the main outcome in a normal population. Thirty-two healthy pregnant Caucasian women without an adverse obstetric history were recruited. They were visited at the same time points and had the same assessments and laboratory tests as the pregnant women with RA. The PARA study was approved by the Medical Ethics Committee, Erasmus MC (Rotterdam, The Netherlands).

Pregnancy outcome

Data on pregnancy outcome included birth weight, gestational age at delivery, and gender of the child. Birth weight was expressed as birth weight SD scores (birth weight SDS), corrected for gestational age and gender[18].

Factors associated with pregnancy outcome

Clinical characteristics were collected by medical record and physical examination. They included maternal age, gynaecological history, medication use and smoking habits.

At every time point, RA Disease Activity Score (DAS28) was calculated by examining 28 joints using 2 variables per joint: number of swollen joints, number of tender joints, and measuring serum C-reactive protein (CRP) level[19]. It has been shown that RA disease activity is most reliably assessed with this modality of the DAS28 during pregnancy[20]. CRP levels were directly measured using the Tina-Quant CRP Immunological Test System (Roche Diagnostics, Almere, The Netherlands). After centrifugation, all samples were frozen at −80°C until assayed. Serum levels of IL-10, IL-6 and TNFα were determined using the immunoassay system, IMMULITE 1000 (Siemens Healthcare Diagnostics, Breda, The Netherlands). The intra-run/inter-run mean±variation coefficient was: IL-10, 27.7±4.6%/23.35±5.4%; IL-6, 105.6±4.9%/ 87.67± 6.1%; TNFα, 105.6±4.9% /89.5±3.8%. The lower limit of quantification was IL-10, 5.0; IL-6, 2.0; TNFα, 2.0. All cytokine levels are presented in pg/ml.

Genetic factors associated with cytokine levels

SNPs selected were either proven to be functional in relation to cytokine level or associated with RA or pregnancy outcome. In addition, a minor allele frequency of >0.10

in the National Center for Biotechnology Information database was required[21]. Eight SNPs were selected, which all proved to be located in the promoter region of the cytokine gene. Blood for DNA isolation was available for 134 patients.

On chromosome 1, the *IL10* gene has three SNPs known to influence serum IL-10 levels: rs1800871, rs1800872 and rs1800896[14]. The last of these SNPs increases the risk of developing RA when it is a homozygous AA carrier[22].

On chromosome 7, the *IL6* gene has two SNPs, rs1800795 and rs1800797, which are known to influence serum IL-6 levels and are associated with risk of preterm birth [23].

On chromosome 6, the *TNFα* gene has three SNPs known to influence serum TNFα levels: rs1800630, rs1800629 and rs1799724[24]. To evaluate rs1800630, we took a tagging SNP in high linkage disequilibrium (rs2844482; r^2 =1.00)[25]. All genetic testing was done using a Sequenom® iPLEX.

Data analysis

Descriptive statistics are presented as numbers, percentages, means and SDs. Spearman rank correlation coefficients were calculated to evaluate correlations between disease activity and serum levels of cytokines.

Univariate linear regression analyses were performed, with birth weight SDS as dependent variable and maternal serum levels of IL-10, IL-6 and TNFα at preconception and in the first and last trimester as independent variables. Even after trans- formation, cytokine levels were not normally distributed and were therefore dichotomised according to their median: 0 when the level was lower or equal to the median (low), and 1 when the level was higher than the median (high). When the median was 0, the median of the detectable cytokine levels was taken. Depending on the trimester, IL-10 was measurable in 11–16% of the women with RA (table 1). Consequently, the median of the measurable levels was taken. For IL-6 and TNFα, the median of all levels was taken. To describe the association between maternal cytokine level and birth weight SDS, RA disease activity was addressed as a confounder because it is known to influence birth weight and cytokine levels[26]. Other potential confounders are maternal age, smoking habit, and use of prednisone and/or sulfasalazine[27]. A multivariate regression model (analysis of covariance) with backward selection was used, and all potential confounders with highest p value were eliminated from the model until all p values were less than 0.2.

For SNP genotype distributions, significant departure from Hardy–Weinberg equilibrium was calculated using the $\chi 2$ test. The Lewontin's D prime (D0) and correlation coefficient (r2) were calculated to assess the presence of linkage disequilibrium (r2≥0.8). The Kruskal–Wallis rank test was used to determine the difference in serum cytokine levels within the three genotype groups. Linear regression, based on allele dose, was per- formed, with birth weight SDS as dependent variable and genotype groups as independent variable. All statistical analyses were performed using Stata software (V.12.0 for Windows). All SNP analyses were performed using Haploview (V.4.2 for Windows) with the CEU trio as reference.

Table 2.1 - Patient and Pregnancy Characteristics.

	RA patients (n= 161)	Reference group (n=32)
Patient Characteristics	**mean (SD)**	**mean (SD)**
Age at delivery in years	32.53 (3.66)	32.11 (4.40)
RA duration at delivery in years	7.90 (6.50)	na
Parity	**%**	**%**
nulliparous	50.3	44
multipara	48.5	56
missing	1.2	na
Smoking during pregnancy	4 (n=154)	10 (n=32)
DAS28 at different time points,		
pre-conception	3.73 (0.98) (n=73)	na
trimester 1	3.71 (1.11) (n=139)	na
trimester 3	3.40 (1.13) (n=158)	na
Use of Medication at different time		
Prednisone	**% (n)**	
pre-conception	29 (n=21)	na
trimester 1	27 (n=37)	na
trimester 3	26 (n=42)	na
Sulfasalazine		
pre-conception	21 (n=15)	na
trimester 1	17 (n=24)	na
trimester 3	17 (n=27)	na
Both		
pre-conception	12 (n=9)	na
trimester 1	11 (n=15)	na
trimester 3	9 (n=15)	na
Pregnancy outcome	**mean (SD)**	**mean (SD)**
Birth weight in kilograms	3.390 (0.58)	3.492 (0.45)
Gestational age in weeks,	39.4 (1.80)	40.1 (1.45)
Birth weight SDS	-0.004 (1.09)	-0.020 (0.96)
Gender of child male, %	57	56
Birth weight SDS in groups[a] (trimester 3)		
DAS28 ≤ 3.2 (low)	0.164 (0.99) (n=72)	
DAS28 3.2-5.1 (intermediate)	-0.119 (1.07) (n=74)	
DAS28 > 5.1 (high)	-0.390 (1.53) (n=12)	
Cytokine levels at different time points [c]	**median (IQR) (detectable %)**	**median (IQR) (detectable %)**
IL-10**:** pre-conception	6.01 (5.45-10.4) (13%)[b]	na
IL-10**:** trimester 1	9.61 (5.95-15.9) (16%)[b]	8.16 (5.72-9.07) (9%)[b]
IL-10**:** trimester 3	9.56 (6.21-12.6) (11%)[b]	6.45 (6.45-13.9) (9%)[b]
IL-6: pre-conception	4.84 (0.00-12.8) (76%)	na
IL-6: trimester 1	2.91 (0.00-7.57) (71%)	0.00 (0.00-2.09) (28%)
IL-6: trimester 3	2.09 (0.00-3.98) (51%)	0.00 (0.00 to 2.23) (32%)
TNFα**:** pre-conception	10.5 (8.78-12.2) (100%)	na
TNFα**:** trimester 1	14.2 (11.8-17.9) (100%)	9.87 (8.59-12.0) (100%)
TNFα**:** trimester 3	11.0 (9.31-13.1) (100%)	10.00 (8.56-13.15) (100%)

Abbreviations: RA, rheumatoid arthritis; na, not applicable; DAS28, RA disease Activity Score in 28 joints with CRP levels; SDS, Standard Deviation Score; [a] disease groups according to EULAR criteria; [b] Median of the detectable levels was taken; [c] All cytokine levels are expressed as pg/ml.

RESULTS

Participants

Mean maternal age at delivery was 32.5 years, and mean RA disease duration was 7.9 years (Table 2.1). Fewer than 4% of the subjects smoked, and 52–55% used medication during pregnancy. Medication use was restricted to prednisone, sulfasalazine or both (Table 2.1). None of the pregnant women used methotrexate or biologic agents before conception or during pregnancy. Only a minority of patients (n=3) used hydroxychloroquine.

Mean birth weight SDS was -0.004 SD (1.09). As expected, higher DAS28 was associated with a lower birth weight[6]. During the third trimester, an increase of 1 point in DAS28 correlated with a decrease in birth weight SDS of 0.21 (p= 0.005).

The 32 healthy pregnant women had a mean age of 32.1 years and 10% (3/32) smoked. Mean birth weight SDS was -0.02 SD (0.96) (Table 2.1).

Cytokine levels & birth weight

All cytokine levels correlated strongly with each other and the levels at different time points were related (Table 2.2). Levels of IL-10, IL-6 but not TNFα, correlated with DAS28 during all time points (first trimester shown in Table 2.2). Cytokine levels in healthy women showed similar correlations, but these were not significant, because of the small sample (data not shown).

Table 2.2 - Correlation of cytokine levels, DAS at first trimester and other time point in RA patients.

	Correlation during 1st trimester *Spearman's rho (p-value)*			**Correlation during 1st and other time point** Spearman's rho (*p-value)*	
Levels	IL-10	IL-6	TNFα	Before pregnancy	Third Trimester
IL-10	-	-	-	0.29 (0.02)	0.49 (<0.001)
IL-6	0.32 (<0.001)	-	-	0.51 (<0.001)	0.54 (<0.001)
TNFα	0.35 (<0.001)	0.24 (0.004)	-	0.20 (0.10)	0.45 (<0.001)
DAS28	0.25 (0.004)	0.40 (<0.001)	0.03 (0.71)	0.48 (<0.001)	0.56 (<0.001)

Abbreviations: DAS28, disease activity score of rheumatoid arthritis in 28 joints with CRP levels

As stated above, cytokine levels were defined as low when the level was lower or equal to the median, and high when the level was higher than the median. When the median was 0, the median of the detectable cytokine levels was taken.

During first trimester, IL-10 was detectable in 16% of the patients with RA (n=21). According to our definition IL-10 was high in 7% of the RA patients (n=10) and low in 93% (n=129). The mean birth weight SDS was 0.55 in the high-IL-10 group and –0.07 in the low-IL-10 group. The high-IL-10 group was associated with higher birth weight SDS than the low-IL-10 group (difference 0.75, p=0.040) after adjustment for confounders (Table 2.3a). No such effect was seen at preconception or in the third trimester.

Table 2.3a - Effect of dichotomised (high vs. low) cytokine levels (crude-adjusted) on birth weight SDS

First trimester (n=139)					
		Crude		**Adjusted**	
		ß (95% CI)	**p-value**	**ß (95% CI)**	**p-value**
IL-10		0.62 (-0.08;1.32)	0.08	0.75 (0.04;1.46)	0.04
	DAS28			-0.13 (-0.29;0.03)	0.11
	maternal age			0.02 (-0.01;0.04)	0.10
	prednisone			-0.28 (-0.68; 0.12)	0.17
IL-6		-0.47 (-0.83;-0.11)	0.01	-0.50 (-0.87;-0.13)	<0.01
	DAS28			-	-
	maternal age			0.01 (-0.00;0.01)	0.10
TNFα		0.04 (-0.34;0.41)	0.85	-	-
	DAS28			-	-
	maternal age			-	-
	prednisone			-0.24 (-0.56;0.08)	0.15

Abbreviations: DAS28, disease activity score of rheumatoid arthritis in 28 joints with CRP levels - not included in the model because p>0.2; Adjusted for RA disease activity, maternal age, smoking, prednisone use and sulfasalazine use

During the first trimester, IL-6 was detectable in 71% of the patients with RA (n=95). It was high in 48% (n=67). The mean birth weight SDS was −0.26 in the high-IL-6 group and 0.21 in the low-IL-6 group. The high-IL-6 group was associated with lower birth weight SDS (difference −0.50, p=0.009) after adjustment for confounders (Table 2.3a). No such effect was seen at the other time points (Table 2.3b). When the effects of IL-6 and IL-10 were analysed simultaneously, the effect was even more pronounced, resulting in a birth weight SDS increase of 0.82 for high IL-10 and a decrease of 0.58 for high IL-6 (Table 2.3c).

High levels of TNFα had no effect on birth weight SDS at any time point (Table 2.3a/b). Cytokine levels did not influence the gestational age (data not shown). In the reference group, cytokine levels were low and often undetectable (Table 2.1). Numbers were too small to allow statistical analysis

Table 2.3b - Effect of dichotomised (high vs. low) cytokine levels (crude-adjusted) on birth weight SDS

Third trimester (n=161)					
		Crude		**Adjusted**	
		ß (95% CI)	**p-value**	**ß (95% CI)**	**p-value**
IL-10		-0.21 (-0.94;0.53)	0.58	-	-
	DAS28			-0.21 (-0.35;-0.07)	<0.01
	maternal age			0.02 (0.01;0.04)	<0.01
	prednisone			-	-
IL-6		-0.23 (-0.57;0.11)	0.19	-	-
	DAS28			-0.22 (-0.35;0.08)	<0.01
	maternal age			0.02 (0.01;0.37)	<0.01
TNFα		0.09 (-0.26;0.43)	0.62	-	-
	DAS28			-0.21 (-0.35;-0.07)	<0.01
	maternal age			0.02 (0.01;0.04)	<0.01
	prednisone			-	-

Abbreviations: DAS28, disease activity score of rheumatoid arthritis in 28 joints with CRP levels - not included in the model because p>0.2; Adjusted for RA disease activity, maternal age, smoking, prednisone use and sulfazalazine use

Table 2.3c - ANCOVA: Effect of dichotomised (high vs. low) cytokine levels in first trimester on birth weight SDS. Levels were put in the model simultaneously.

First trimester					
		Crude (n=134)		**Adjusted (n=131)**	
		ß (95% CI)	**p-value**	**ß (95% CI)**	**p-value**
IL-10		0.795 (0.10; 1.49)	<0.03	0.819 (0.12; 1.52)	0.02
IL-6		-0.542 (-0.91; -0.18)	<0.01	-0.575 (-0.94; -0.21)	<0.01
	maternal age			0.006 (-0.00; 0.01)	0.13

Adjusted for RA disease activity, maternal age, smoking, prednisone use, sulfazalazine use

Single-Nucleotide Polymorphisms

Genotypes across all eight SNPs had no effect on the maternal cytokine levels even after correction for disease activity or medication use. Two SNPs were in Hardy-Weinberg disequilibrium and two had linkage disequilibrium (Table 2.4). Mothers who were homozygous for A in the polymorphism rs1800896 had new-borns with a significantly (p= 0.021) higher birth weight SDS, 0.27 (SD ± 1.02) compared with mothers homozygous for G, -0.34 (SD ± 1.03). Mean birth weight SDS in the heterozygous group was -0.14 (SD ± 1.11).

Table 2.4 - Effect of genotype on cytokine levels and on birth weight SDS.

SNP		MAF (n=134)	MAF (n=120)	HWE	Rank test[c]	mean birth weight sds (n)			Linear[d]
	WT/var	our pop	HapMap	p	p	Wt	Hetero	var	p
IL-10									
rs1800871[a]	C/T	0.24 (T)	0.21 (T)	0.58	0.97	-0.23 (85)	0.08 (39)	0.12 (10)	0.13
rs1800872[a]	C/A	0.24 (A)	0.21 (A)	0.58	0.97	-0.23 (85)	0.08 (39)	0.12 (10)	0.13
rs1800896	G/A	0.49 (A)	0.47 (A)	0.98	0.49	-0.34 (38)	-0.14 (69)	0.27 (27)	0.03
IL-6									
rs1800795[b]	G/C	0.40 (C)	0.47 (G)	0.002	0.53	0.16 (34)	0.07 (50)	-0.26 (50)	0.58
rs1800797[b]	G/A	0.38 (A)	0.48 (G)	0.01	0.64	-0.16 (30)	0.08 (51)	-0.27 (53)	0.47
TNFα									
rs2844482 [e,f]	G/A	0.18 (A)	0.16 (A)	0.52	0.39	0.00 (87)	-0.26 (44)	-1.19 (2)	0.08
rs1800629	G/A	0.20 (A)	0.22 (A)	0.56	0.42	-0.08 (87)	-0.13 (40)	-0.39 (7)	0.55
rs1799724	C/T	0.08 (T)	0.10 (T)	1.00	0.56	-0.09(110)	-0.25 (23)	0.74 (1)	0.78

Abbreviations: SNP; Single-Nucleotide Polymorphism, WT; wild type, var; variant allele; MAF, Minor Allele Frequency; HWE, Hardy-Weinberg equilibrium; Hetero, Heterozygote: [a,b] SNPs are in linkage disequilibrium ($r^2 \geq 0.8$); [c] probability of the equality-of-populations using the Kruskal-Wallis rank test (chi-squared with ties); [d] Linear regression, based on allele dose, dependent variable: birth weight SDS, independent variable; genotype groups; [e] To evaluate the *TNFα* -863 (rs1800630) the tagged SNP ID rs2844482 was taken; [f] could not be determined in all patients

DISCUSSION

We hypothesised that high maternal serum cytokine levels influence fetal growth in pregnant women with RA. This hypothesis appears to be true. In our prospective study, we observed that both IL-10 and IL-6 levels influence fetal growth in pregnant women with RA. During the first trimester, high IL-10 levels are associated with higher birth weight SDS, and high IL-6 levels are associated with lower birth weight SDS. This association was still present after correction for disease activity, maternal age, smoking, and use of prednisone and/or sulfasalazine during pregnancy. Unlike most studies, our study focused not only on the last trimester of the pregnancy, but also preconception and the first trimester, emphasising the importance of cytokine levels at the beginning of pregnancy.

Birth weight SDS was 0.75 higher in the high IL-10 group than in the low-IL-10 group. Birth weight SDS was 0.50 lower in the high IL-6 group than the low-IL-6 group. The effect of high levels became even more prominent when IL-10 and IL-6 levels were analysed simultaneously. Birth weight SDS was 0.82 higher in the high-IL-10 group and 0.58 lower in the high-IL-6 group. A difference of 0.5 SDS is considered to be of clinical

relevance, and therefore our findings are of relevance in women with high IL-10 and high IL-6 at the beginning of their pregnancy.

In our study, cytokine levels were independent of their functional SNPs. We explored the possibility of predicting birth weight based on preconception cytokine levels or functional variations in the relevant cytokine genes. However, no relation was found. These gene factors therefore describe insufficient variance to be useful in prediction. In addition, we observed that only the IL10 gene polymorphism, rs1800896—which in other studies is related to higher IL-10 levels[14 22], was associated with birth weight, but it cannot be excluded that this is attributed to multiple testing.

To evaluate the effects of high cytokine levels, we studied an RA population that is often characterised by high serum cytokine levels. In our RA population, 16% had a detectable IL-10 level comparable with literature values[28]. Even though the literature indicates that different diseases may be associated with specific cytokine profiles during pregnancy[29], the results of this study still may produce a better understanding of the consequences of high cytokine levels during pregnancy in general, but particularly in the first trimester.

A comparison group of 32 healthy pregnant women were included in the study. The main purpose of this reference group was to observe maternal serum cytokine levels in normal pregnancies. The number is too small to draw conclusions.

This study highlights the influence of cytokines throughout the first trimester of pregnancy, during which placentation occurs. The role of interleukins has not yet been fully clarified, but we hypothesise that cytokines influence placentation in various ways, by directly influencing placental growth factor (PlGF) and vascular endothelial growth factor (VEGF) and by indirectly influencing extracellular matrix (ECM) degradation. During normal placentation, cytotrophoblasts from the fetal stem cells differentiate and invade the uterine wall by destroying and displacing the ECM, which anchors the placenta to the uterus and provides maternal blood to the fetus. The presence and function of PlGF and VEGF are critical during placentation[30]. Anti-inflammatory cytokines such as IL-10 are considered to have a PlGF-enhancing effect[31]. Pro-inflammatory cytokines such as TNFα and IL-6 have adverse effects on cytotrophoblast growth and development[31].

One of the main mediators of ECM degradations is metalloproteinase-9 (MMP-9)[32]. Overexpression of MMP-9 is suggested to contribute to ECM degradation in the fetal membrane and placenta, thereby reducing placental function and even causing fetal membrane rupture or placental detachment[33]. Previous research has shown that IL-10 inhibits the expression of MMP-9 in cytotrophoblasts[34]. Other studies have shown elevated levels of MMP-9 in pregnant women with RA[35] In our population, maternal IL-10 levels were more often detectable because of their RA. High IL-10 levels positively influence fetal growth, and therefore our data may support the idea that IL-10 participates in the regulation of trophoblasts during human pregnancy by inhibiting MMP-9, thereby creating a more vital placenta. It also supports the idea that the MMP-9 content in first-trimester trophoblasts is more important than in third- trimester cells.[34].

High RA disease activity is associated with lower birth weight[6]. There is a theory that high IL-10 levels are responsible for the improvement in RA during pregnancy[28]. One could speculate that the effect of IL-10 levels on birth weight is not related to their effect on placentation, but acts indirectly by reducing maternal disease activity during pregnancy. However, in our cohort, high IL-10 levels were not associated with an improvement in RA during pregnancy; patients with high IL-10 had higher DAS28 during both in the first and third trimester than those with low IL-10.

Our main findings on IL-10 levels are consistent with mice studies. IL10 knockout mice were found to have more adverse pregnancy outcomes[36]. Fetal loss and fetal growth restriction have been shown to be attenuated by the administration of IL-10 to mice with endotoxin-induced IUGR[10]. These workers also showed that serum concentrations of TNFα and IL-6 were considerably higher in IL10 knockout mice than in the wild- type. This suggests that IL-10 modulates resistance to inflammatory stimuli by down regulating expression of pro-inflammatory cytokines such as TNFα and IL-6, protecting against inflammation-induced pathology[37].

Finally, our study has some limitations. First, the biological effects of cytokines are determined by the interplay of cytokines and their soluble receptors. The levels of these soluble receptors were not determined. Second, it is known that body mass index may influence cytokine levels as well as pregnancy outcome and therefore it could be a potential confounder[38]. The body mass index of the participants was not recorded in this study.

In conclusion, our study shows that high levels of maternal IL-10 and IL-6 influence the birth weight of babies born to pregnant women with RA. The effect of high IL-10 and IL-6 levels is most prominent in the first trimester when placentation occurs. As high IL-10 and IL-6 levels influence birth weight, they may influence the future development of the child.

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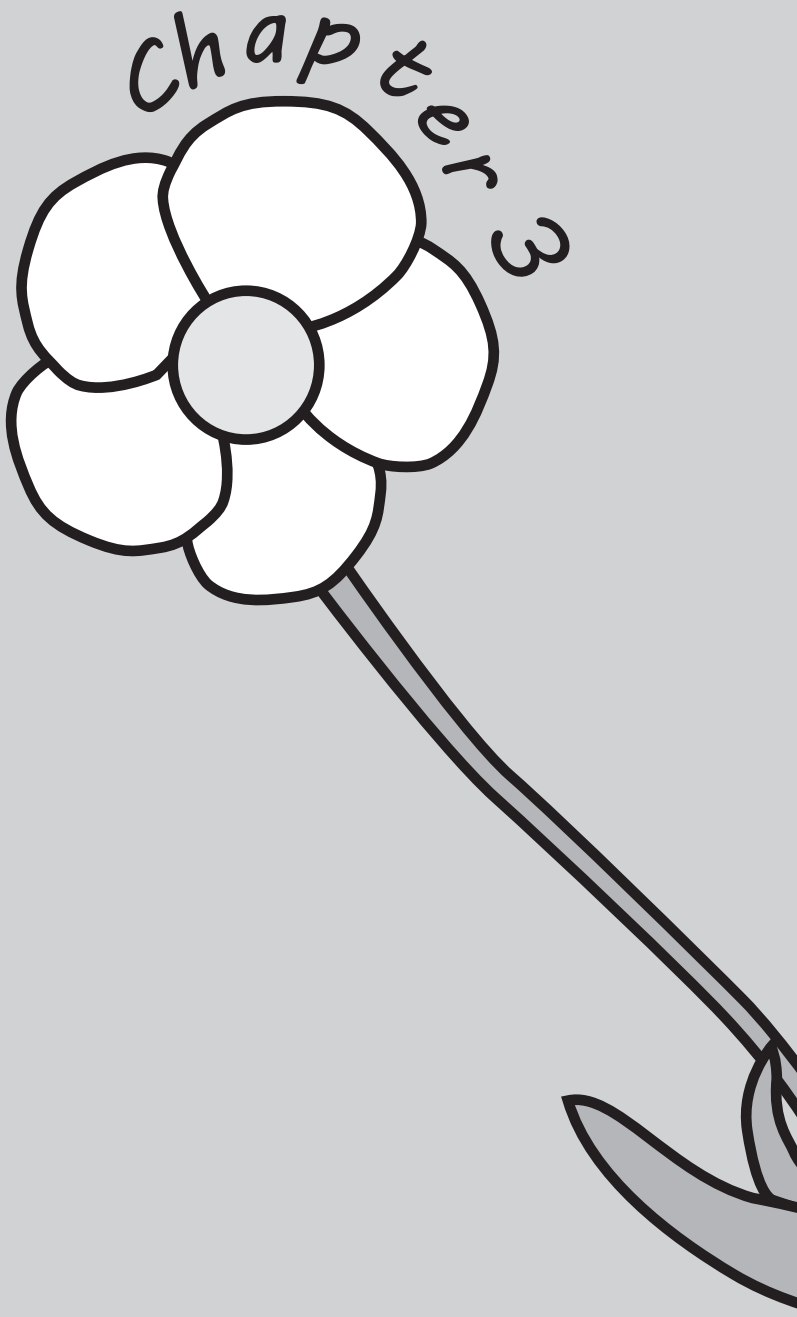
Chapter 3

Rheumatoid arthritis during pregnancy and the postnatal catch-up growth in the offspring

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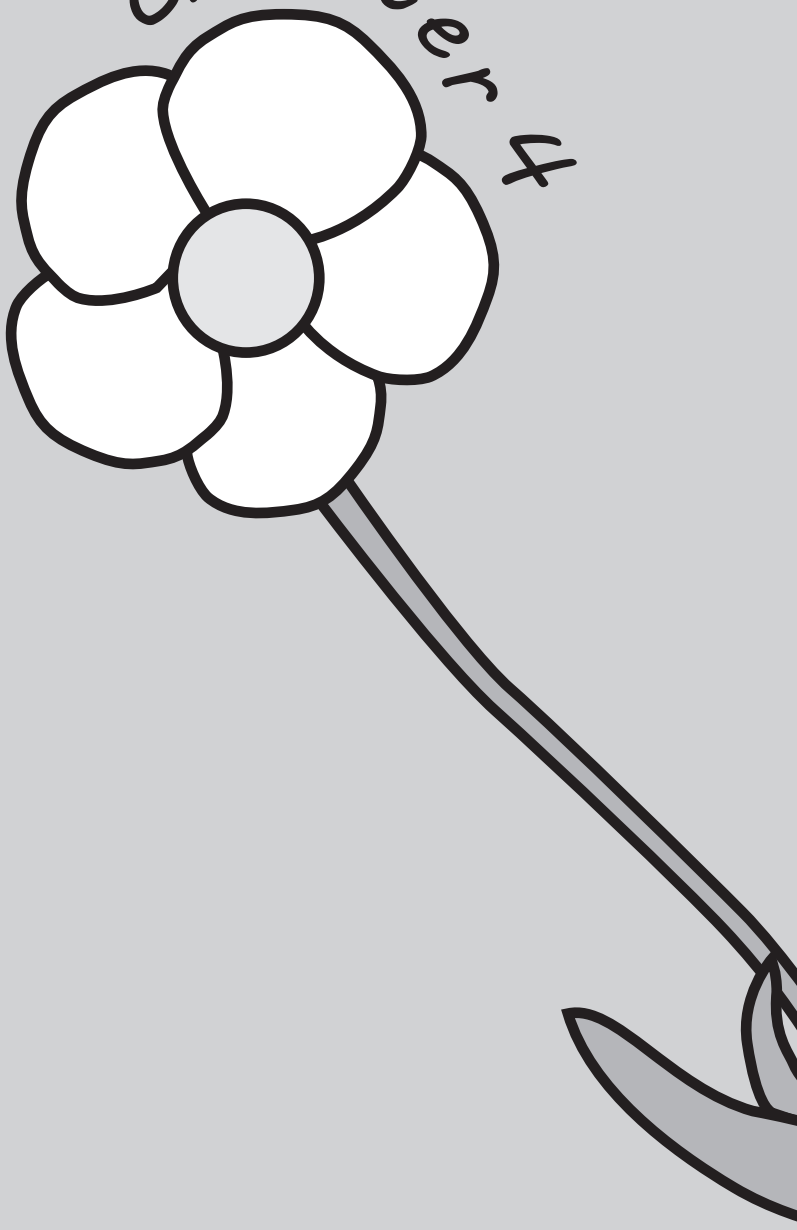
Chapter 4



Does medication or disease activity during pregnancy in patients with rheumatoid arthritis (RA) influence bone density of their 7-year-old offspring?

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ABSTRACT

Background
Prednisone treatment and rheumatoid arthritis (RA) disease activity are both associated with a decrease in bone mineral density (BMD) of the patients. Both prednisone use and high disease activity may be inevitable in pregnant women with RA, but it is unknown if these variables influence the BMD of their offspring.

Objectives
To investigate whether medication or disease activity during pregnancy in patients with RA, influence the BMD of their prepubertal offspring.

Methods
Mothers (n=255) participated in a prospective cohort study on RA and pregnancy. Their children, age 5-10 years, were included in the follow-up study (n=108). The BMD of the children was assessed by Dual-Energy X-ray Absorptiometry. The following variables of the child were taken into account because they are known to influence the BMD, calcium intake, physical activity, serum 25-hydroxyvitamin D concentration, gender, length and weight. Pre- and postnatal variables known to influence the BMD are gestational age, maternal smoking, birth weight, postnatal tempo of growth and type of feeding. The independent variables were prednisone, sulfasalazine and RA disease activity during pregnancy.

Results
No association was found between prednisone use or RA disease activity during pregnancy and BMD of 7-years-old offspring, even after correcting for all known associated variables. Sulfasalazine use had a positive effect on the whole body BMD (difference in SDS=0.53; p=0.005).

Conclusions
Neither medication use nor high RA disease activity during pregnancy is associated with a decreased BMD of the 7-year-old offspring. The maternal benefit of RA medication during pregnancy outweighs the effect on the bone mineral density in the offspring.

INTRODUCTION

Medication use and the disease activity of rheumatoid arthritis (RA) may vary during pregnancy. Medication use during pregnancy is mainly restricted to prednisone, sulfasalazine and sometimes hydroxychloroquine. Prednisone is notorious for depressing the bone mineral density (BMD) of the patient. Nevertheless, in a male RA population sulfasalazine has been correlated with higher BMD[1]. Treatment is essential for pregnant women with RA, because decline of disease activity occurs in only half of women during pregnancy[2]. Elevated disease activity is also involved in the development of low BMD of the RA patient[3,4]. While it is known that medication and RA disease activity influence the BMD of the patient, it is unknown if they also influences the BMD of the offspring when present in pregnancy.

In general, many variables have been reported to influence the BMD during childhood. These variables can be divided into two groups, child-related variables and pre- and postnatal variables. Child-related variables are calcium intake, physical activity, serum 25-hydroxyvitamin D (25-OHD) concentration, gender, length, weight and body mass index (BMI)[5,6]. Pre- and postnatal variables that influence the BMD of the child are maternal smoking during pregnancy, gestational age, birth weight, mode of feeding in early postnatal life and tempo of growth in the first years of life[7,8]. Insufficient maternal serum 25-OHD levels has been associated with a lower BMD of the offspring, but this finding could not be confirmed in a large study on pregnancy outcome of healthy women[9]. It is unknown if pre- and postnatal variables associated with RA, like medication use or disease activity influence the BMD of the children.

The aim of the study was to determine, whether in addition to the already known factors, maternal medication use or RA disease activity during pregnancy are negatively associated with the BMD of their prepubertal offspring.

PATIENTS AND METHODS

Study Population

Our study cohort consisted of healthy children born to mothers participating in the PARA-study (Pregnancy-induced Amelioration of Rheumatoid Arthritis), a prospective, nationwide cohort study on women with RA[2]. In the PARA-study, women with RA were visited by a research nurse at their home address at preconception, 3 times during pregnancy and 3 times postpartum. During these visits, blood was collected, information on medication recorded and RA disease activity (DAS28) calculated by examining 28 joints using 2 variables for each joint: number of swollen joints, number of tender joints, and measuring serum C-reactive protein (CRP) level[10,11]. Medical records were used to confirm gestational age. Birth weight was expressed as birth weight standard deviation scores (SDS), correcting for gestational age and gender[2 12].

After participating in the PARA-study, the parents of all children (n=255) that were born in the PARA-study were contacted by mail. When the children were at least 5 years of age they were invited to visit the Sophia Children's Hospital, Erasmus MC, Rotterdam. During this visit a questionnaire was completed by the parents e.g. to determine food intake and physical activity of the child. Blood samples were drawn from the children, anthropometric measurements were performed and the BMD (in grams/cm^2) was measured by a DXA-scan (Dual-Energy X-ray Absorptiometry scan, type Lunar-Prodigy; GE Healthcare, Chalfont St. Giles, UK). All scans were made with the same machine, and quality assurance was performed daily. The coefficient of variation was 0.5% for total body BMD (BMD_{TB}) and 1.0% for lumbar spine BMD (BMD_{LS})[13-15]. To adjust for differences in bone size, a BMAD (Bone Mineral Adjusted Density; grams/cm^2) was assed. The $BMAD_{LS}$ was calculated by the model BMD_{LS}/ [4/(π / width)][16], with the width as the mean width of the second to fourth lumbar vertebral body. All the BMD values were transformed into SDS for sex and chronological age, according to Dutch reference values[17,18].

Mothers and children were considered to be vitamin D insufficient when their serum 25-OHD concentration was below 50 nmol/L[9]. The serum 25-OHD concentrations of the children at prepubertal age (5-10 year) were determined. Maternal levels were determined during early pregnancy. We used a 25-OHD 125I Radio Immuno Assay kit by DiaSorin, Stillwater, Minnesota 55082-0285, USA. The intra- and inter-assay coefficients of variation for samples were less than 11.7% and 9.4%. Our study was approved by the Medical Ethics Committee, Erasmus MC (Rotterdam). All parents gave their written informed consent.

Statistical analysis

Descriptive statistics are presented as numbers, percentages and means (SD). One-sample t test was used to compare BMD SDS results with zero (mean value for age and sex matched references). Differences between the study population and the non-participating group were investigated with *t*-tests for continuous variables measured and with χ^2 tests for categorical variables.

First (step 1), univariate analyses were executed between BMD_{TB}SDS or BMD_{LS}SDS and all associated variables known to influence the BMD of the child. This included child-related variables, like calcium intake of the child, physical activity, insufficient 25-OHD, gender, length, weight and BMI and variables related to the pre- and postnatal period, like maternal smoking during pregnancy, maternal insufficient 25-OHD, gestational age, birth weight, breast feeding after 12 weeks and tempo of growth in the first years of life Univariate analyses were also executed between BMD_{TB}SDS or BMD_{LS}SDS and the RA associated variable during pregnancy; maternal prednisone or sulfasalazine use and RA disease activity

Secondly (step 2), multivariate regression models were designed for BMD_{TB}SDS and for BMD_{LS}SDS to assess the effect of the RA associated variables during pregnancy. In this

multivariate regression model, all associated variables in the univariate analysis (step 1) with a p-value<0.2 were used. To test for multicollinearity between the dependent variables, a variance inflation factor (VIF) was calculated[19]. Variables with a VIF>5 were excluded from the model. Once the model was produced the RA associated variables were imputed separately.

Finally (step 3), a multivariate regression model was fitted using backward selection starting with all known associated variables (step 1) and RA associated variables (step 2) with a p-value less than 0.2. Variables with the highest p-value were eliminated from the model until all p-values were less than 0.2. All statistical analyses were performed using STATA software (version 12.0 for Mac; StataCorp LP, Texas, USA). Statistical significance was defined as p< 0.05.

RESULTS

Fifty-five percent (108/196) of the eligible children participated in the study, including 3 twins (Figure 1). Of the 45% (88/196) that did not participate in the study, 15% (13/88) were lost-to-follow-up and 85% (75/88) were unwilling to participate. The main reasons for non-participation was traveling to the hospital (38%) and the fact that parents felt that our investigations were too much of a burden for their child (30%). No significant differences were found for all variables between the study group (n=108) and the non-participating group (n=88) (Supplementary Table; S4).

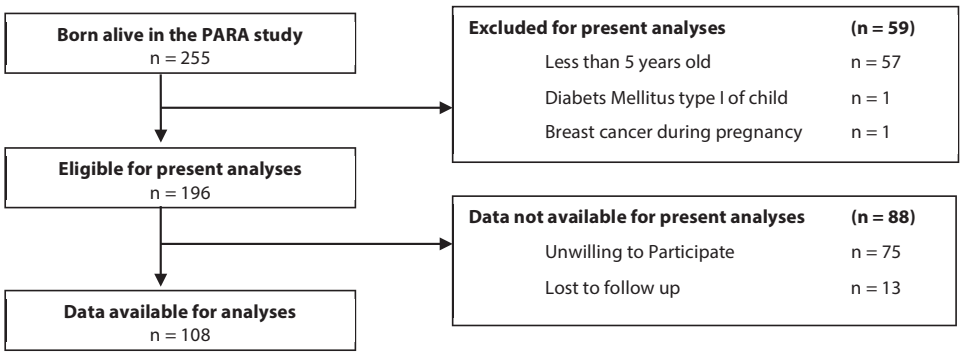

Figure 4.1 - Flowchart of the population studied

In the study group, the mean age of the children was 6.90 years (1.24) and the mean BMI SDS was -0.18 (0.88). The mean BMD_{TB}SDS was -0.11 (0.10), which was comparable to zero and therefore equal to healthy controls. The mean BMD_{LS}SDS was -0.20 (0.99) (not comparable to zero; p=0.04), but within the normal range. The mean BMD_{LS} adjusted for differences in bone size, the mean $BMAD_{LS}$SDS was -0.05 (1.03) (comparable to zero) (Table 4.1).

Table 4.1 - Characteristics of Mother and Child

	Mean (SD)
Clinical Characteristics Mother (n=105)	
age at delivery (years)	32.5 (3.81)
RA duration at delivery (years)	7.49 (6.33)
insufficient 25-OHD (< 50 nmol/L)	22 (20)
DAS28CRP	
first trimester	3.65 (1.19)
third trimester	3.33 (1.18)
Use of Medication during pregnancy	
no medication	43 (41)
only Prednisone	27 (26)
only Sulfasalazine	17 (16)
only Hydroxychloroquine	1 (1)
combination Prednisone Sulfasalazine	16 (15)
combination Sulfasalazine	1 (1)
Clinical Characteristics Child (n=108)	
At Birth	
gender (M/F)	60/48
birth weight (kilograms)	3.37 (0.8)
gestational age (weeks)	39.4 (1.8)
birth weight SDS	0.03 (1.13)
birth length SDS	0.36 (1.24)
target Height SDS	0.88 (0.86)
At 7-years-old	
age (years)	6.90 (1.24)
weight SDS	0.07 (1.70)
height SDS	0.05 (0.98)
BMI SDS	-0.18 (0.88)
BMD_{TB} SDS	-0.11 (0.10)
BMD_{LS} SDS	-0.20 (0.99)
$BMAD_{LS}$ SDS	-0.05 (1.03)
milk intake (servings/d)	1.7 (0.83)
physical activity (hrs/wk)	2.44 (1.79)
insufficient 25-OHD (< 50 nmol/L)	21 (20)

All data are expressed as mean (SD) or number (%). Abbreviations: RA, rheumatoid arthritis; DAS28CRP, RA Disease Activity Score in 28 joints with CRP levels; SDS, Standard Deviation Score; BMD, Bone Mineral Density; TB, total body; LS, lumbar spine; BMAD, bone mineral adjusted density; 25-OHD, 25-hydroxyvitamin D

Child-related variables

The mean calcium intake of the child, expressed in daily milk intake, was 1.7 servings/day (0.83), the mean physical activity was 2.4 hours/week (1.8). Five children refused to give blood. A total of 20% (21/103) had insufficient serum 25-OHD concentration (lower than 50 nmol/L) (Table 4.1). In the univariate analyses, these variables had no significant effect on the BMD of the child.

All anthropometric measurements of the child, like weight, height and BMI were associated with the BMD_{TB}SDS and BMD_{LS} SDS (Table 4.2).

Pre- and postnatal variables

Mean gestational age was 39.4 weeks and less than 5% (5/105) of the mothers smoked during pregnancy. Both variables had no association with the BMD of the child at 7-years of age. Serum 25-OHD concentration was insufficient in 20% (22/108) of the mothers without any association on the BMD of the offspring. Mean birth weight SDS was 0.03 (1.8) and birth weight SDS was associated with BMD_{TB}SDS (β=0.23, p=0.006) (Table 4.2) and BMD_{LS}SDS (β=0.22, p=0.009) (Table 4.2).

Thirty-four percent of the mother's breast-fed their child for more than 12 weeks. There was no significant association between the duration of breastfeeding and the BMD. Tempo of growth in weight for length in the first year of life had no effect on the BMD at 7 years (Table 4.2).

RA-related variables

Medication was used during pregnancy in 58% (63/108) of the mothers and restricted to prednisone (26%), sulfasalazine (16%), hydroxychloroquine (1%), a combination of prednisone and sulfasalazine (15%) or a combination of hydroxychloroquine and sulfasalazine (1%) (Table 4.1).

The median dose of prednisone was 6.2 mg/day with an interquartile range (IQR) of 1-15mg/day. The median (IQR) dose of sulfasalazine was 2000 (500-4000) mg/day.

There was no significant association between prednisone use during pregnancy and the BMD of the offspring (Table 4.2). Sulfasalazine use had a positive effect on the BMD_{TB}SDS of the child at 7 years (β= 0.50; p= 0.008), but had no effect on BMD_{LS}SDS (Table 4.3). There was no dose-effect present for prednisone or sulfasalazine use on the outcome (data not shown).

The RA disease activity (DAS28) decreased from 3.65 (SD 1.19) in the first to 3.33 (SD 1.18) in the third trimester. DAS28 during pregnancy had no effect on the BMD of the child (Table 4.2).

Table 4.2 - Univariate analyses- Effect of the variables on the BMD_{TB} and BMD_{LS} (step 1)

	BMD_{TB}SDS		BMD_{LS}SDS	
	β	**p-value**	**β**	**p-value**
Child-related variables				
Calcium intake (servings/day)	0.17	**0.14**	0.13	0.27
Physical activity (hours/week)	0.04	0.46	-0.07	0.22
Insufficient 25-OHD (child)*	-0.08	0.74	-0.04	0.88
Gender (m/f)*	0.03	0.88	-0.26	**0.18**
Weight (kg)	0.04	<0.01†	0.05	<0.01**†**
Height (cm)	0.03	**<0.01**	0.03	**<0.01**
BMI (sds)	0.26	**0.02**	0.38	**<0.01**
Pre- and postnatal variables				
Maternal smoking (y/n)*	-0.03	0.59	-0.01	0.90
Insufficient 25-OHD (mother)*	-0.33	**0.17**	-0.23	0.34
Gestational age (wks)	0.02	0.70	0.06	0.27
Birth weight (sds)	0.23	**<0.01**	0.22	**<0.01**
Breastfeeding (y/n)*	-0.25	**0.13**	-0.02	0.88
Tempo height	0.03	0.74	0.04	0.71
Tempo weight	-0.04	0.46	0.02	0.78
RA-related variables				
Prednisone*	0.28	0.15	-0.10	0.61
Sulfasalazine*	0.55	<0.01	0.33	0.11
DAS28- Trimester 1	0.02	0.83	-0.05	0.52
DAS28- Trimester 3	-0.02	0.76	-.010	0.25

*variable analysed categorical; **†**variable was excluded from analysis VIF>10; Abbreviations: SDS, Standard Deviation Score; BMD, Bone Mineral Density; $_{TB}$, total body; $_{LS}$, lumbar spine; BMI, body mass index; Insufficient 25-OHD, 25-hydroxyvitamin D; DAS28, RA disease activity score in 28 joints with CRP levels. All **bold** variables were used for multivariate analyses for the BMD mentioned (step2)

Multivariate regression model

For the multivariate model with BMD_{TB}SDS as dependent variable, the following variables were included based on their p-value ($p<0.2$) in the univariate analysis (step1); calcium intake, height of the child, BMI, birth weight SDS, mode of feeding and insufficient maternal 25-OHD level. Weight of the child was excluded from analysis due to a VIF of 33.17. When the RA variables were imputed separately (step 2), only maternal sulfasalazine had a p-value less than 0.2 and was therefore used in the final BMD_{TB} model (step 3). This final model showed a significant association between BMD_{TB}SDS and the height of the child ($\beta=0.02$; $p=0.043$) and sulfasalazine use ($\beta = 0.53$; $p=0.005$) (Table 4.4). This indicates that an increase in height of 10 cm or maternal sulfasalazine use results in a higher BMD_{TB} of 0.2 or 0.5 SDS, respectively.

Table 4.3 - Multi-variate analyses-Effect of RA-related variables on the BMD_{TB} and BMD_{LS} (step 2)

	$BMD_{TB}SDS$ [a]		$BMD_{LS}SDS$ [b]	
	β	**p-value**	**β**	**p-value**
RA-related variables				
Prednisone*	0.24	0.20	0.01	0.96
Sulfasalazine*	0.50	<0.01	0.29	0.12
DAS28- Trimester 1	0.03	0.72	-0.03	0.73
DAS28- Trimester 3	0.01	0.89	-0.03	0.68

*variable analysed categorical; [a] all adjusted for calcium intake; height, body mass index birth weight, breast feeding and 25-OHD (mother); [b] all adjusted for gender, height, BMI and birth weight; Abbreviations: SDS, Standard Deviation Score; BMD, Bone Mineral Density; TB, total body; LS, lumbar spine; DAS28, RA Disease Activity Score in 28 joints with CRP levels.

For the multivariate $BMD_{LS}SDS$ model, the following variables were included based on the univariate analysis; gender of the child, height, BMI, birth weight SDS. Weight of the child was excluded from analysis due to a VIF of 32.4. When the RA variables were entered, only sulfasalazine was used in the final BMD_{LS}model. This final model showed a significant association between $BMD_{LS}SDS$ and height (β=0.03; p=0.013) and BMI SDS of the child (β=0.25; p=0.026) (Table 4.4). In the final $BMD_{LS}SDS$ model, the association with maternal sulfasalazine was not significant.

Table 4.4 - Multivariate analyses-Association on BMD_{TB} and BMD_{LS} using backward selection (step 3)

	$BMD_{TB}SDS$		$BMD_{LS}SDS$	
Variables	**β**	**p-value**	**β**	**p-value**
Sulfasalazine*	0.53	<0.01	0.29	0.13
Calcium	0.19	0.07		
Gender (m/f)*			-	-
Height (cm)	0.02	0.04	0.03	0.01
BMI (sds)	-	-	0.25	0.03
Birth weight (sds)	0.14	0.10	-	-
Breastfeeding (y/n)*	-0.28	0.07		
Insufficient 25-OHD	-0.29	0.18		

*variable analysed categorical; Abbreviations: SDS, Standard Deviation Score; BMD, Bone Mineral Density; TB, total body; LS, lumbar spine; BMI, body mass index; 25-OHD, 25-hydroxyvitamin D; -, variable not included in model (limit for inclusion p<0.2).

DISCUSSION

Our study indicated that prednisone use or elevated RA disease activity during pregnancy in women with RA had no influence on the total body or lumbar spine bone density of their 7-year-old offspring. Furthermore, sulfasalazine had no negative influence on the bone mineral density and even showed a positive association of 0.5 SDS with the total body bone density of the child. Our hypothesis that maternal medication or RA disease activity during pregnancy could be negatively associated with the current BMD of the 7-year-old offspring is therefore refuted.

Prednisone creates bone loss by affecting the precursors of osteoblasts and osteoclasts and by lowering serum calcium and vitamin D levels through decreasing intestinal absorption of calcium[3,20]. Prednisone does not pass the placenta, but the placenta is not yet fully developed at 12 weeks of pregnancy, so in this stage the fetus may be exposed. During this early period of embryonic development the skeleton begins as a cartilaginous scaffold, followed by the primary ossification between 8th and 12th weeks. Most of the bone mineralization is completed during the third trimester of pregnancy[21]. In previous studies we already showed that prednisone shortens the gestational age, but no influence was found on the pre- or postnatal growth of the child (see also Chapter 3)[22]. With current findings, we cannot rule out that maternal prednisone use might influence this prenatal process of embryonic development. We do conclude that maternal prednisone use has no effect on the BMD of the 7-year old offspring.

It has been reported that sulfasalazine use correlates with higher BMD, however it was unknown whether maternal sulfasalazine use during gestation influences the BMD of the offspring[1]. Sulfasalazine passes the placenta and is present in breast milk. The ratio of maternal to cord plasma (or breast milk) for most sulfasalazine metabolites is approximately 1:1-1:2[23]. Therefore, an infant may be exposed to sulfasalazine for a long pre- and postnatal period. A large nationwide study found no increase in congenital malformations when pregnant women were using sulfasalazine[24]. It is in line with literature that sulfasalazine has a positive influence on BMD, but until now it was unknown that maternal use has also an effect on the BMD of the 7-year-old offspring.

In this study the focus of the BMD measurement was on two different bone sites, total body (BMD_{TB}) and lumbar spine (BMD_{LS}). Total body is mainly composed of cortical bone and when mechanical stress is increased, cortical bone increases in size, but not in density. Any effect during pre- and postnatal period will probably be on the cortical bone and not the trabecular bone. This is comparable to our results because pre- and postnatal variables, including sulfasalazine use during pregnancy, seem to affect the BMD_{TB} more than the BMD_{LS}. Lumbar spine is composed of trabecular bone and this will increase in density when mechanical stress like weight and physical activity is enhanced[25,26]. Our results also support this hypothesis based on the stronger association found between height, weight and BMI with BMD_{LS} than BMD_{TB} outcomes [5,6].

Our previous study did show that elevated RA disease during pregnancy is associated with a lower birth weight and with rapid catch-up in weight for length during the first year of life [2,27]. Both have been related to an unfavorable cardiovascular and metabolic profile in adults. We therefore want to emphasize that elevated RA disease activity should be avoided during pregnancy highlighting the important role for medication like prednisone and sulfasalazine in reaching this goal.

Finally, our study had some limitations as only 55% of all eligible children participated in the study. Although there was no statistical difference between the participating and non-participating group, there could be a bias due to missing data..

In conclusion, our study shows that neither medication nor elevated RA disease activity during pregnancy is associated with a lower whole body or lumbar spine BMD in 7-year-old offspring. The aim of the study was reached because our study shows that maternal medication use has no effect on the bone density of the 7-year old offspring.

Supplementary Table S4 - Characteristics of the population studied and those lost-to-follow-up

	study population (n=105)	lost-to-follow-up (n=88)*	
	mean (SD)	**mean (SD)**	**p-value**
Clinical Characteristics Mother			
age at delivery (years)	32.6 (3.81)	32.3 (3.55)[a]	0.63
RA duration at delivery (years)	7.49 (6.33)[b]	6.29 (6.08)[c]	0.19
Disease activity during pregnancy			
first trimester	3.65 (1.19)[d]	3.70 (1.03)[e]	0.77
third trimester	3.33 (1.18)	3.35(1.04)[f]	0.88
Use of Medication during pregnancy	**n (%)**	**n (%)**	**p-value**
no medication	44 (42)	41 (48)[g]	
prednisone	26 (25)	24 (28)[g]	0.33
only Sulfasalazine	17 (16)	14(16)[g]	
combination of two	18 (17)	7 (8)[g]	
Clinical Characteristics Child	**(n=108)**	**(n=88)**	**p-value**
birth weight (kilograms)	3.422	3.255[a]	0.06
gestational age (weeks)	39.5	39.1[a]	0.22
birth weight SDS	0.05	-0.20[a]	0.11
gender (M/F)	57/48	51/34[a]	0.43
Mode of feeding 12 weeks post-partum	**%**	**%**	**p-value**
only breast	34 (36/105)	40 (34/85)	0.50
only formula	61 (64/105)	53 (45/85)	
both	5 (5/105)	7 (6/85)	

All data are expressed as mean (SD) or percentage * lost-to-follow-up (n=13) + unwilling to participate (n=75) (see also figure 1; flowchart); [a] n =85; [b] n =104; [c] n=84; [d] n= 89; [e] n=71; [f] n=82; [g] n=86. Abbreviations: RA, rheumatoid arthritis; DAS28, RA Disease Activity Score in 28 joints with CRP levels; SDS, Standard Deviation Score

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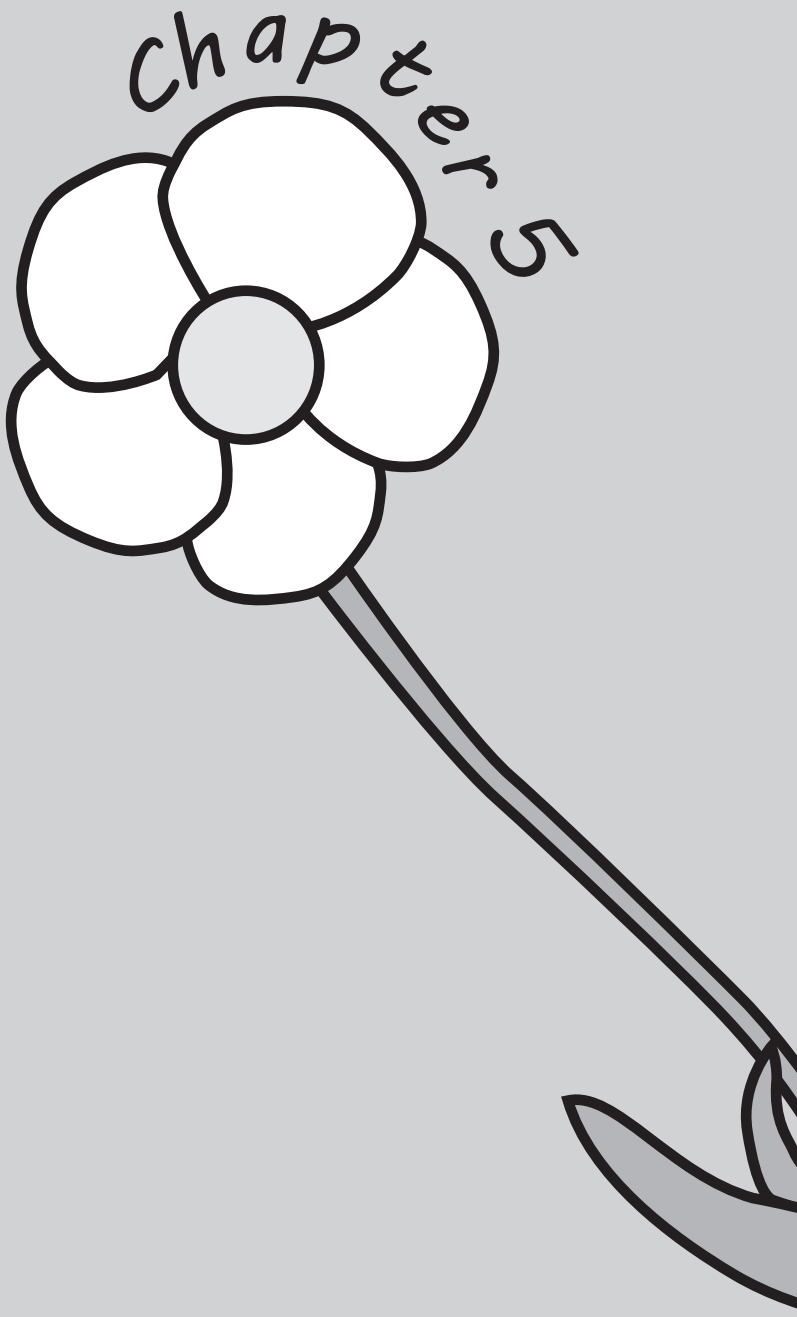
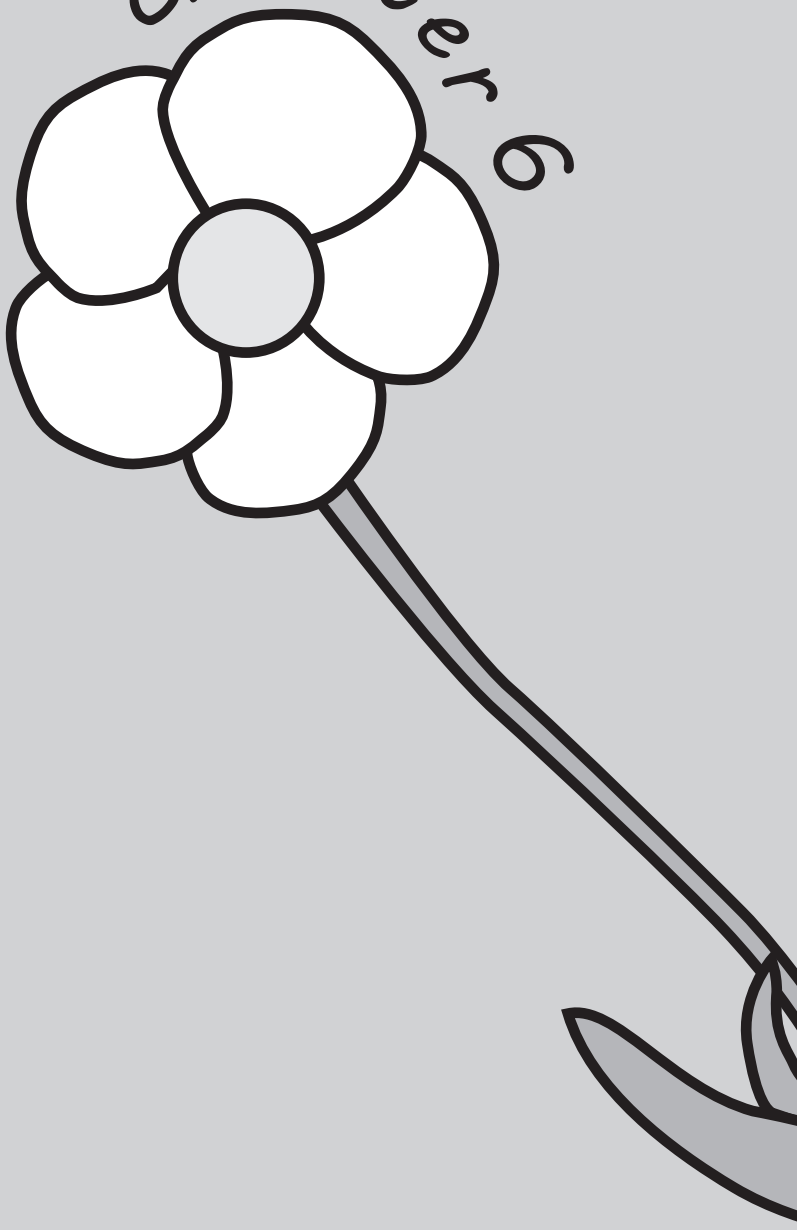

Chapter 5

Does prednisone use or disease activity in pregnant women with rheumatoid arthritis influence body composition of their offspring?

F.D.O. de Steenwinkel
R.J.E.M. Dolhain
J.M.W. Hazes
A.C.S. Hokken-Koelega

Submitted

Chapter 6



Does prednisone use during pregnancy induce insulin resistance in the offspring?

F.D.O. de Steenwinkel
R.J.E.M. Dolhain
J.M.W. Hazes
A.C.S. Hokken-Koelega

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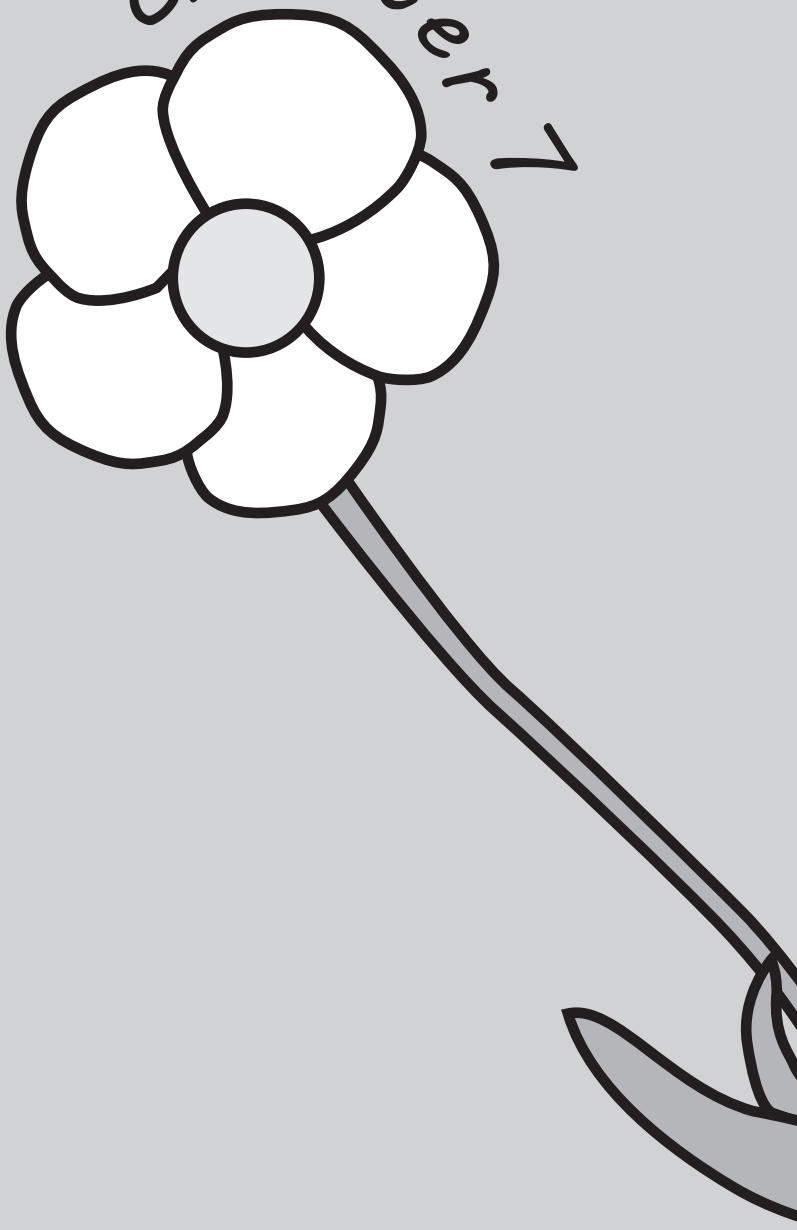
Chapter 7



The influence of fetal prednisone exposure on the cortisol levels in the offspring.

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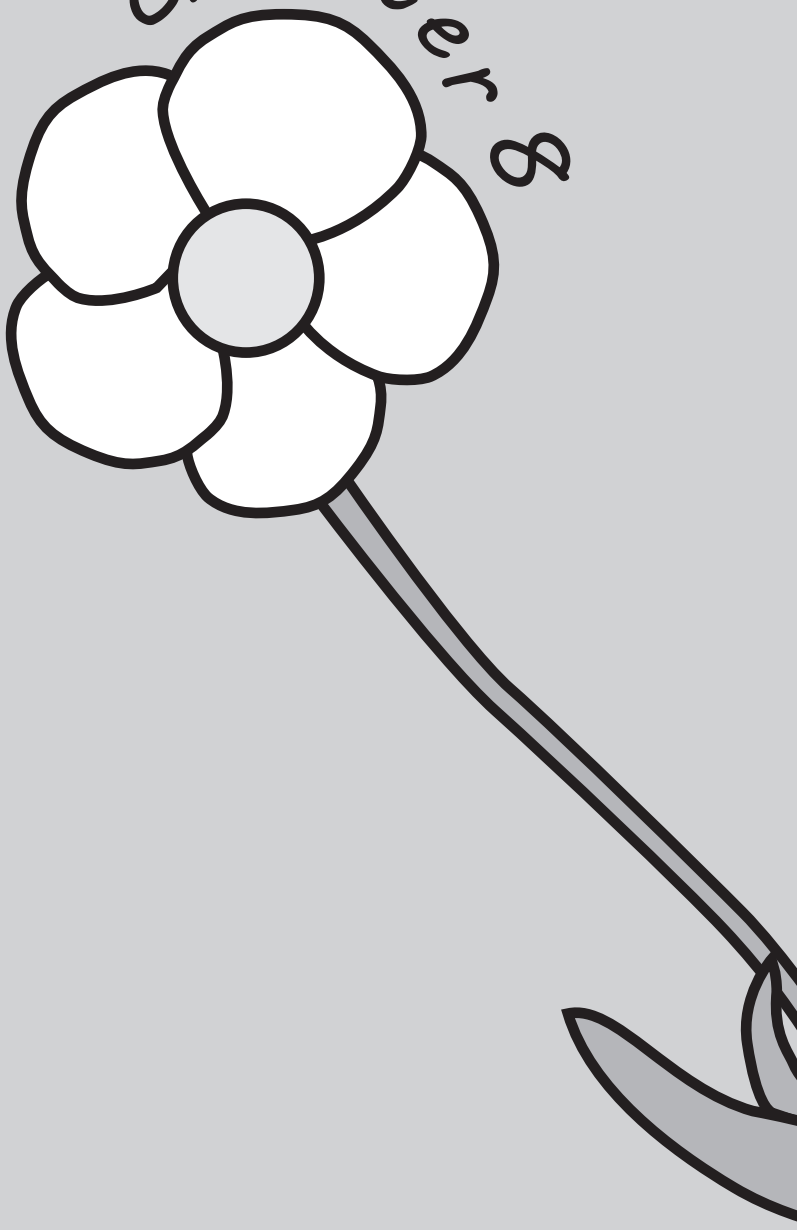
Chapter 8




General discussion

In this thesis, we describe findings in a large group of children aged 5-10 years. The mothers of these children were all diagnosed with rheumatoid arthritis (RA) and were prospectively followed during and after their pregnancy. This thesis presents RA associated variables during pregnancy, mainly RA disease activity and RA medication, and the associations with determinants of adult health and diseases in the offspring. Determinants of adult health being present at an early age include body composition, lipids, blood pressure, cortisol levels and insulin resistance. These determinants influence the risk on diseases in adulthood, like cardiovascular diseases (CVD) or type 2 diabetes mellitus (T2DM)[1-4].

In the present chapter each paragraph covers a different period in early life. Beginning with the fetal period (I), followed by the postnatal period during the first year (II), and finally the prepubertal period from 5-10 years (III) (Figure 8.1). The main findings concerning the determinants of adult health are described in the beginning of each paragraph and discussed at the end of the paragraph. Recent literature and clinical implications will be discussed where possible. At the end of this chapter, an overall interpretation of the results and direction for future research are given. Finally, a recommendation for treatment of pregnant women with RA will be specified.

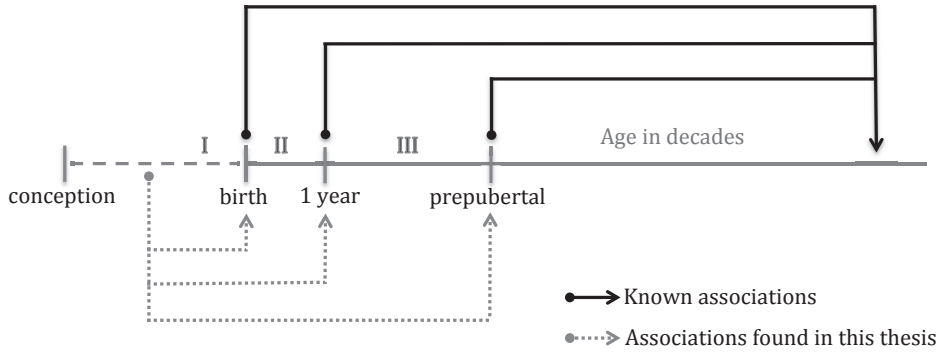


Figure 8.1 - Associations found between RA variables during pregnancy, and early risk factors of diseases in adulthood. These risk factors can be present during fetal period (I), first year of life (II), or at prepubertal age (III).

FETAL PERIOD (I)

Circulating cytokines

The fetal period is one of, if not the most, critical phase in life of every human being. Many factors can influence the fetal growth of the child, like maternal malnutrition, smoking, alcohol abuse, maternal weight, gestational weight gain and maternal hypertension[5-8]. Birth weight is a proxy for the fetal growth and function of the placenta.

Birth weight was transformed into a birth weight standard deviation score (birth weight SDS), which corrects the birth weight for gestational age and gender[9].

Elevated RA disease activity during pregnancy has been shown to negatively influence the birth weight SDS of the child[10]. The mechanism of how a systemic disease, like RA, in which mainly synovial tissues are targeted, affects the growth of the fetus is unknown. Due to the fact that elevated RA disease activity is often accompanied by elevated cytokines, we hypothesized that circulating cytokines affect fetal growth. By assessing two pro-inflammatory cytokines, TNFα and IL-6 and one anti-inflammatory cytokine, IL-10, we wanted to explain how elevated RA disease activity during pregnancy is associated with low birth weight SDS of the child[10].

Based on animal studies we postulated that elevated pro-inflammatory cytokines in pregnant women with RA will negatively influence the fetal growth, and elevated anti-inflammatory cytokines will positively influence the fetal growth[11,12].

Maternal cytokine levels were measured at pre-conception and during the first and third trimesters. We also genotyped certain functional Single-Nucleotide Polymorphisms (SNPs), which are known to influence the concentration levels of IL-10, IL-6 and TNFα. The genotyping of these functional SNPs was executed to explore the possibility to predict birth weight based on pre-conception cytokine levels or functional variations in the relevant cytokine genes.

We observed that in pregnant women with RA, IL-10 and IL-6 levels influence the fetal growth of the child. During the first trimester, high anti-inflammatory IL-10 levels are associated with higher birth weight SDS, and high pro-inflammatory IL-6 levels are associated with lower birth weight SDS[13]. This association remained present after correction for RA disease activity, maternal age, smoking, prednisone or sulfasalazine use during pregnancy.

Furthermore, the association was only present in the first trimester and not in the third or at preconception. High levels of pro-inflammatory TNFα had no effect on birth weight SDS. Furthermore, the circulating cytokines levels of IL-10, IL-6 and TNFα were not related with the corresponding SNPs.

Discussion and clinical implication

Our finding, that an elevated level of IL-10 is associated with higher birth weight SDS of the child is consistent with animal studies. IL-10 knockout mice showed more adverse pregnancy outcome than wild-type mice[12]. We also found that elevated IL-10 levels positively influenced birth weight independent of RA disease activity. This suggests a protective role for IL-10 during pregnancy concerning the fetal growth of the child and also supports the idea that IL-10 participates in the regulation of trophoblasts during human pregnancy, creating a more vital placenta[14,15].

That elevated IL-6 levels were found in association with lower birth weight SDS is also in line with animal studies, which have highlighted that elevated pro-inflammatory

cytokines, such as IL-6 negatively influence the function and growth of the placenta[11,13]. Additionally, they can have adverse effects on the growth and development of cytotrophoblasts, which are crucial for placentation[14]. Our results partly support this conclusion, because the elevated IL-6 levels had a negative influence on birth weight and the association was only found in the first trimester of pregnancy, in which the placentation occurs. No association was found with TNFα.

Circulating cytokine levels at pre-conception or during the third trimester did not associate with birth weight SDS. One of the explanations could be that during the third trimester, the cytokine levels are not only determined by the RA disease activity, but also by other mechanisms. Nevertheless, it does highlight the importance of cytokines throughout the first trimester of pregnancy in which placentation occurs.

In our study, cytokine levels were not related with the functional SNPs. We explored the possibility to predict birth weight based on pre-conception cytokine levels or functional variations in the relevant cytokine genes, but no relation was found.

RA medication, such as prednisone and sulfasalazine may influence the level of circulating cytokines. Questions can be raised concerning new RA therapies, involving the use of biologicals, which are mainly, though not exclusively, therapeutic antibodies targeting pro-inflammatory cytokines, including TNFα and IL-6[16-18]. Therapy with recombinant human IL-10 was not effective in suppressing RA disease activity in non-pregnant RA patients[19]. Nevertheless, recombinant human IL-10 is very effective in suppressing Crohn's disease activity[20]. Biologicals targeting IL-6, like Tocilizumab, are not yet considered safe during pregnancy. Therefore, at this moment, it is clinically not possible to rebalance IL-10 and IL-6 levels in pregnant women. Perhaps anti-TNF medication can create a rebalance in circulating cytokines. TNF-blockers have been categorized as “group B” by the Food and Drug Administration (FDA), meaning that animal reproduction studies have failed to demonstrate a risk to the fetus, but no adequate and well-controlled studies in pregnant women have been done. This implicates that no firm conclusions can be drawn about the safety of anti-TNF therapy during pregnancy[21].

Our findings indicate that rheumatologists should be aware that elevated cytokine levels could influence the birth weight SDS of the child, which is a proxy for the fetal growth. Nevertheless, measuring cytokines throughout the pregnancy or even before conception is not implicated. Adequate RA therapy remains crucial during pregnancy, not to diminish RA disease activity and to reduce pro-inflammatory cytokine levels.

FIRST YEAR PERIOD (II)

Tempo of postnatal growth

Recent studies showed that rapid postnatal catch-up growth in weight for height is related to unfavorable cardiovascular and metabolic profiles later in life[1,2]. This

association is even stronger when the most prominent increase in weight occurs during the first three months of life, which is called rapid catch-up[1]. Accelerated weight gain during this specific period negatively affects health profile at 21 years[1].

When a child is born with a lower birth weight, catch-up growth in weight and height may occur and the tempo of this catch-up is crucial. Elevated RA disease activity during pregnancy is associated with low birth weight SDS, but no association is present between RA medication and birth weight SDS[10]. We therefore hypothesized that children, who were exposed to elevated RA disease activity *in utero*, will experience more catch-up growth in weight, but no such effect will be present in children who were exposed to RA medication *in utero*.

In our study we found that with every point increase in RA disease activity (DAS28, range 0-10), the odds for rapid catch-up growth in weight increased with factor 1.44. This means that when a mother has high RA disease activity (DAS28 > 5.1) during pregnancy, the child has 7.5 times higher chance of a less favorable health profile in early adulthood than expected.

The growth in weight for height at the age of two years did not differ between the children with high or low disease activity during pregnancy. Although this might appear reassuring, it has been shown that rapid catch-up in weight in the first 3 months of life is often followed by a normal growth pattern during infancy, but nevertheless associated with a less favorable health profile in the future[2]. As hypothesized, children who were exposed to prednisone or sulfasalazine *in utero* had no different growth pattern during the first year than those who were not exposed.

Discussion and clinical implication

We concluded that elevated RA disease activity during pregnancy increased the risk of rapid catch-up in weight in the first year. This is an indication that active RA disease activity during pregnancy might be a risk factor for unfavorable cardiovascular and metabolic profile later in the lives of the offspring. Our study emphasizes the importance of having low RA disease activity, but more importantly our study showed that medication use, including prednisone, during pregnancy had no influence on the tempo of growth. Based on our findings, low maternal disease activity should be pursued during pregnancy. Not only is it beneficial for the pregnant women, it could also result in a healthier postnatal gain in weight in the infant.

Our data shows that clinicians should always consider combination therapy when treatment with only prednisone, sulfasalazine, or hydroxychloroquine is failing. More than half of the mothers in this study used medication during pregnancy, but only one fifth used a combination of medication. In other studies combination therapy is even lower[22]. Continuation, or extension of RA medication will not only improve the maternal health during pregnancy, but this study also shows that it could affect future health of the unborn child.

At such a young age of 5-10 years, the increased risk due to rapid catch-up might be reduced if the child creates a healthy lifestyle. Creating a healthy lifestyle by education and counselling is always important, but perhaps even more important in children with an increased risk due to rapid catch-up in weight.

It is vital that for a child born with a low birth weight SDS, due to the elevated RA disease or any other reason, that the tempo of weight gain is kept moderate, especially during the first 3 months. Adequate monitoring of the weight and height in the post-natal period is crucial[2].

While underlining the importance of our findings we do want to emphasize the relative magnitude of our results. Although we found significant associations between disease activity and the tempo of postnatal weight gain, the growth of the majority of the children was within the normal range. However, our findings may have greater consequences in other countries were RA disease activity is less well controlled. The overall DAS28 in The Netherlands is 3.1, while this is 4.4 in Non-European countries[23].

PREPUBERTAL PERIOD (III)

Growing evidence suggests that 're-programming' of the fetus occurs during early development in response to *in utero* variations in environment. This 're-programming' may be involved in the presence of risk factors at young age, leading to diseases in adulthood[24]. Adverse *in utero* environment variations may be generated by RA disease activity or RA medication during pregnancy. This may then lead to fetal adaptions, which re-program the hormone secretion and metabolism of the fetus[25]. This fetal 're-programming' can be beneficial on the short term, but may increase the risk of several diseases later in life[24,26]. One example is maternal malnutrition, creating low intrauterine supply of nutrients, which could lead to fetal re-programming. Also prolonged fetal exposure to synthetic glucocorticoids is assumed to create an adverse effect on the fetal programming by re-setting the fetal hypothalamic-pituitary-adrenal (HPA) axis[25-30]. Animal studies showed a reduced, negative feedback of the HPA axis when exposed to synthetic glucocorticoids *in utero*. This re-setting of the fetal HPA axis led to elevated glucocorticoid levels and high blood pressure in the offspring at adult age[31].

In the first two paragraphs we investigated the risk during the fetal period and the first year of life to develop a less favorable health profile in adulthood. Obviously, to be certain if children develop diseases in adulthood we have to wait a few more decades. We determined risk factors, which were already present at prepubertal age. We therefore determined the effect of maternal RA disease activity and medication use on the following parameters.

Daytime cortisol levels

Prednisone exposure *in utero* could have long-term consequences for the child, resulting in elevated cortisol levels or early signs of insulin resistance[27-30]. We found that children who were prednisone-exposed *in utero* had a slightly elevated daytime cortisol level compared to the non-exposed and compared to age-specific references[32]. This association was independent of age, gender and RA disease activity. We therefore concluded that prednisone-exposure *in utero* is associated with a higher overall daytime cortisol level at the prepubertal age.

Metabolic syndrome

To assess the risk of adult diseases, like cardiovascular disease (CVD), insulin resistance, or obesity at an early age, several determinants can be measured, like components of the metabolic syndrome (MetS). This is a combination of 5 closely related cardiovascular risk factors which have been adapted for the use in children: abdominal obesity, high triglyceride (TG), low high-density lipoproteine (HDL), high blood pressure and high fasting glucose[3]. When 3 or more components are present the child has MetS. No association was found between elevated RA disease activity or RA medication and increased prevalence of MetS. Also the five components of MetS were not increased when the children were exposed to elevated RA disease activity or prednisone *in utero.*

Abdominal obesity

The total amount and distribution of the fat mass can be assessed using skinfold thicknesses or DXA-scan (Dual-Energy X-ray Absorptiometry). A higher amount of total fat mass during childhood tends to track into adulthood, increasing the risk for obesity and insulin resistance[33-35]. Abdominal distribution of body fat is associated with insulin resistance and dyslipidemia[33]. In our study we found no differences in the total amount or distribution of body fat in the children who were exposed to elevated RA disease activity or prednisone *in utero.*

Insulin resistance

Insulin resistance can be a precursor of the MetS and T2DM and can be evaluated with the Homeostasis Model of Assessment Insulin Resistance (HOMA-IR) and adiponectin concentration insulin resistance [36-40]. Neither elevated RA disease activity, nor RA medication were associated with the prevalence of insulin resistance or adiponectin level in the prepubertal offspring.

Dyslipidemia

High levels of triglycerides, cholesterol and low levels of high-density lipoprotein can all be early indicators for a unfavorable health profile later in life. We found no association between elevated RA disease activity or RA medication and an unfavorable lipid profile in the offspring.

Bone mineral density

Prednisone is notorious for depressing bone mineral density (BMD), but sulfasalazine has been correlated with higher BMD in a male RA population[41]. We found that RA medication does not negatively influence BMD of the offspring, 5-10 years after exposure *in utero.* Maternal prednisone use was not associated with lower BMD and sulfasalazine medication even showed a significantly higher BMD in the offspring.

Discussion and clinical implication

During the prepubertal period, we did not find an association between RA disease activity or RA medication and increased risk for diseases in adulthood. Nonetheless, follow-up studies need to be performed to assess whether these risk factors remain absent in the future.

Children who were prednisone-exposed *in utero* had a slightly elevated daytime cortisol level compared to the children who were not prednisone-exposed and compared to the mean cortisol levels of the age-specific references[32]. Cortisol levels were higher, but within the normal range. The cortisol elevation of the prednisone-exposed children was not associated with higher BMI or blood pressure. The latter is reassuring, but does not exclude possible long-term consequences of persistently higher daytime cortisol levels. Our results warrant follow-up of our study population to evaluate the consequences of higher cortisol levels in adolescence.

The fact that both elevated RA disease activity and prednisone-exposure *in utero* did not negatively influence the health profile of the prepubertal offspring is reassuring for now and does not lead to any clinical implications considering medication use in pregnant women with RA. Nevertheless, it does create concern about the effects of prednisone-exposure *in utero* when higher synthetic glucocorticoids doses are given.

GENERAL DISCUSSION

At the end of this chapter, we discuss the strength and limitation of our study and the overall interpretation of the results concerning RA disease activity and RA treatment during pregnancy. Recommendations for clinicians concerning pregnant women with RA are given as well as recommendations for future research.

Strength and limitations of our study population

The main strength of this study is that all pregnancies were prospectively followed. Medication intake and RA disease activity was followed throughout the entire pregnancy and always assessed by the same research assistants. Furthermore, all data concerning the prepubertal children were measured and collected by one doctor to limit the variation in measurements. Only 55% of all eligible children participated in the

study. Nevertheless, there was no statistical difference in independent variables between the participating (n=108) and non-participating group (n=88).

We conducted a nationwide study, but all children were examined at the Sophia Children's Hospital in Rotterdam. The main reason for unwillingness to participate was the distance to the hospital (38%), or that parents felt that the investigations were too much of a burden for their child (30%). We contacted most of the parents and only 5% (13/255) were lost to follow-up.

Overall interpretation concerning RA disease activity during pregnancy

In this thesis, we showed that the adverse fetal environment, due to elevated RA disease activity, is associated with fetal growth retardation leading to low birth weight SDS[13]. We also showed the association between elevated RA disease activity and rapid catch-up growth in weight in the first year of life. Both phenomena are associated with an increased risk for diseases in adulthood, like CVD and T2DM[2]. Nevertheless, at the age of 5-10 years, there was no association between elevated RA disease activity and the presence the presence of MetS, abdominal obesity, insulin resistance or dyslipidemia. All might be risk factors of diseases in adult life. This can be explained in different ways.

At the age of 5-10 years, additional factors become more important in determining the presence of early risk factors for future disease, like weight, height and body mass index (BMI) of the child. In line with literature, we did find an association between birth weight SDS and height, weight, BMI and lean body mass at the age of 5-10 years[1]. If the cohort would have been larger we might have found an association between RA disease activity and these variables as well.

Another explanation for the fact that no association was found between elevated RA disease activity during pregnancy and early risk factors at 5-10 years could be that the association is only detectable later in life. During puberty, the child will physically change and the body and the body composition will fully develop. Literature shows that an early rapid catch-up in weight is often followed by a normal growth pattern during childhood, but is nevertheless associated with a less favorable health profile in the future[2].

A third explanation could be the non-invasive methods that were chosen in this study to determine risk factors, because of the young age of the children. For example, the preferred method to quantify insulin resistance is hyperinsulinemic euglycemic clamp (HEC) or the intravenous glucose tolerance test (IGTT). We selected a less invasive and therefore more ethically approved approach. In that respect, it is of great importance that all children are seen in the future in a follow-up study until the children have reached adulthood and are preferably tested by HEC or IGTT.

Due to the fact that we found associations between elevated RA disease activity during pregnancy and some risk factors, we would advise to diminish the RA disease activity during pregnancy as much as possible (Figure 8.2). The association between elevated RA disease activity during pregnancy and low birth weight SDS, including

rapid catch-up growth in weight, may have such an impact on the future health of the child that elevated RA disease activity during pregnancy should be avoided. The child will benefit when born with a higher birth weight and less risk to experience rapid catch-up in weight for length in the first year of life. Furthermore, the mother will strongly benefit during pregnancy when the disease activity is lower, which will lead to less morbidity and disability and a better condition after delivery when she has to take care of her child.

Overall interpretation concerning RA treatment during pregnancy

The use of RA medication during pregnancy, and especially prednisone, will always be controversial. In this thesis we only found an association between prednisone use during pregnancy and a higher daytime cortisol level in the prepubertal offspring. Those higher cortisol levels were, however, within the normal range and not accompanied by clinical outcomes, like higher BMI or blood pressure. The latter is reassuring, but does not exclude possible long-term consequences of persistently higher daytime cortisol levels. There were no other associations between prednisone use during pregnancy and the presence of early risk factors on adult disease at this young age, like the presence of MetS, abdominal obesity, insulin resistance or dyslipidemia. It has to be mentioned that the administrated prednisone dose was a relatively low dose of approximately 6.0 mg/day (range 1-15mg).

Some studies demonstrated that pregnant women with RA, who discontinued medication, had unfavorable outcomes in terms of preterm delivery, than women who continued their medications[22]. Late-preterm or early term infants have more medical complications than infants born between 39 and 40 weeks of gestation[42]. Furthermore, low RA disease activity during conception is relevant for a stable, low level of disease activity during pregnancy[43,44]. All these studies support medication use to achieve a healthier pregnancy outcome (Figure 8.2).

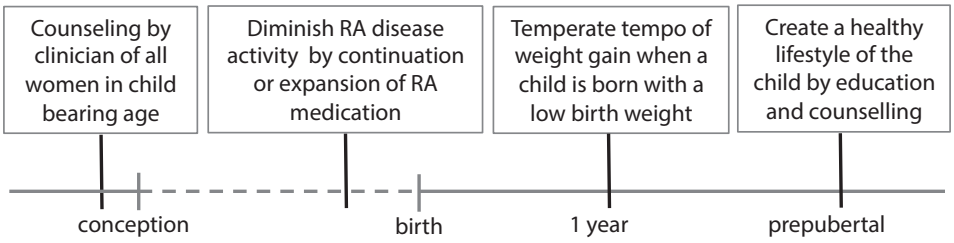


Figure 8.2 – Recommendation for clinicians concerning pregnant women with RA

Besides continuation of RA medication we also recommend counseling and guidance of women with RA who have a pregnancy wish by a clinician (Figure 8.2). The association found between elevated RA disease activity during pregnancy and early risk factors on adult diseases is most prominent in early pregnancy. It is therefore crucial that at preconception, all potential perinatal risk factors, including RA disease activity and RA medication, are evaluated by a clinician and discussed with the patient. Change in medication, but also lifestyle interventions, prior to pregnancy could lead to improved pregnancy outcome[45]. However, as nearly 50% of pregnancies in the United States are unplanned, clinicians, most importantly rheumatologist, should be aware of safe alternatives concerning RA medication in women of childbearing age[46].

None of the mothers in our study population used TNF-blockers, because TNF-blockers were a novelty at the time of this study and not yet prescribed during pregnancy. Only recently, TNF-blockers have been categorized as "group B" by the FDA and are therefore more frequently prescribed during pregnancy to control active RA. TNF-blockers pass the placenta and when given during pregnancy, inoculation of the offspring with live vaccines is contraindicated until the biologic agent is no longer detectable in the child's circulation[47,48]. A case of fatal disseminated mycobacterial infection has been reported in an infant of a mother who was receiving Infliximab for Crohn's disease throughout her pregnancy[47]. Being born healthy and receiving BCG vaccine at 3 months, he died 1.5 months later from disseminated BCG infection.

Still, there is no evidence that TNF-blockers are associated with embryotoxicity, teratogenicity or increased pregnancy loss, compared with pregnancies unexposed to biologics in RA or other diseases, like inflammatory bowel disease (IBD)[49,50]. In case of IBD, a recent systematic review found no association between administration of TNF-α inhibitors and adverse pregnancy outcome, congenital abnormalities, or increased relative risk of infections in the first year of life of the offspring[49]. Their advice was that biologics should be discontinued during pregnancy solely if the IBD is in remission using the same stop criteria as for patients with IBD in general, as uncontrolled activity of IBD may expose the mother and child to a risk greater than those only potentially coming from the use of TNF-α inhibitors[49].

Based on literature, we would advise to use TNF-α inhibitors during pregnancy only when highly necessary, preferably only in the first trimester and always under the supervision of a rheumatologist or other physicians. Future studies concerning large registries of pregnant women using biologials will be necessary before firm conclusions can be drawn.

Recommendations for future research

Not all components of health in the offspring who were exposed to prednisone have been explored. This study did not address the mental and cognitive development of the prednisone-exposed children. Prednisone has been given for many decades and in some studies adverse effects have been described on fetal programming resulting in

impairment of fetal growth[27]. Other studies showed less fetal brain development and neurologic consequences, some still present in the 6 to 10-years-old-offspring[28-30]. It is strongly recommended that the investigators evaluate the psychological development of the children, including reading or attention disorders, when the children of our current study population are seen in a follow-up study. The higher daytime cortisol level found in the *in utero* prednisone-exposed offspring also warrant continued follow-up to evaluate the long-term consequences of higher cortisol levels on the health profile in adolescence and adulthood.

In conclusion, the main findings of our study show that circulating IL-10 and IL-6 influence the birth weight SDS in pregnant women with RA, elevated RA disease activity increases the risk of fast catch-up growth in weight during the first year, and prednisone use increases daytime cortisol levels at prepubertal age (Figure 8.3). More importantly this thesis highlights the fact that many early determinants for increased risk of diseases in adulthood are not present when RA medication is given. The main conclusion of this thesis might therefore be that elevated RA disease activity is more damaging to mother and child than the use of RA medication. Physician should strive to avoid high RA disease activity during pregnancy. Continuation, or extension of RA medication will not only improve the maternal health during and after pregnancy, but it may also affect future health of the unborn child.

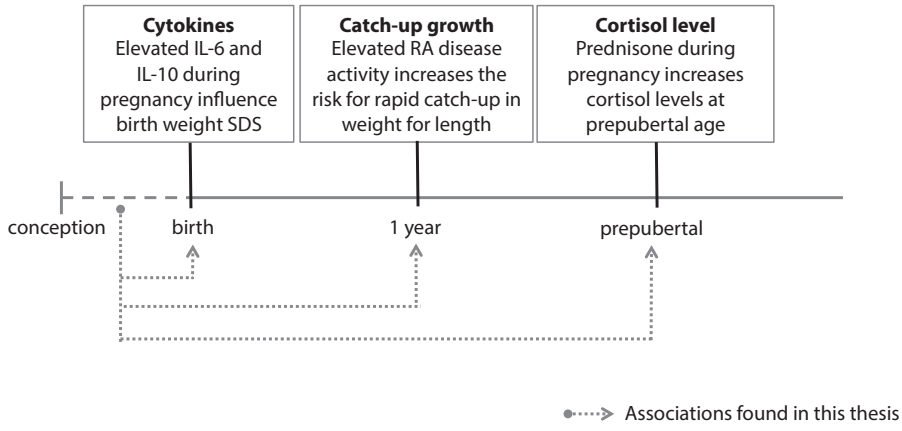


Figure 8.3 – The main associations found in this thesis between maternal RA determinants during pregnancy and early determinants of adult diseases in the offspring.

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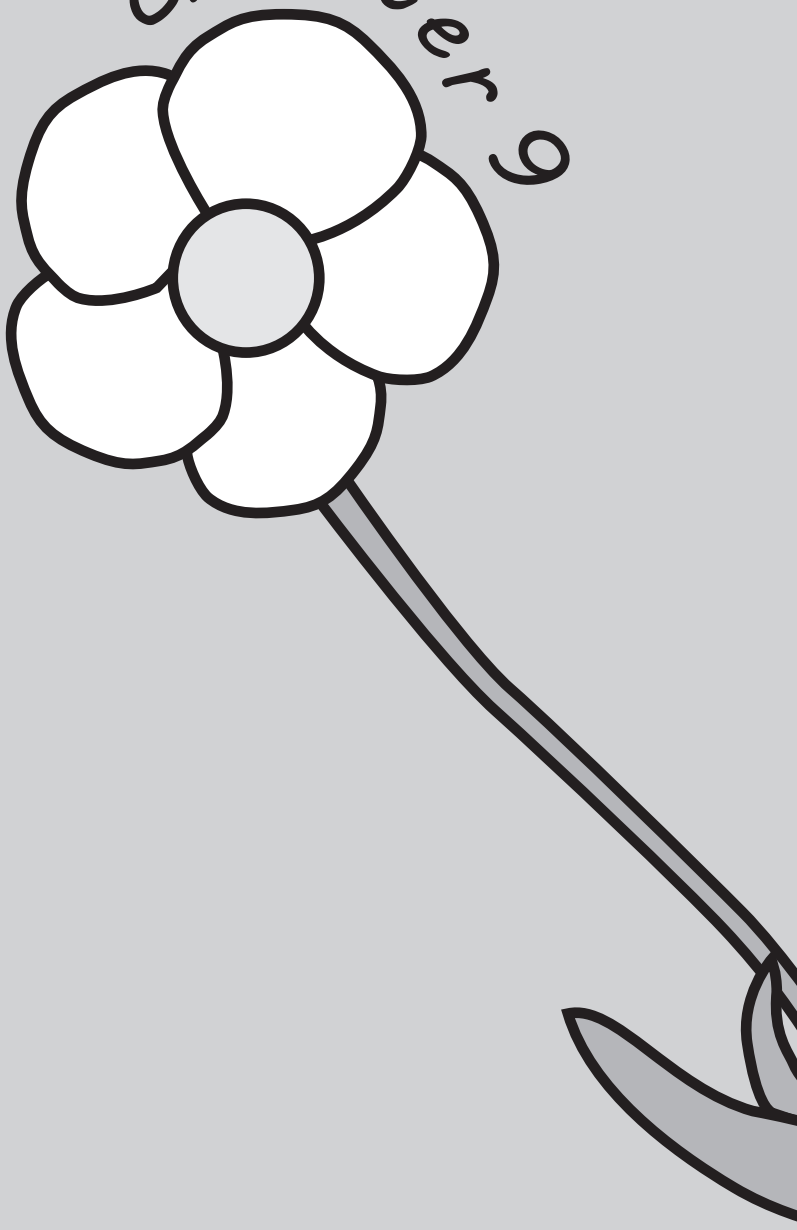
Chapter 9




summary

This doctoral dissertation describes the results found in the FEPRA-study (FEtal Programming in Rheumatoid Arthritis). In this study, detailed description is given on RA associated variables during pregnancy, namely prednisone use and RA disease activity, and the consequences of these variables on the offspring.

Chapter 1

This chapter gives an overview of definitions, prevalence and medical treatment of rheumatoid arthritis (RA). It provides a general introduction on RA during pregnancy and birth outcome. Furthermore, it describes different hypotheses with regard to the influence of birth size and childhood growth on adult diseases and their determinants. The main aim of the study was to assess if RA determinants, like RA disease activity, medication use and the presence of RA autoantibodies, are associated with the prevalence of early life determinants of adult diseases in the offspring. These early life determinants are described during 3 different periods in early life: the fetal period (I) in **Chapter 2**, the postnatal period during the first year (II) in **Chapter 3** and the period before puberty (III) in **Chapter 4-7**.

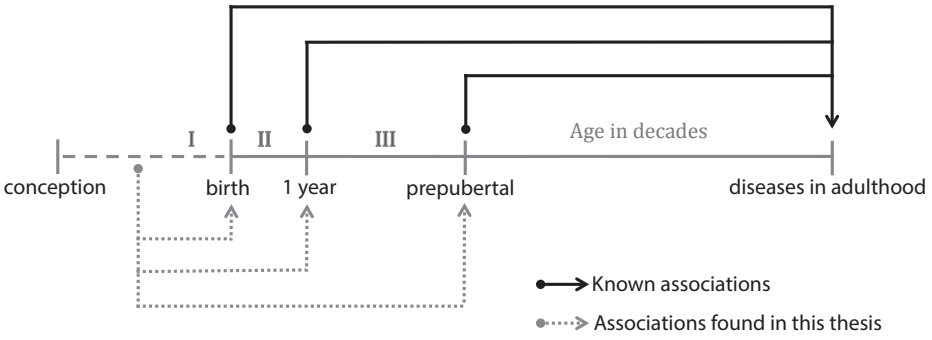


Figure 9.1 - Associations found between RA variables during pregnancy, and early risk factors for diseases in adulthood. These risk factors can be present during fetal period (I), first year of life (II), or at prepubertal age (III).

Chapter 2

This chapter defines the association between elevated serum cytokine levels in the pregnant women with RA and the birth weight of the child. Birth weight is expressed in birth weight standard deviation scores (birth weight SDS), corrected for gestational age and gender. A difference of 0.5 SDS is considered to be of clinical relevance.

During first trimester, maternal serum levels of interleukin-10 (IL-10) and interleukin-6 (IL-6) were associated with fetal growth, independent of the RA disease activity. In the high IL-10 group the birth weight SDS was 0.75 higher compared to the low IL-10 group. In the group of women with high IL-6 the birth weight SDS was 0.50 lower compared to the low IL-6 group.

When analysed simultaneously the effect was even more pronounced, resulting in a birth weight SDS increase of 0.82 for high IL-10 and a decrease of 0.58 for high IL-6. No such correlation was seen with tumor necrosis factor-alpha (TNFα) or in third trimester of pregnancy.

Chapter 3

Previous studies in children born to healthy mothers have shown that rapid catch-up in weight during the first 3 months after birth is related to unfavorable cardiovascular and metabolic profiles later in life. To investigate the effect of RA during pregnancy on the future development of the child we analyzed the tempo of this postnatal weight.

We found that elevated RA disease activity during pregnancy is associated with rapid catch-up in weight independent of medication or the presence of RA associated antibodies. Therefore, elevated RA disease activity should be avoided during pregnancy because it could be a risk factor for unfavorable cardiovascular and metabolic profile later in life.

Medication during pregnancy, including prednisone, had no effect on growth. From this it can be concluded that continuation or extension of medication aimed at acquiring low maternal disease activity during pregnancy, will not only improve maternal health during pregnancy, but may also affect the future health of the unborn child.

Chapter 4

In this chapter the associations between RA medication and RA disease activity of the mother during pregnancy and the bone mineral density (BMD) of the child is described. Prednisone is notorious for depressing the BMD of the patient, but sulfasalazine has been correlated with higher BMD in a male RA population.

We concluded that neither medication use nor high RA disease activity during pregnancy is associated with a decreased BMD of the 7-year-old offspring. The maternal benefit of RA medication during pregnancy outweighs the effect on the bone mineral density in the offspring.

Chapter 5

To investigate whether prednisone or elevated RA disease activity in pregnant women with RA were associated with the presence of early determinants for cardiovascular diseases (CVD) or type 2 diabetes mellitus (T2DM) we assessed the body composition of the child and the components of the metabolic syndrome (MetS) adjusted for children.

MetS is a combination of 5 closely related cardiovascular risk factors: abdominal obesity, high triglyceride (TG), low high-density lipoproteïne (HDL), high blood pressure and high fasting glucose.

The body composition of the child was assessed by anthropometric measurement and by a dual-energy X-ray absorptiometry-scan (DXA), showing the total amount and distribution of lean body mass and fat mass

Our study shows that children born from mother with RA have no elevated risk on early determinants for CVD and T2DM based on the presence of MetS. Furthermore, we concluded that prednisone and RA disease activity in pregnant women with RA had no influence on the body composition of their 7-years-old offspring.

Chapter 6

In this chapter we focused on whether prednisone use in pregnant women with RA is associated with an increased prevalence of insulin resistance of their prepubertal offspring. The difference in insulin resistance between the exposed and the non-exposed was based on the fat distribution of the child, fasting blood samples including the Homeostasis Model of Assessment Insulin Resistance (HOMA-IR) and adiponectin levels. HOMA-IR is a method used to quantify insulin resistance using fasting levels of glucose and insulin. Healthy children have a HOMA-IR of 1.

We concluded that children who were exposed to prednisone in utero had no increase in insulin resistance compared to the non-exposed children. Furthermore, the prednisone-exposed children had similar fat distribution and similar adiponectin levels.

Chapter 7

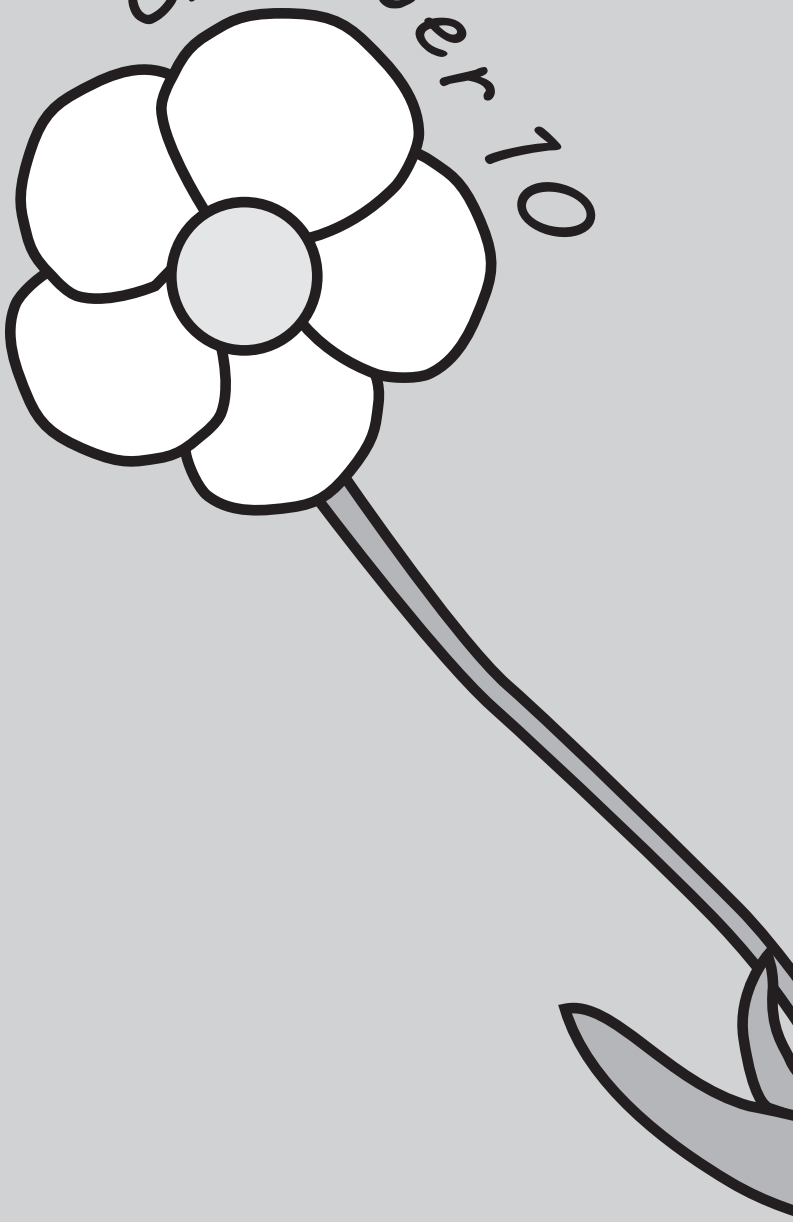
To assess whether fetal prednisone exposure influences the HPA axis activity of the offspring (5-10 years)multiple salivary cortisol samples were taken during the day: at awakening, 30 minutes after awakening, afternoon, bedtime. Children who were exposed to prednisone *in utero* had higher daytime cortisol levels compared to the non-exposed children. The difference was small and not associated with a higher body mass index or blood pressure of the children. Therefore, these findings will not lead to revision of therapeutic treatment of pregnant women with RA who need prednisone.

Still, it does create concern about prednisone exposure *in utero,* especially when higher doses of prednisone are given than in current study. Our results warrant further studies concerning higher doses of prednisone use during pregnancy and the long-term effects in the offspring.

Chapter 8

The general discussion is dedicated to the main findings of the FEPRA-study described in this thesis. Conclusions are explained in the light of current literature and clinical implications of the results are given including recommendations for future research.

Chapter 10

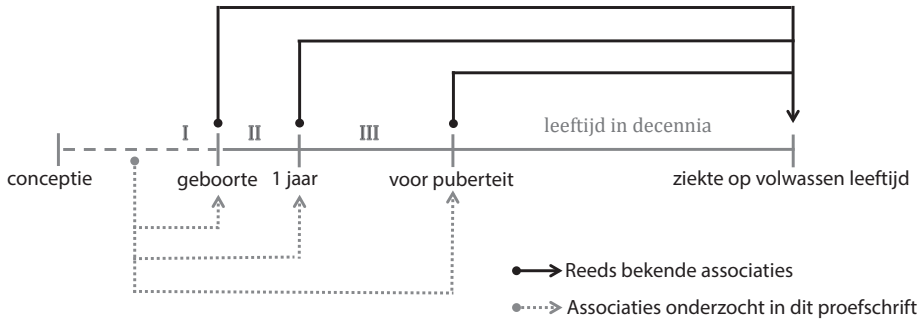



Samenvatting

In dit proefschrift worden de resultaten weergegeven die gevonden zijn in de FEPRA-studie (FEtal Programming in Rheumatoid Arthritis-studie). Het doel van de studie was te onderzoeken of er een associatie is tussen de ziekteactiviteit van reumatoïde artritis (RA) inclusief medicatiegebruik tijdens de zwangerschap en de aanwezigheid van vroege risicofactoren op toekomstige ziektes in het nageslacht (5-10 jaar).

Hoofdstuk 1

Dit hoofdstuk geeft een overzicht van de definitie, prevalentie en behandeling van reumatoïde artritis (RA). Het geeft een algemene introductie van RA tijdens de zwangerschap en de invloed van RA op het ongeboren kind. Verder worden hypotheses besproken waarin verschillende voorspellende factoren op toekomstige ziektes worden uitgelegd. Deze voorspellende factoren kunnen zijn een laag geboortegewicht, de snelheid waarin een kind groeit in het eerste jaar, en de opeenstapeling van vet in het lichaam. Deze voorspellende factoren zijn tijdens verschillende periodes in het leven aanwezig, daarom worden er 3 verschillende periodes in het vroege leven van het kind beschreven: de foetale periode (I) in **hoofdstuk 2**, de postnatale periode (II) in **hoofdstuk 3** en de periode voor de puberteit, (III) in **hoofdstuk 4-7**.



Figuur 10.1 –Associaties onderzocht in dit proefschrift tussen RA tijdens de zwangerschap en de reeds bekende risicofactoren op toekomstige ziektes Deze risicofactoren kunnen aanwezig zijn tijdens de foetale periode (I), het eerste levensjaar (II) of vlak voor de puberteit (III).

Hoofdstuk 2

Dit hoofdstuk beschrijft de associatie tussen verhoogde serumconcentraties van cytokines in de zwangere vrouwen met RA en het geboortegewicht van het kind. Geboortegewicht is uitgedrukt in geboortegewicht standaard deviatie score (SDS). Dit betekent dat het gewicht van het kind is gecorrigeerd voor de duur van de

zwangerschap en het geslacht van het kind. In de kliniek wordt een verschil van 0.5 SDS beschouwd als relevant.

De studie laat zien dat een verhoogde serumconcentratie van interleukine-10 (IL-10) en van interleukine-6 (IL-6) tijdens het eerste trimester van de zwangerschap geassocieerd zijn met geboortegewicht van het kind. De gevonden associatie was onafhankelijk van de ziekteactiviteit van de RA. In de groep met hoog IL-10 was het geboortegewicht SDS 0.75 hoger in vergelijking tot de groep met laag IL-10. In de groep met hoog IL-6 was het geboortegewicht SDS 0.50 lager in vergelijk tot de groep met laag IL-6. Wanneer beide cytokines tegelijkertijd in een model werden geanalyseerd was het effect op het geboortewicht zelfs groter. Zo'n gecombineerd model resulteerde in een toename van het geboortegewicht SDS met 0.82 in de groep met hoog IL-10 en een afname van het geboortegewicht SDS met 0.58 voor de groep met hoog IL-6. Er werd geen relatie gevonden tussen de serumconcentratie van tumor-necrosis factor-alpha (TNFα) en het geboortegewicht.

Hoofdstuk 3

Eerdere studies naar kinderen van gezonde vrouwen hebben laten zien dat een snelle inhaalgroei in gewicht tijdens de eerste drie maanden na de bevalling kan leiden tot cardiovasculaire en metabole aandoeningen op volwassen leeftijd. Om het effect van RA tijdens de zwangerschap op de toekomstige ontwikkeling van het kind te analyseren hebben we de inhaalgroei in de eerste drie levensmaanden onderzocht

We vonden dat een verhoogde ziekteactiviteit van de RA tijdens de zwangerschap geassocieerd is met een snelle inhaalgroei in gewicht tijdens de eerste drie maanden. Deze gevonden associatie was onafhankelijk van het gebruik van medicatie of de aanwezigheid van RA geassocieerde antistoffen. Op grond van deze bevinding zou verhoogde RA-ziekteactiviteit vermeden moeten worden tijdens de zwangerschap, omdat het een verhoogd risico zou kunnen geven op cardiovasculaire en metabole aandoening op volwassen leeftijd bij het kind. Medicatie tijdens de zwangerschap, inclusief het gebruik van prednison, vertoonde geen relatie met de snelheid van de groei van het kind.

De conclusie van dit hoofdstuk is dat continueren of uitbreiden van medicatie tijdens de zwangerschap met als doel het verlagen van de ziekteactiviteit van de RA de latere gezondheid van het ongeboren kind positief zou kunnen beïnvloeden.

Hoofdstuk 4

In dit hoofdstuk wordt beschreven hoe het gebruik van medicatie en de ziekteactiviteit van RA tijdens de zwangerschap geassocieerd zijn met de botdichtheid van het

nageslacht in de leeftijd van 5 tot 10 jaar. Een bekende bijwerking van het gebruik van prednison is dat het de botdichtheid van de patiënt vermindert. Ook een verhoogde ziekteactiviteit is geassocieerd met een verminderde botdichtheid in RA-patiënten. Bij sulfasalazinegebruik daarentegen kan een verbetering van de botdichtheid optreden. In hoeverre de ziekteactiviteit van de RA of medicatiegebruik van de moeder tijdens de zwangerschap van invloed is op de botdichtheid van hun kinderen, is niet bekend.

In onze populatie vonden we geen associatie tussen enerzijds prednisongebruik of RA ziekteactiviteit van de moeder tijdens de zwangerschap en anderzijds een vermindering van de botdichtheid van het kind in de leeftijd van 5 tot 10 jaar. Sulfasalazine liet een verbetering zien in de totale botdichtheid van het kind. Gezien het ontbreken van nadelige effecten op de botdichtheid bij het kind, is er geen reden is om, vanuit dit oogpunt, zwangeren met RA de gangbare medicatie voor hun ziekte te onthouden.

Hoofdstuk 5

Om te bepalen of er een verhoogd risico is op cardiovasculair ziektes of type 2 diabetes mellitus werd de vetverdeling van het kind gemeten en werd de eventuele aanwezigheid van het metabolisch syndroom (MetS) onderzocht. Een kind voldoet aan de criteria voor het MetS als van een combinatie van vijf gerelateerde cardiovasculaire risicofactoren er drie aanwezig zijn. Deze vijf risicofactoren zijn: abdominale obesitas, verhoogde triglyceride, verlaagde high-density lipoproteïne, hoge bloeddruk en een verhoogde nuchtere glucose spiegel.

De vetverdeling van een kind kan bepaald worden met behulp van antropometrische metingen, maar kan ook bepaald worden door middel van een dual-energy X-ray absorptiometry-scan, waarbij de totale hoeveelheid vet en de verdeling van het vet kunnen worden bepaald. In dit hoofdstuk zijn beide methodes gebruikt om de invloed van prednisongebruik en die van de RA ziekteactiviteit tijdens de zwangerschap op de lichaamssamenstelling van het nageslacht te onderzoeken.

De conclusie van het onderzoek is dat de aanwezigheid op MetS geen relatie heeft met prednisongebruik of RA ziekteactiviteit tijdens de zwangerschap. Verder is de vetverdeling van de kinderen niet verschillend. Er zijn dus geen vroege aanwijzingen voor cardiovasculair ziektes of type 2 diabetes mellitus aanwezig bij het nageslacht van vrouwen met RA in de leeftijd van 5 tot 10 jaar.

Hoofdstuk 6

In dit hoofdstuk richten we ons op de vraag of prednisongebruik tijdens de zwangerschap door vrouwen met RA, de insulineresistentie van hun kinderen op 5-10 jarige leeftijd beïnvloedt.

Een voorteken van de insulineresistentie is een verlaagde serumconcentratie van adiponectine. Adiponectine is een hormoon dat afgegeven wordt door vetweefsel. De insulineresistentie in een kind kan ook worden bepaald met het Homeostasis Model of Assessment Insulin Resistance (HOMA-IR). Deze index geeft de verhouding weer tussen de nuchtere glucose- en nuchtere insulineserumconcentratie. Gezonden kinderen hebben een HOMA-IR van 1.

Er werd geen verschil gezien in serumconcentratie van adiponectine tussen de kinderen die wel of niet waren blootgesteld aan prednison intra-uterien. Ook was er geen verschil in de HOMA-IR index. Zodoende wordt er in dit hoofdstuk geconcludeerd dat prednisongebruik tijdens de zwangerschap geen verhoogde kans op insulineresistentie geeft bij het nageslacht als de kinderen 5 tot 10 jaar oud zijn.

Hoofdstuk 7

Eerdere studies bij dieren hebben laten zien dat foetale blootstelling aan prednison de activiteit van de hypothalamus–hypofyse–bijnier-as (in het Engels: hypothalamic-pituitary-adrenal-axis; HPA-as) van het nageslacht kan beïnvloeden. Cortisol is één van de eindproducten van deze HPA-as. Cortisol kan gemeten worden in het serum, maar ook in het speeksel.

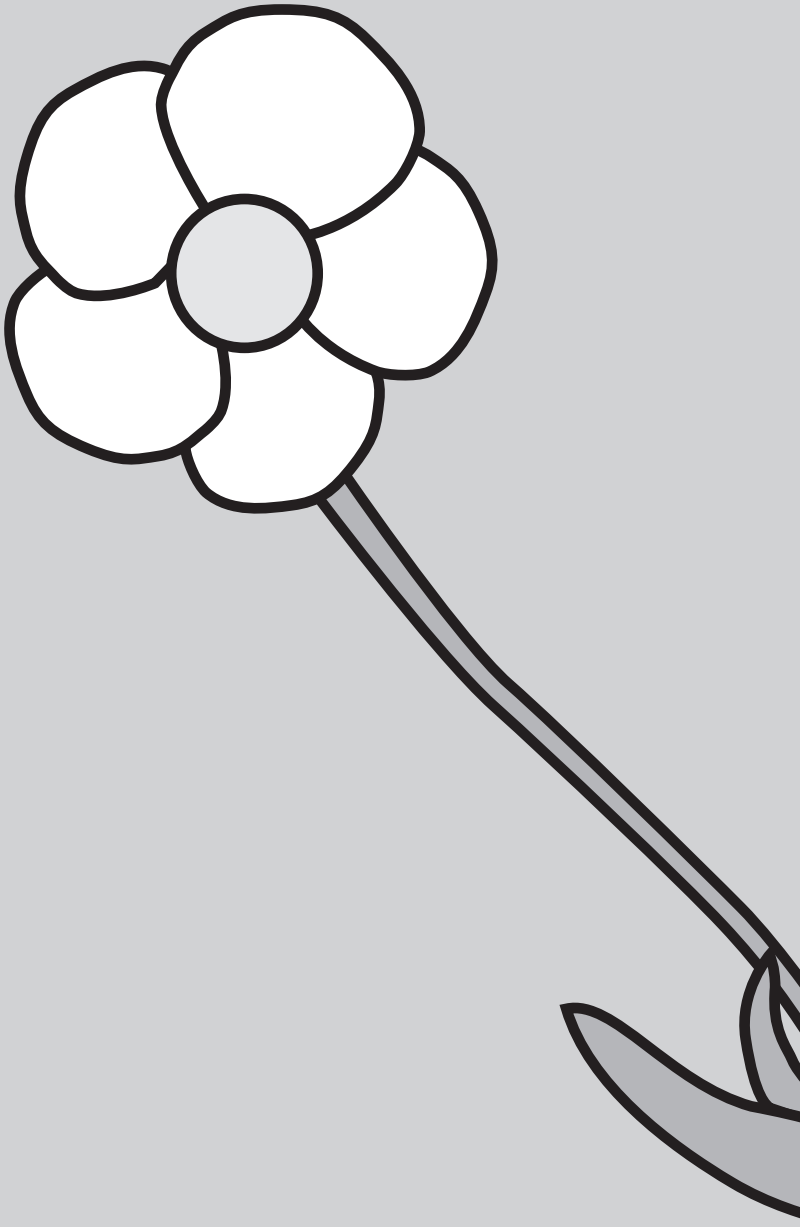
Om de activiteit van de HPA-as in de kinderen van de FEPRA-studie te analyseren zijn er meerdere speekselcortisoltesten gedaan bij de kinderen. Deze afnames waren 4 keer gedurende een dag: bij het wakker worden, 30 minuten na het wakker worden, om 13.00 en vlak voordat de kinderen naar bed gingen.

De kinderen die foetaal waren blootgesteld aan prednison hadden een hogere cortisol dag-spiegel dan de kinderen die niet waren blootgesteld aan prednison. Het verschil in de cortisol waarden was klein. Er waren ook geen klinische kenmerken van verhoogde cortisol aanwezig, zoals een hogere body mass index of een hogere bloeddruk.

De conclusie van dit hoofdstuk is dat op grond van deze studie er geen reden is om zwangere vrouwen het gebruik van prednison te ontraden. Echter, vervolgonderzoek naar de effecten van prednison bij kinderen die hieraan intra-uterien zijn blootgesteld is van belang. Hierbij is het aan te bevelen om ook kinderen te onderzoeken die aan hogere doses prednison zijn blootgesteld dan de kinderen in dit proefschrift.

Hoofdstuk 8

In de discussie worden de belangrijkste bevindingen uit de FEPRA-studie herhaald en vergeleken met andere bevindingen uit de literatuur. Bovendien worden de klinische implicaties van de bevindingen beschreven en worden aanbevelingen voor toekomstig onderzoek in dit vakgebied gedaan.



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LIST OF ABBREVIATIONS

ACPA	Anti-Citrullinated Protein Antibody
AUC	Area Under the Curve
BMD_{TB}	Bone Mineral Density total body
BMD_{LS}	Bone Mineral Density lumbar spine
BMI	Body Mass Index
BP	Blood Pressure
CAR	Cortisol Awakening Response
CRP	C-reactive protein
CVD	Cardiovascular Diseases
DAS28	Disease Activity Score of Rheumatoid Arthritis in 28 joints with CRP levels
DXA	Dual-Energy X-ray Absorptiometry
ECM	Extracellular Matrix
FEPRA	FEtal Programming in Rheumatoid Arthritis
FGIR	Fasting Glucose/Insulin Ratio
FM	Fat Mass
HEC	Hyperinsulinemic Euglycemic Clamp
HDL	High-Density Lipoproteïne
HOMA-IR	Homeostasis Model of Assessment - Insulin Resistance
HPA	Hypothalamic–Pituitary–Adrenal
IL-6	Interleukin-6
IL-10	Interleukin-10
IQR	Inter Quartile Range
LBM	Lean Body Mass
LDL	Low-Density Lipoproteïne
MetS	Metabolic Syndrome
MMP-9	Metalloproteinase-9
OGTT	Oral Glucose Tolerance Test
PARA	Pregnancy-induced Amelioration of Rheumatoid Arthritis
PlGF	Placental Growth Factor
RA	Rheumatoid Arthritis
RF	Rheumatoid Factor
SDS	Standard Deviation Score
T2DM	Type 2 diabetes mellitus
TG	Triglyceride
TH	Target Height
TNFα	Tumor Necrosis Factor-alpha
VEGF	Vascular Endothelial Growth Factor
QUICKI	Quantitative Insulin Sensitivity Check Index
25-OHD	25-hydroxyvitamin D

ABOUT THE AUTHOR

Florentien de Steenwinkel was born on February 27th 1981 in Leidschendam, the Netherlands. After graduating in 2000 at the Maerlant Lyceum in Den Haag, she started pre-medical school at the College of Art, Science and Technology, Chichester, United Kingdom (2001) and received her preparatory instruction in psychology at the University of Amsterdam (2002).

In 2002 she began her medical study at the Erasmus University Rotterdam. She received her doctoral degree in 2006 after she finished her graduate research at the Hospital Central in Maputo, Mozambique. In that same year she started her internships at the University of Amsterdam. She obtained her medical degree in January 2009, upon which she started her residency in Pediatric-Surgery (ANIOS) at the Sophia Children's Hospital, Rotterdam

In September 2009 she started to work at the research project described in this thesis at the department of Rheumatology of the Erasmus MC, Rotterdam under the supervision of Prof. dr. J.M.W. Hazes (Rheumatology), Prof. dr. A.C.S. Hokken-Koelega (Pediatric-Endocrinology) and dr. R.J.E.M. Dolhain (Rheumatology).

In those years many Journal Clubs and Research Meetings followed. During one of those meetings she met Maurits de Rotte, to whom she is now engaged.

In august 2013 Florentien graduated from the Master of Science - Clinical Epidemiology at the Netherlands Institute of Health Sciences (NIHES).

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- Classical Methods for Data-analysis
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- Methods of Public Health Research
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- Pharmaco – epidemiology
- Markers and Prognostic Research

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- Clinical Epidemiology
- Introduction to Clinical Research
- Principles of Epidemiologic Data-Analysis
- Maternal and Child Health
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- Case-control Studies
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Meeting of the European League Against Rheumatism (EULAR), Madrid (poster)
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Meeting of the European League Against Rheumatism (EULAR), Madrid (poster)
Nederlandse Ver. Reumatologie (NVR), Papendal, (poster)
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Publications (this thesis)

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F.D.O. de Steenwinkel, A.C.S. Hokken-Koelega, J.M.W. Hazes, R.J.E.M. Dolhain. Does medication and disease activity during pregnancy in patients with rheumatoid arthritis (RA), influence bone density of the prepubertal offspring? *Arthritis and Rheumatism* 2013

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F.D.O. de Steenwinkel, R.J.E.M. Dolhain, J.M.W. Hazes, A.C.S. Hokken-Koelega. Does prednisone use during pregnancy induce insulin resistance in the offspring? *Submitted*

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Publications (others)

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Een feest is niet compleet zonder mooie, dansende vrouwen. Het is een algemeen feit dat de mooiste meidengroep van het Sophia Kinderziekenhuis, die van de Endo is.

Lieve Annemieke, Elbrich, Gerthe, Judith, Laura, Lidewij, Lin, Manouk, Nienke, Petra, Renske, Sinddie, Yvonne en Suzanne (geen Endo maar zeker zo mooi op de dansvloer), ik ben jullie dankbaar voor het samen dansen, het samen drinken, het samen zeilen, het samen skiën, maar vooral voor het samen delen van zorgen, frustraties en mooie momenten.

De mooiste van meidengroep is zonder enig twijfel dr Renes. Lieve Judith, jij was de lithium van mijn promotieonderzoek, de alcohol van dit feest. Ik ben je dankbaar voor al je steun en vertrouwen and yes Babe, I do love you more than chicken salad.

Waar gedanst wordt, wordt gedronken en dan komen we op dit feest al snel bij de afdeling Reumatologie uit.

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En dan natuurlijk die mensen op het feest die eigenlijk niet te gast zijn, want ze horen er altijd bij. Je houdt zoveel van ze dat het feest zonder hen wordt afgelast of verplaatst. In mijn geval zijn dat mijn ouders, mijn zusje en broertje, mijn Damesch, mijn Biatch, Jacoba's, Takkie en mijn aanstaande.

Lieve ouders, ik ben jullie dankbaar voor het eindeloos geloof in mijn kwaliteiten, voor jullie hoop en steun om al mijn dromen waar te maken, maar boven alles voor jullie onvoorwaardelijke liefde, die ik al mijn hele leven ken. "Ons resten geloof, hoop en liefde, deze drie, maar de grootste daarvan is de liefde."(Korinthiërs 13:13)

Lieve Cathelijne, met weemoed denk ik soms terug aan onze tijd in het Amsterdamse, de Jacob, de Overtooom. Je bent er altijd voor me geweest en ik ben blij met zo'n een fantastische grote zus.

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Lieve Maurits, jij bent het dierbaarste in mijn leven, nooit meer in me leentje. Ik houd zo oneindig veel van je. Samen kunnen we de hele wereld aan, want één plus één is meer dan twee. Mijn leven is een feest door jou en we zijn pas bij de openingsdans.

Et puis seulement quand c'est fini, alors on danse.