# Folic Acid, Dietary Patterns and Perinatal Health
## The Generation R Study

Sarah Timmermans

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In 1895 isoleerde Sir Frederick Hopkins een helder geel kleurpigment uit de vleugels van de luzernevlinder (Colias Hyale; familie Pieridae). Hij noemde het pteridine, naar het Griekse *pteron,* “vleugel”.
Enkele jaren voordat Sir Frederick Hopkins zijn ontdekkingen publiceerde in het wetenschappelijke tijdschrift *“Philosophical Transactions of the Royal Society”,* werd Lucy Wills geboren. Ten tijde van haar geboorte wist nog niemand dat zij later aan de basis van één van de belangrijkste ontdekkingen in de medische geschiedenis zou staan. Het waren namelijk haar bevindingen in 1931 welke lieten zien dat pteridine niet alleen van belang was als geel kleurpigment maar ook, als onderdeel van foliumzuur, bijdroeg aan belangrijke gezondheidseffecten.
Tijdens haar werkzaamheden begin jaren dertig ontdekte Lucy Wills dat gistextract een positieve rol zou kunnen vervullen bij bloedarmoede bij zwangere Hindoestaanse vrouwen. Het bleek dat het een bepaalde voedingsfactor in gist was die voor dit effect verantwoordelijk was. Deze voedingsfactor werd aanvankelijk de *“Wills Factor”* genoemd. In 1941 werd het door Herschel Mitchell voor het eerst geïsoleerd uit spinaziebladen. Hij gaf het de naam waaronder wij het tegenwoordig kennen: foliumzuur (naar het Latijnse *folium,* "blad"), ook wel bekend als 2-NH2-4-OH-*pteridine* P-aminobenzoic acid L-glutamic acid: onder meer naar de pteridine-ring waaruit het bestaat.

**Folic Acid, Dietary Patterns and Perinatal Health**

The Generation R Study

Thesis, Erasmus University Rotterdam, The Netherlands

The first phase of The Generation R Study has been financed by the Erasmus MC Rotterdam, the Erasmus University Rotterdam and The Netherlands Organization for Health Research (ZonMw).
The printing of this thesis has been financially supported by The Department of Obstetrics and Gynaecology, Erasmus MC Roterdam, The Generation R Study Group Rotterdam, the Erasmus University Rotterdam, Nederlandse Vereniging voor Obstetrie en Gynaecologie, the J.E. Jurriaanse Stichting and The Netherlands Heart Foundation.

Further financial support for this dissertation was kindly provided by:
Bayer Health Care BV
BMA BV
Bronovo Research Fonds
Danone Research – Centre for Specialised Nutrition
Johnson & Johnson Medical BV / Women's Health and Urology
Stichting Metabolica
Medical Dynamics
Memidis Pharma
Nutricia Nederland BV
Philips Nederland BV / Healthcare
Vitamine Informatie Bureau

Cover & chapter design: Jelle Mollema, vraag_en_teken@hotmail.com
Lay-out: Ontwerpbureau SIS, Amsterdam & Nijmegen, The Netherlands
Print: Ipskamp Drukkers B.V. Enschede, The Netherlands
ISBN / EAN 978-90-9025610-8



# Folic Acid, Dietary Patterns and Perinatal Health

The Generation R Study

## Foliumzuur, voedingspatronen en perinatale gezondheid

Het Generation R Onderzoek

**Proefschrift**

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus
Prof.dr. H.G. Schmidt

en volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op
woensdag 15 september 2010 om 13:30 uur

door

**Sarah Timmermans**

geboren te Nijmegen

ERASMUS UNIVERSITEIT ROTTERDAM

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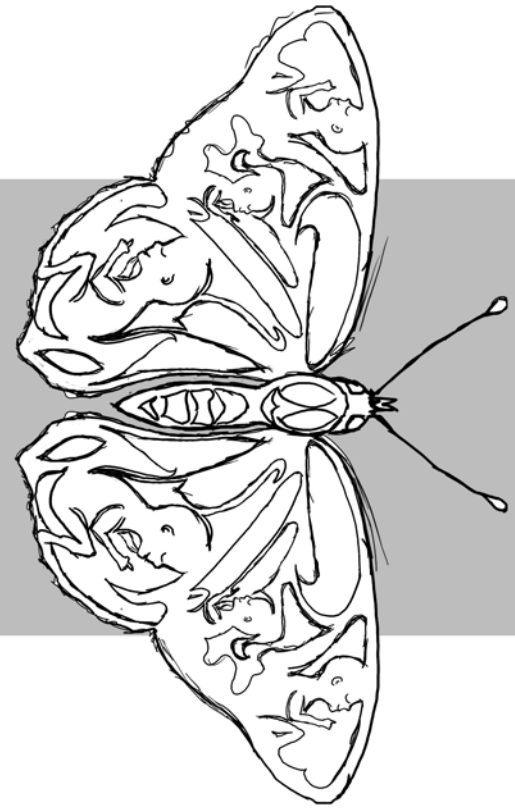
Financial support by The Netherlands Heart Foundation for the publication of this thesis is gratefully acknowledged

## Contents

# Chapter 1

# Introduction



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## Rationale

The perinatal mortality rate in The Netherlands is among the highest in the European Union, with one in 100 babies dying during pregnancy, at birth or shortly thereafter.[1] Low birth weight, preterm birth, congenital anomalies, perinatal asphyxia, and pre-eclampsia are major contributors to perinatal mortality. Moreover, pre-eclampsia is not only a major driver for perinatal mortality but plays a significant role in maternal mortality as well.[1, 2]

Maternal nutrition has been recognised as one of the most important environmental factors influencing the development of the embryo, fetus, and placenta, as well as maternal health.[3-5] Since pregnancy is characterised by different stages representing a continuum, the timing of a nutritional insult may impact differentially on pregnancy course and outcome. For this reason, the early pregnancy period, i.e. the period before the tenth pregnancy week, is important since several pregnancy complications have their onset during this particular phase.[3-7]

Increasing evidence indicates a role for micronutrients in the pathophysiology of some major perinatal and maternal outcomes.[8] In this respect, folate, being the most investigated nutrient in reproductive medicine, is of interest. It is an essential substrate for intermediates of cell multiplication and cell differentiation, and an important source of methylgroups. Additionally, folate plays an important role in the homocysteine metabolism.[9] During periods of rapid cell growth and division maternal folate demand increases dramatically. A deficient folate supply may result in impaired deoxyribonucleic acid (DNA) synthesis and an inability to methylate cellular proteins, lipids, and DNA. For this reason pregnant women with a folate deficiency are at a substantial risk for a variety of pregnancy complications, including neural tube defects.[10, 11] As a consequence, most European countries, including The Netherlands, recommend all women planning pregnancy to use 0.4 – 0.5 mg folic acid per day from at least one month before until three months after conception in addition to a healthy diet.[12]

Recently, the identification of dietary patterns has emerged as a constructive and innovative new tool to elucidate relationships between nutrition and disease. Analysing food consumption in the form of dietary patterns offers a perspective that is different from conventional approaches focusing on a single nutrient, or a few nutrients or foods. Because nutrients and foods are generally consumed in combination (meals) and according to habits, possible complex interactive or synergistic effects may best be investigated by considering the dietary pattern.[13, 14] This could provide important insight possibilities for dietary changes, and may facilitate translation of the findings into public health recommendations.

In elucidating associations between maternal nutrition and perinatal and maternal outcome, both synthetic folic acid intake and dietary patterns have been examined with varying results.[15-34] The majority of studies focused on the effects of folic acid in mid- and late pregnancy. Likewise, studies on dietary patterns and pregnancy outcomes are sparse. Thus, despite the fact that early pregnancy is a critical period for placental development,

embryogenesis, and embryonic programming, relatively little is known about the implications of maternal nutrition during this particular period on pregnancy course and outcome.

In addition to maternal nutrition other important extraneous stimuli including lifestyle may also contribute to maternal and perinatal inequalities. In this respect, growing attention has been focused on the impact of residential neighbourhood on pregnancy course and outcome. Recent numbers have shown considerable geographic differences in pregnancy outcomes with increased risks for pregnancies from large urban areas.[35, 36] It has been suggested that perinatal and maternal health inequalities are the result of differential accumulation of lifestyle, medical and constitutional factors.[37, 38] These geographic effects on perinatal and maternal health may have important policy implications, given the possibility of designing preventive strategies. However, information on the effect of residential neighbourhood environment on pregnancy outcome as well as individual risk factors is limited.[35, 36]

Against this background the questions to be addressed in this thesis are:

- **Folic acid** – To what extent does synthetic folic acid intake in the periconception and / or early pregnancy period affect pregnancy course and outcome?
- **Dietary pattern**s – What are the associations between maternal dietary patterns in early pregnancy, and perinatal and maternal outcomes?
- **Urban perinatal health** – Can perinatal and maternal health inequalities for women living within a large urban area be related to individual lifestyle, medical and constitutional risk factors?

## Setting

All studies described in this thesis were embedded within the framework of The Generation R Study, an ongoing population-based cohort study designed to identify early environmental, biological, and social determinants of growth, development, and future health.[39, 40] The Generation R Study is conducted in Rotterdam, the second largest city in The Netherlands comprising about 585,000 inhabitants. Study participants were pregnant Rotterdam women expected to deliver between April 2002 and January 2006. In total 8880 women enrolled prenatally, of whom 80% during the early pregnancy period. Information regarding maternal folic acid use, nutritional intake, maternal folate status, and sociodemographic, lifestyle, obstetrical, and health-related determinants was obtained from questionnaires, physical examinations, and biological samples assessed in early, mid-, and late pregnancy. Several overlapping sources such as our own ultrasound facilities but also obstetric caregivers, and Municipal Health Services, provided information about perinatal and maternal outcomes, including intrauterine growth, placental parameters (uteroplacental vascular resistance and placental weight), low birth weight, preterm birth, congenital anomalies, perinatal asphyxia, and gestational hypertension and pre-eclampsia. Currently, the study encompasses approximately 7000 actively participating children aged 4 - 8 years, together with their parents. At the age of 5 - 6 years these children are being invited to visit The Generation Research Center to study their growth, development, and health using innovative and detailed tools.[41] The Generation R Study has been approved by the Medical Ethical Committee of the Erasmus MC, University Medical Center Rotterdam, and the medical ethical review boards of all participating hospitals. All participants provided written informed consent. The Generation R Study follows the STROBE guidelines.[41, 42]

## Outline of the thesis

**Folic acid** – The first part of this thesis assesses the associations between folic acid, fetal growth and maternal haemodynamic adaptation mechanisms during pregnancy. In Chapter 2.1 we examine whether self-reported periconception folic acid use affects fetal and placental growth, and the risks of low birth weight. Chapter 2.2 focuses on the association between self-reported periconception folic acid use, uteroplacental vascular resistance, maternal blood pressure during pregnancy, and the risks of gestational hypertension and pre-eclampsia.
**Dietary patterns** – The second part of this thesis focuses on dietary patterns in early pregnancy and the association with fetal and placental growth, and maternal blood pressure patterns during pregnancy. In Chapter 3.1 we evaluate the effects of dietary patterns in early pregnancy on intrauterine growth, placental growth, and low birth weight. In Chapter 3.2 the association between major dietary patterns in early pregnancy, maternal systolic and diastolic blood pressure, and pre-eclampsia is assessed.
**Urban perinatal health** – The final part of this thesis studies individual risk factors in relation to urban perinatal health. The prevalence of adequate preconception start of folic acid use and potential determinants are investigated in Chapter 4.1. In Chapter 4.2 the association between accumulation of perinatal risk factors and adverse perinatal outcomes is examined on a neighbourhood level.
Finally, in Chapter 5 in light of the main findings of this thesis clinical implications and suggestions for future research are presented. A summary is provided in Chapter 6.

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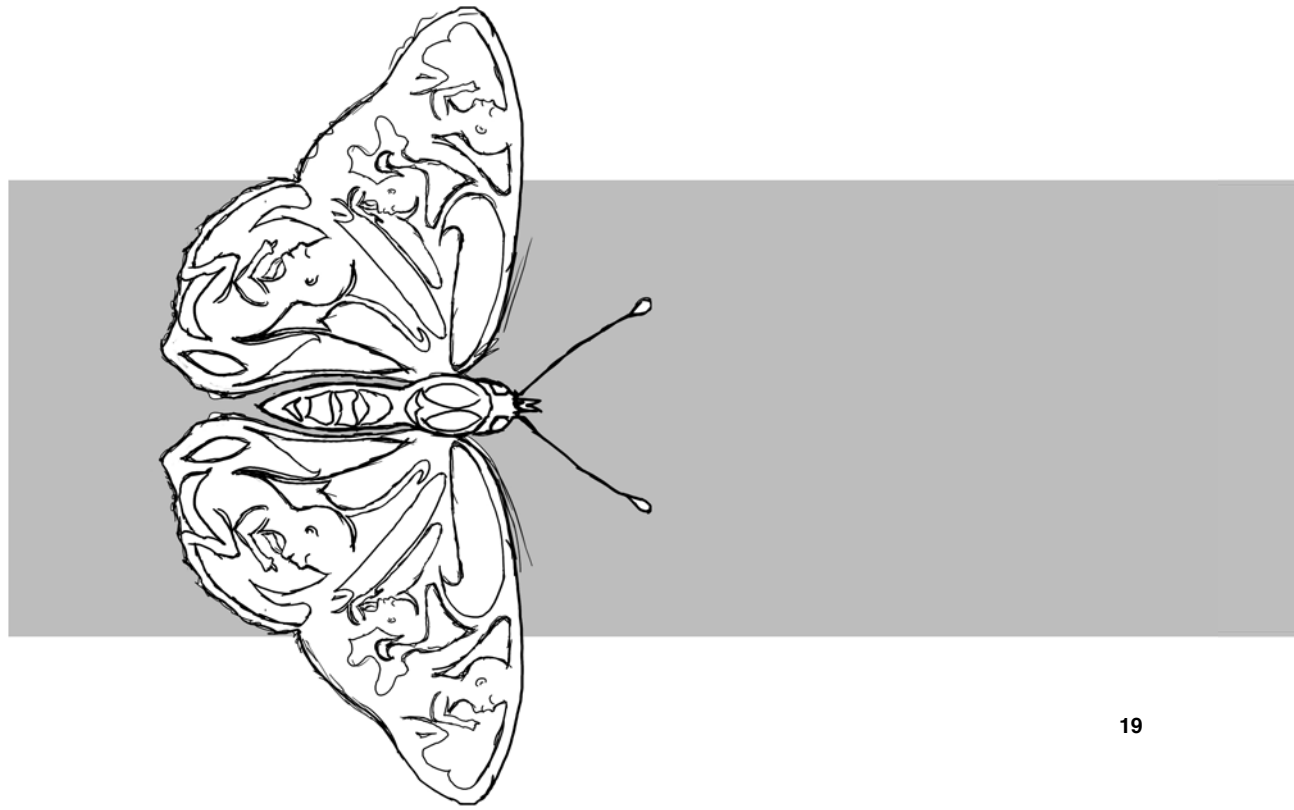
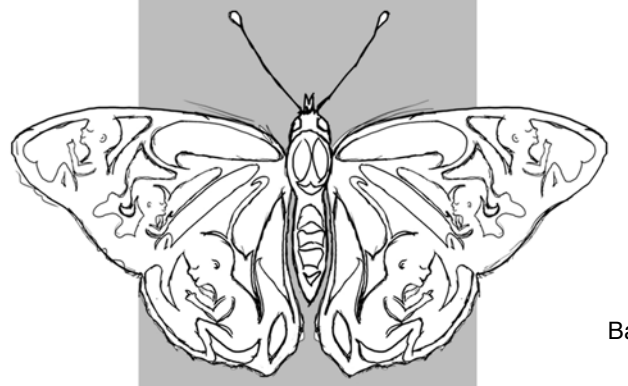
# Chapter 2

# Folic acid



# Chapter 2.1

# Periconception folic acid use, fetal growth, and the risks of low birth weight and preterm birth



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Based on British Journal of Nutrition 2009,
102: 777 - 85

## Abstract

Countries worldwide recommend women planning pregnancy to use folic acid during the periconception period. These recommendations mainly focus on the prevention of neural tube defects despite increasing evidence that folic acid may also influence birth weight. Within the framework of The Generation R Study Rotterdam, The Netherlands, we examined whether periconception folic acid use affects fetal growth and the risks of low birth weight, being small for gestational age at birth (SGA), and preterm birth. Data on 6353 pregnancies were available. Main outcome measures were fetal growth measured in mid- and late pregnancy by ultrasound, birth weight, placental weight, SGA, and preterm birth in relation to self-reported periconception folic use. Overall, self-reported periconception folic acid use was positively associated with fetal growth. Preconception folic acid intake was associated with a 68 grams higher birth weight (95% Confidence Interval (CI) [37.2; 99.0]) and a 13 grams higher placental weight (95% CI [1.1; 25.5]) compared to no folic acid use. In these analyses parity significantly modified the effect estimates. Start of folic acid use after pregnancy confirmation was associated with a reduced risk of low birth weight (odds ratio (OR) 0.61, 95% CI [0.40; 0.94]). Similarly, reduced risks for low birth weight and SGA were observed for women who started folic acid use preconceptionally compared to those who did not use folic acid (OR 0.43, 95% CI [0.28; 0.69], and OR 0.40, 95% CI [0.22; 0.72]). In conclusion, periconception folic acid use is associated with increased fetal growth resulting in a higher placental and birth weight, and decreased risks of low birth weight and being SGA.


## Introduction

Low birth weight, as a proxy for fetal growth, has been associated with various chronic diseases later in life.[1] Fetal growth depends on multiple genetic factors and environmental exposures derived from the mother. In this respect maternal nutrition during pregnancy has shown to play a critical role.[2, 3]

Because developing organ systems directly respond with permanent adaptations to the availability of nutrients during critical periods of rapid development, timing of adequate maternal nutrition is important to determine the effects both in the fetus and child.[4, 5] Moreover, first evidence has suggested that fetal growth is vulnerable to maternal nutrition especially during the preconception period and first weeks of gestation since it has the potential to affect epigenetic mechanisms in both the placenta and fetus.[2, 6-8]

For this reason, folate is of great interest. Together with vitamin B12 it plays a critical role in the homocysteine (tHcy) metabolism. The folate dependent tHcy pathway is important for protein, lipid, and deoxyribonucleic acid (DNA) synthesis. In addition, folate provides methylgroups for the synthesis of methionine and its derivate S-adenosyl-methionine. The latter is the most important methyldonor in the human body for DNA methylation and represents one of the best known epigenetic mechanisms.[9]

During pregnancy, folate demand increases due to placental and fetal growth and development. For this reason pregnant women with a folate deficiency are at increased risk for various reproductive failures including neural tube defects.[10, 11] As a consequence, most European countries recommend women planning pregnancy to use folic acid during the periconception period in addition to a healthy diet.[12, 13]

Several studies have shown positive associations between maternal folic acid intake and fetal growth.[14-22] However, the majority of these studies was focused on the effects of folic acid use in mid- and late pregnancy on fetal growth. Only a few studies assessed the associations in early pregnancy and these failed to be consistent.[15, 23] Thus, despite the fact that early pregnancy is the most important period for placental development, embryogenesis, and fetal programming, relatively little is known about the implications of folic acid use during this particular pregnancy period on fetal growth.[7, 8, 24]

The aim of the present study was to investigate the effects of self-reported periconception folic acid use on fetal and placental growth in low risk singleton pregnancies. In addition, we studied the associations between periconception folic acid use, low birth weight, small for gestational age (SGA), and preterm birth.

## Materials and Methods

***Study design***

The study was embedded in The Generation R Study Rotterdam, The Netherlands, a population-based prospective cohort study from early pregnancy onwards.[25, 26] The Generation R Study was designed to identify early environmental and genetic determinants of growth, development, and health from fetal life until young adulthood. The study is conducted in Rotterdam, the second largest city in The Netherlands. Eligible women were those who were resident in the study area and delivered between April 2002 and January 2006. The study aimed to enrol women in early pregnancy (gestational age < 18 weeks) but enrolment was possible until birth of the child. All midwifery practices and three hospitals located in the study area participated during the prenatal phase. The overall response rate was about 61% and was based on the number of children born to eligible mothers during the inclusion period.[25, 26]

***Folic acid use***

Pregnant women were asked by a questionnaire at enrolment (median 14.6 weeks of gestation, interquartile range 5.0) whether they had used folic acid periconceptionally and when folic acid use was started. Self-reported folic acid use was categorised into three groups: 1) preconception start: defined as start of folic acid intake at any moment prior to conception; 2) start before eight weeks: defined as start of folic acid intake from the moment that pregnancy was recognised but before the eighth week of gestation; and 3) no use: defined as no use of folic acid at all. Because our interest was mainly focused on start, and use, of folic acid during the period just before and after conception, i.e. the periconception period, we did not include women who started folic acid use after the eighth week of gestation in the main analysis (n = 46). However, because this group may represent an interesting comparison group we did include them in a sensitivity analysis. About 15% of the women reported to have used folic acid as part of a multivitamin supplement regimen. The doses of folic acid in these multivitamins were approximately 0.4 - 0.5 mg per day.

***Fetal growth***

Fetal ultrasound examinations were carried out in early pregnancy (median 13.5 week of gestation, interquartile range 4.9), mid-pregnancy (median 20.6 weeks of gestation, interquartile range 1.4), and late pregnancy (median 30.4 weeks of gestation, interquartile range 1.1). The ultrasound examinations were used to establish both gestational age and fetal growth characteristics.[27] Fetal biometry, including head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC), and femur length (FL), was measured transabdominally during each ultrasound examination. Crown-rump length (CRL) was measured in early pregnancy. Dating of pregnancy was performed using the first ultrasound measurement of either CRL (gestational age until 12 weeks and 5 days of gestation, and CRL measurement smaller than 65 mm), or BPD (gestational age from 12 weeks and 5 days of gestation onwards, and BPD larger than 23 mm).[27] Estimated fetal weight (EFW) was calculated in mid- and late pregnancy using the formula of Hadlock.[28] Longitudinal growth curves and gestational-age-adjusted standard deviation (SD) scores were constructed for all fetal growth measurements. These gestational-age-adjusted standard deviation scores were based on reference growth curves from the whole study population and represent the equivalent of z-scores.[27]

Medical records completed by community midwives and obstetricians were used to obtain information about gestational age at birth (weeks), birth weight (grams), fetal gender, and placental weight (grams), and to calculate placental index (placental weight / birth weight).[29, 30] Prematurity was defined as the birth of an infant before 37.0 weeks of gestation and low birth weight was defined as a birth weight < 2500 grams. SGA was defined as an SD-score < -2.0 and based on SD-curves derived from this cohort.[27]

***Covariates***

Information regarding maternal age, educational level, ethnicity, smoking habits, alcohol consumption, and parity was obtained from the questionnaire at enrolment in the study. Educational level was assessed by the highest completed educational level of the mother and classified into two categories: 1) primary school; and 2) secondary school, university or college.[31] Ethnic background was defined from information of the country of birth of the woman herself and her parents and categorised as follows: 1) Western, including Dutch, European, North American, and Oceanean; 2) Moroccan; 3) Turkish; 4) Antillean and Surinamese; and 5) other non-Western, including African, Asian and South- and Central American.[32] At the first ultrasound examination maternal height (m), and weight (kg) were measured and body mass index (BMI; in kg / m2) was calculated. Information on fertility treatment and pregnancy complications including pre-eclampsia and gestational diabetes was obtained from midwives and obstetricians. Women who became pregnant after fertility treatment (in vitro fertilization (IVF) or Intracytoplasmatic Sperm Injection (ICSI)) were excluded from all analyses.

***Data analysis***

Associations of self-reported folic acid use with fetal growth characteristics and birth characteristics were assessed using simple and multiple linear regression models. The linear regression analyses that were based on fetal growth characteristics were restricted to mothers that enrolled and had their pregnancies dated in early pregnancy (78%). The consideration of confounding variables was determined a priori and based on earlier literature. These included time of enrolment in study, gestational age, maternal age, height, weight, parity, ethnicity, fetal gender, educational level, smoking habits, alcohol consumption, primary or secondary antenatal care, gestational diabetes, and pre-eclampsia. Potential confounders were then selected as a result of exploratory analyses and included in the analyses if the effect estimates of the fetal growth parameters changed more than 10%. By using this approach type of antenatal

care, alcohol consumption, pre-eclampsia, and gestational diabetes were not included in the final multiple regression model. In the multiple regression models, with respect to missing values, the missing indicator method was used.[33] To analyse the associations of folic acid use with the risks of low birth weight, SGA, and preterm birth, we used simple and multiple logistic regression models with a similar approach to select confounders and to deal with missing values. Effect modification was tested by multiplying self-reported folic acid use with the covariates educational level, ethnicity, parity and BMI. Subsequently, and under the condition of a p - value < 0.1, multiple linear regression analyses were performed in strata of that specific determinant. Last, to test possible associations of folic acid use started after the eighth week of gestation with fetal growth we also performed analyses with the cohort categorised into four groups,: 1) preconception start; 2) start before eight weeks; 3) start from eight weeks onwards; and 4) no folic acid use. All statistical analyses were performed using SPSS version 15.0 for Windows (SPSS Inc, Chicago, IL, USA).

## Results

8880 women enrolled during pregnancy in The Generation R Study.[26] Forty-six women (0.5%) started using folic acid after the eighth week of gestation and complete information on folic acid use was not available in 25% of the women (n = 2274). Of the remaining 6560 women 47 IVF or ICSI pregnancies, 63 twin pregnancies, and 77 fetal deaths were identified, and 20 women were loss to follow-up. Data on 6353 low risk singleton pregnancies (71.6%) were available.

Characteristics of the women per folic acid category are presented in Table 1. Of all women 39.2% (n = 2493) reported to have started folic acid use preconceptionally. Approximately 31% of all women (n = 1983) reported to have started folic acid use after pregnancy recognition, and 29.5% of the women (n = 1877) reported not to have used folic acid at all. The age of the cohort ranged from 15.3 to 46.3 years with a median of 29.8 years. The lowest median age was found in women who did not use folic acid (27.8 years). The percentage of women with a higher educational level was also lowest in this group (26.9%). In the whole cohort the largest ethnic groups were the Dutch and other Western (63% together). The percentage of women who did not use folic acid was highest (71.6%) among non-Western women including Moroccan, Turkish, Surinam and Antillean women.

**Table 1 Characteristics of participants in the study stratified by category of folic acid use**

| | Folic acid use | | |
|---|---|---|---|
| | No use | Start before eight weeks | Preconception start |
| | n = 1877 | n = 1983 | n = 2493 |
| **Mean maternal age (years)** | 27.8 (5.8) | 29.7 (5.0) | 31.5 (4.2) |
| **Median height (cm)** | 167.5 (5.5) | 167.5 (7.0) | 168.0 (7.0) |
| **Median weight (kg)** | 68.9 (8.0) | 68.8 (10.0) | 68.9 (11.2) |
| **Multipara (%)** | 55.8 | 37.4 | 39.1 |
| **Educational level (%)** | | | |
| Primary school | 26.9 | 6.9 | 3.0 |
| Secondary school, university or college | 73.1 | 93.1 | 97.0 |
| **Ethnicity (%)** | | | |
| Western | 28.4 | 69.0 | 83.9 |
| Moroccan | 15.5 | 3.8 | 2.1 |
| Turkish | 17.4 | 7.2 | 3.8 |
| Surinam and Antilles | 19.6 | 12.0 | 5.9 |
| Non-Western otherwise | 19.1 | 8.0 | 4.3 |
| **Smoking habits any time in pregnancy (%)** | 29.4 | 31.8 | 16.5 |
| **Alcohol consumption any time in pregnancy (%)** | 32.0 | 57.9 | 58.3 |
| **Enrolment in study in early pregnancy (%)** | 67.4 | 80.0 | 84.7 |
| **Antenatal care during first trimester (%)** | | | |
| Primary care | 90.5 | 92.5 | 92.1 |
| Secondary care | 7.9 | 7.5 | 9.5 |
| **Fetal growth ultrasound** | | | |
| Mid-pregnancy (%) | 91.8 | 95.5 | 95.7 |
| Mean Head circumference (mm) | 179.1 (13.5) | 178.7 (13.2) | 179.0 (12.6) |
| Mean Abdominal circumference (mm) | 156.6 (14.0) | 156.3 (13.8) | 156.7 (12.9) |
| Mean Femur length (mm) | 33.6 (3.4) | 33.3 (3.2) | 33.3 (3.1) |
| **Fetal growth ultrasound** | | | |
| Late pregnancy (%) | 94.5 | 96.5 | 96.9 |
| Mean Head circumference (mm) | 283.2 (12.3) | 284.5 (12.2) | 286.1 (11.9) |
| Mean Abdominal circumference (mm) | 262.0 (16.6) | 262.8 (16.1) | 265.5 (2.9) |
| Mean Femur length (mm) | 57.3 (3.0) | 57.4 (2.9) | 57.5 (2.9) |
| **Birth outcomes** | | | |
| Median gestational age at birth (weeks) | 39.7 (2.0) | 39.9 (1.9) | 39.9 (1.9) |
| Mean birth weight (grams) | 3340.2 (563.6) | 3424.7 (557.9) | 3484.1 (553.7) |
| Placental weight (grams) | 628.7 (149.5) | 633.5 (146.0) | 644.4 (149.4) |
| Placental index | 0.190 (0.036) | 0.187 (0.035) | 0.186 (0.035) |
| Male gender (%) | 51.8 | 50.9 | 48.6 |

Values are mean ( SD), median (interquartile range) or percentages (%) within column

The associations between self-reported folic acid use and fetal growth characteristics in mid- and late pregnancy are presented in Table 2. Periconception folic acid use was associated with trends towards a larger head circumference and abdominal circumference in mid- and late pregnancy, compared to women who did not use folic acid. In addition, similar trends towards a larger femur length in mid- and late pregnancy were observed for women who periconceptionally used folic acid, compared to women who did not use folic acid. However, these effect estimates were not significant.

**Table 2 Associations between periconception folic acid use and fetal growth measured by ultrasound**

| | Head circumference 20 wks | | Abdominal circumference 20 wks | | Femur length 20 wks | |
|---|---|---|---|---|---|---|
| **Folic acid use** | Crude* | Adjusted† | Crude* | Adjusted† | Crude* | Adjusted† |
| No use | Reference | Reference | Reference | Reference | Reference | Reference |
| Start before eight weeks | 0.54 | 0.10 | 0.53 | 0.05 | -0.04 | 0.01 |
| | [0.07; 0.99] | [-0.41; 0.60] | [-0.67; 1.13] | [-0.61; 0.71] | [-0.17; 0.09] | [-0.14; 0.16] |
| Preconception start | 1.26 | 0.61 | 1.31 | 0.41 | -0.02 | 0.04 |
| | [0.82; 1.70] | [0.09; 1.12] | [0.75; 1.88] | [-0.27; 1.08] | [-0.14; 0.11] | [-0.11; 0.19] |

| | Head circumference 30 wks | | Abdominal circumference 30 wks | | Femur length 30 wks | |
|---|---|---|---|---|---|---|
| **Folic acid use** | Crude* | Adjusted† | Crude* | Adjusted† | Crude* | Adjusted† |
| No use | Reference | Reference | Reference | Reference | Reference | Reference |
| Start before eight weeks | 1.33 | 0.63 | 0.85 | 0.32 | 0.07 | 0.03 |
| | [0.63; 2.04] | [-0.12; 1.38] | [-0.13; 1.84] | [-0.75; 1.39] | [-0.10; 0.24] | [-0.16; 0.21] |
| Preconception start | 2.78 | 1.34 | 3.32 | 1.71 | 0.15 | 0.02 |
| | [2.11; 3.45] | [0.57; 2.11] | [2.39; 4.25] | [0.61; 2.80] | [-0.01; 0.31] | [-0.18; 0.20] |

Results from simple and multiple linear regression analysis. Values are regression coefficients (95% Confidence Interval) and reflect the difference in growth in mm for each characteristic (HC, AC, FL) measured in mid-pregnancy (median 20.6 weeks, interquartile range 1.4) and late pregnancy (median 30.4 weeks, interquartile range 1.1), compared to no folic acid use

*Crude: adjusted for gestational age at time of ultrasound measurement

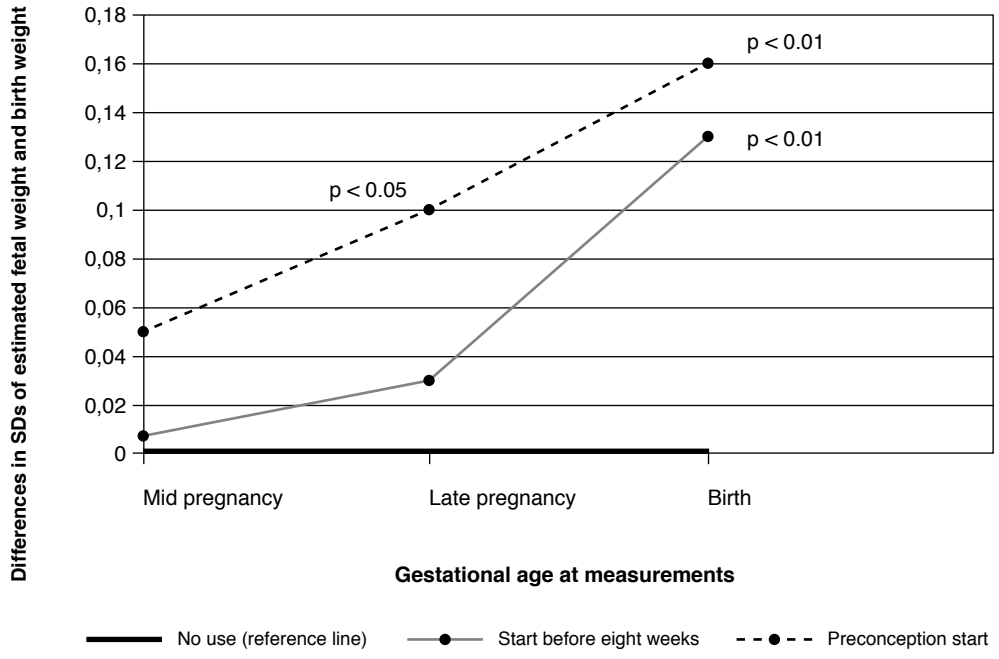
†Adjusted: additionally adjusted for maternal age, height, weight, parity, ethnicity, fetal gender, educational level and smoking habits

Figure 1 shows the differences in SD scores of fetal weight from mid-pregnancy until birth between the three folic acid categories. No differences in SD scores between the folic acid use groups were observed for estimated fetal weight in mid-pregnancy. These effects changed over time with a higher estimated fetal weight in late pregnancy for women who preconceptionally started folic acid use (difference = 0.10, 95% Confidence Interval (CI) [0.02; 0.19]), and a higher birth weight for both women who preconceptionally started folic acid use and women who started after pregnancy recognition, compared to no folic acid use at all



(difference = 0.16, 95% CI [0.09; 0.23] and difference = 0.13, 95% CI [0.06; 0.20], respectively). After adjustment for potential confounders birth weight was 68 grams higher in women who preconceptionally started folic acid use (95% CI [37.2; 99.0]) and 53 grams higher in women who started use after pregnancy recognition (95% CI [23.6; 83.1]), compared to birth weight of newborns of women who did not use folic acid.

**Figure 1 Associations between periconception folic acid use and estimated fetal weight and birth weight**




Results from multiple linear regression analysis. Values are regression coefficients and reflect the difference in standard deviation scores (SD) of estimated fetal weight (EFW) in mid-pregnancy (median 20.6 weeks, interquartile range 1.4) and late pregnancy (median 30.4 weeks, interquartile range 1.1); and the difference of SDs in birth weight, in infants of women who used periconception folic acid, compared to women did not use folic acid. Values are adjusted for gestational age, maternal age, height, weight, parity, ethnicity, fetal gender, educational level and smoking habits

Table 3 shows the associations between self-reported folic acid use and placental weight. Placental weight was approximately 10 grams more in women who preconceptionally started using folic acid (95% CI [0.32; 20.49]), compared to placental weight of women who did not use folic acid. After adjusting for potential confounders, this effect estimate did not change. For placental index a trend was observed towards a smaller index in both women who used preconception folic acid (difference = -0.004, 95% CI [-0.007; -0.002]) as well as in women who started after pregnancy recognition but before the eighth week of gestation (difference

= -0.003, 95% CI [-0.006; -0.001]), compared to women who did not use folic acid. However, this trend was not significant anymore after adjusting for potential confounders.

Table 4 shows the associations between folic acid use and birth outcomes. Preconception start of folic acid was associated with a decreased risk of low birth weight (odds ratio (OR) 0.47, 95% CI [0.33; 0.68]) as well as a decreased risk of SGA (OR 0.38, 95% CI [0.23, 0.63]) compared to women who did not use folic acid. After adjustment for potential confounders these effect estimates did not further change. In addition, after adjustment for potential confounders, start of folic acid use after pregnancy recognition was also associated with a decreased risk of having a child with low birth weight (OR 0.61, 95% CI [0.40; 0.94]), compared to women who did not use folic acid. Folic acid use (either preconception start or start after pregnancy confirmation) was not significantly associated with the risk of preterm birth after controlling for potential confounders.

**Table 3 Associations between periconception folic acid use, placental weight and placental index**

| | Placental weight | | Placental index | |
|---|---|---|---|---|
| **Folic acid use** | Crude* | Adjusted† | Crude* | Adjusted† |
| No use | Reference | Reference | Reference | Reference |
| Start before eight weeks | 0.37 | 6.84 | -0.003 | -0.001 |
| | [-10.20; 10.90] | [-4.81; 18.48] | [-0.006; -0.001] | [-0.004; 0.001] |
| Preconception start | 10.41 | 13.28 | -0.004 | -0.001 |
| | [0.32; 20.49] | [1.08; 25.48] | [-0.007; -0.002] | [-0.004; 0.002] |

Results from simple and multiple linear regression analysis. Values are regression coefficients (95% Confidence Interval) and reflect the difference of each characteristic (placental weight in grams), compared to no folic acid use
*Crude: unadjusted. Placental weight adjusted for gestational age at birth
†Adjusted: additionally adjusted for maternal age, height, weight, parity, ethnicity, fetal gender, educational level and smoking habits

**Table 4 Associations between periconception folic acid use and pregnancy complications**

| | Low birth weight | | SGA | | Prematurity | |
|---|---|---|---|---|---|---|
| **Folic acid use** | Crude* | Adjusted† | Crude* | Adjusted† | Crude* | Adjusted† |
| No use | Reference | Reference | Reference | Reference | Reference | Reference |
| Start before eight weeks | 0.75 | 0.61 | 0.82 | 0.69 | 0.75 | 0.75 |
| | [0.52; 1.07] | [0.40; 0.94] | [0.53; 1.25] | [0.42; 1.14] | [0.58; 0.99] | [0.55; 1.02] |
| Preconception start | 0.47 | 0.43 | 0.38 | 0.40 | 0.77 | 0.88 |
| | [0.33; 0.68] | [0.28; 0.69] | [0.23; 0.63] | [0.22; 0.72] | [0.60; 0.99] | [0.63; 1.21] |

Results from simple and multiple logistic regression analysis. Data are odds ratios (95% Confidence Interval)
*Crude: unadjusted. Low birth weight adjusted for gestational age at birth
†Adjusted: additionally adjusted for maternal age, height, weight, parity, ethnicity, fetal gender, educational level and smoking habits



Parity significantly modified the effect of periconception folic acid use on birth weight ($p < 0.01$) (Figure 2). After adjustment for potential confounders preconception start of folic acid use among multiparous women was associated with 240 grams higher birth weight (95% CI [195.1; 282.9]), compared to nulliparous women who did not use folic acid. In contrast, preconception start folic acid among nulliparous women was associated with a 55 grams higher birth weight (95% CI [15.4; 94.6]). We did not observe further significant effect modification on the additive scale by educational level, ethnicity, or BMI.

Last, the analysis performed to test associations of folic acid use started after the eighth week of gestation with fetal growth did not reveal differences between A) non-users, preconception users, and women who started before eight weeks, and B) folic acid use from eight weeks onwards, and similarly no association of this group with fetal growth (likely due to small numbers in this latter group; n = 46).

## Comment

In this prospective population-based cohort study we demonstrate that self-reported low dose periconception folic acid use is associated with increased fetal growth compared to non-users and that parity significantly modifies this effect. Self-reported low dose periconception folic acid use is also associated with reduced risks of having a child with low birth weight or being SGA at birth. No significant association was observed between periconception folic acid use and preterm birth.

Our study was embedded in a large prospective cohort study with a significant number of measurements performed in the mothers which increases the accuracy of our effect estimates. However, some limitations should be addressed. First, because the response rate of The Generation R study was approximately 61%, selective participation of pregnant women may have influenced the observed associations.[34] In addition, complete information on folic acid use was missing in approximately 25% of the women. This non-response could lead to selection bias if the association between periconception folic acid use and fetal growth would differ between those with and without complete data. Even though this seems unlikely it cannot be fully excluded. Second, the use of questionnaires to assess folic acid use encompasses several limitations including information bias and giving desirable answers. Yet, studies show that self-reported intake of folic acid correlates to folate serum levels.[35] Moreover, we aimed to assess folic acid use in early pregnancy to minimise possible recall bias. However, even though a clear definition of folic acid use was used in the questionnaire misclassification especially between those who started using folic acid preconceptionally and those who started after pregnancy recognition should always be considered. Last, folic acid use is related

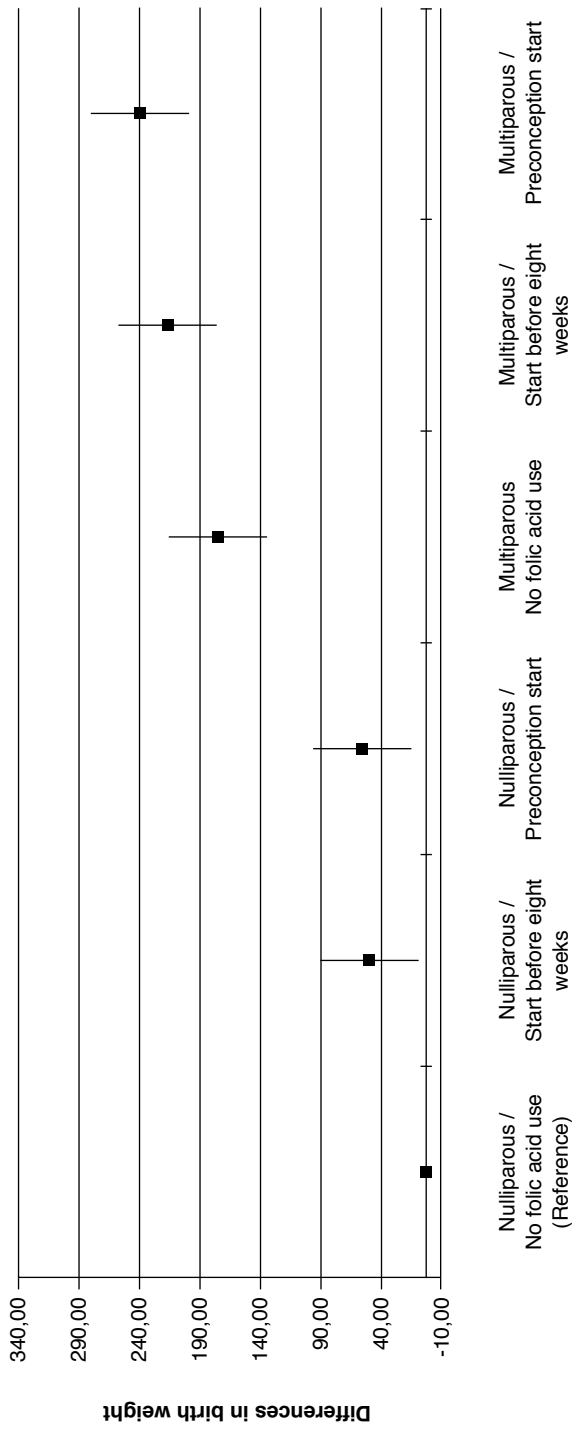



**Figure 2 Associations between periconception folic acid use and birth weight stratified by parity**

Results from multiple linear regression analysis stratified per parity / folic acid subgroup. Values are regression coefficients (95% Confidence Interval) and reflect the difference in birth weight in grams, compared to nulliparous women who did not use folic acid. Values are adjusted for gestational age at birth, maternal age, height, weight, ethnicity, fetal gender, educational level and smoking habits



to socioeconomic status (SES) and health behaviours (dietary habits, smoking).[36] Even though we were able to control for a large number of potential confounders available from our questionnaires, residual confounding is always of particular concern in vitamin supplement studies and should for this reason be taken into account.

Folate is a B vitamin that serves as a substrate in many underlying pathways of cellular processes including cell multiplication, apoptosis, intracellular signaling, and programming. All these processes are implicated in fetal and placental growth and development.[9] Therefore, additional supply of folic acid during pregnancy is likely to influence fetal growth. Furthermore, an optimal placental function is of main importance for the growth and development of the fetus. Thus, when folate directly affects placental growth and development it also may indirectly affect fetal growth.[8, 21, 29, 30] Previous studies support our findings and show positive associations between increased folic acid intake, higher birth weight, and reduced risks of low birth weight and SGA. [14-22] However, the majority of these studies was focused on increased folic acid intake in mid- and late pregnancy. Since fetal growth is relatively great in the second half of pregnancy and a larger supply of folic acid during this particular period may directly serve as a substrate for increased cellular synthesis their results were not really unanticipated. Only few studies assessed the associations between folate in early pregnancy and fetal growth.[15, 23] Rolschau et al.[15] found an increased birth weight in infants of mothers who preconceptionally started folic acid use. Interestingly, they reported this effect only to be present in the 43th week of gestation. They also reported a reduced prevalence of low birth weight and SGA in newborns of women who preconceptionally started use and in contrast with our study a reduced risk for preterm birth. However, like most other studies that investigated associations between folic acid use and fetal growth, they used very high doses of folic acid (up to 2.5 mg) compared with the self-reported low doses of 0.4 - 0.5 mg per day in our study.[15, 16, 18-20]

The association between periconception folic acid use and fetal growth could also be explained by optimisation of the folate dependent tHcy pathway. Reduced folate status is associated with elevated tHcy and higher serum levels of tHcy have been associated with decreased fetal growth and placental vasculopathy.[37] In most cases hyperhomocysteinaemia can be treated by low dose folic acid. Additionally, it has been shown that folate has the potential to improve endothelial function independent from tHcy status.[38]

Previous studies have shown that folic acid use of 5 mg in mid- and late pregnancy increases placental size, cell number (DNA), and protein content.[20, 21] In our study women who preconceptionally started taking folic acid did not only have significantly larger newborns but larger placentas as well, compared to women who did not use folic acid. However, this association was not found in women who started folic acid after pregnancy recognition.

These findings could implicate that periconception folic acid use influences placental growth via other pathways rather than through improved placental vasculogenesis or its role in placental nucleic acid synthesis. The results as shown in our study on self-reported periconception folic acid use might suggest that fetal and placental programming are affected by additional folic acid supply at different time frames throughout the periconception period. Folate is essential for DNA methylation, an important epigenetic mechanism that plays a regulatory role in genome programming and imprinting during pregnancy.[9] Differences in quantitative methylation may affect genes implicated in embryogenesis and fetal growth.[39, 40] It has been shown that variations in preconception exposure to folic acid can lead to epigenetic modifications of the genome in the offspring associated with adiposity, insulin resistance, and high blood pressure in adulthood.[7, 24] This might also apply to our results suggesting that periconception folic acid use may cause epigenetic modifications in the preimplantation embryo which may result in differential placental and fetal growth patterns. However, at this moment these speculations need to be studied in further detail.

An important finding from our analysis was the modifying effect of parity. The positive effect of parity on birth weight has been well established.[29, 30] However, to our knowledge effect modification by parity on the association between periconception folic acid use and fetal growth has not been reported before. In the past Kloosterman[30] suggested that multiparous women offer a more favourable environment for placental development and function in subsequent pregnancies, through remodeling of the maternal vascular structure in former pregnancies.[29,30] From this respect it can be hypothesised that periconception folic acid use interacts with these vascular remodeling processes in multiparous women thereby affecting placental and subsequent fetal growth. However, this needs to be studied further by other investigators.

In conclusion, self-reported periconception folic acid use is significantly associated with increased fetal growth resulting in higher placental and birth weight, and decreased risks of having a child with low birth weight or being SGA. The effects are most pronounced in women who preconceptionally start using folic acid and are modified by parity. To investigate the underlying pathways in more detail and possible consequences for postnatal growth and development future studies are necessary.



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## Chapter 2.2

# Folic acid is positively associated with uteroplacental vascular resistance

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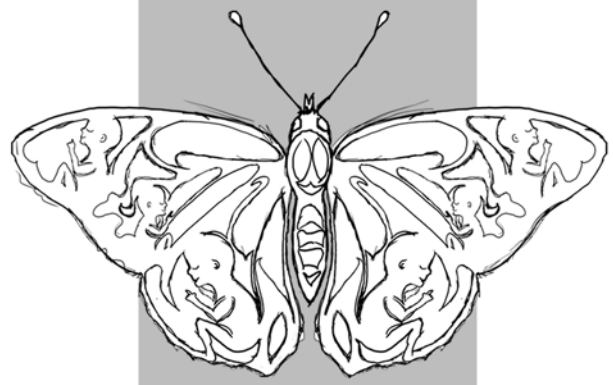


Based on Nutrition, Metabolism, and Cardiovascular Diseases 2009: Oct 9. Epub ahead of print

## Abstract

Periconception folic acid use may influence early placentation and thereby the occurrence of hypertensive pregnancy disorders. For this reason, we examined the associations between periconception folic acid use and uteroplacental vascular resistance, maternal blood pressure, and the risks of gestational hypertension and pre-eclampsia in 5993 pregnant women participating in a population-based cohort study. Folic acid use was assessed by questionnaire. Mean pulsatility index (PI) and resistance index (RI) of the uterine (UtA) and umbilical arteries (UmA) were measured by Doppler ultrasound in mid- and late pregnancy. Systolic and diastolic blood pressures (SBP, DBP) were measured in early, mid-, and late pregnancy. Compared to women who did not use folic acid, preconception folic acid users had a slightly lower UtA-RI in mid-pregnancy (difference = -0.02, 95% Confidence Interval (CI) [-0.03; -0.01]) and late pregnancy (difference = -0.02, 95% CI [- 0.03; -0.001]), a lower UtA-PI in mid-pregnancy (difference = -0.06, 95% CI[ -0.1; -0.03) and late pregnancy (difference = -0.03, 95% CI [-0.05; -0.01]), as well as tendencies towards a lower UmA-PI in mid-pregnancy (difference = -0.02, 95% CI [-0.04; -0.001]) and late pregnancy (difference = -0.01, 95% CI [-0.02; 0.01]). Additionally, these women had a slightly higher SBP and DBP throughout pregnancy. Neither the patterns of blood-pressure change during pregnancy nor the risk of gestational hypertension and pre-eclampsia differed between the folic acid categories. In conclusion, periconception folic acid use is associated with a lower uteroplacental vascular resistance and a higher blood pressure during pregnancy. The effects are small and within physiologic ranges and seem not associated with the risk of hypertensive pregnancy disorders. These findings may suggest that periconception folic acid use affects early placentation possibly through improved placentation and /or decidual vascular remodeling processes.




## Introduction

Hypertensive pregnancy disorders have been associated with substantial morbidity and mortality. The origin is thought to be related to atypical early placentation resulting in an enhanced peripheral vascular resistance and a subsequent increase in maternal blood pressure.[1] Most established risk factors such as maternal age, ethnicity, and parity are not modifiable. However, it has been suggested that early placentation is vulnerable to periconception exposures since they may affect inflammatory responses, antioxidant defenses, rapid cell division, and DNA and protein synthesis.[2, 3]

In this respect folate could be of interest. It is an essential substrate for intermediates of cell multiplication and cell differentiation and an important source of methylgroups for the methylation of proteins, lipids, and deoxyribonucleic acid (DNA). In addition, folate plays an essential role in the homocysteine (tHcy) metabolism.[4] It is known that pregnant women with a folate deficiency are at increased risk for several reproductive failures.[5] For this reason most European countries recommend that women planning pregnancy, independent of their folate status, should periconceptionally use folic acid in addition to a healthy diet. Moreover, it has been shown that folate deficiency is associated with increased human cytotrophoblast cells apoptosis thereby possibly influencing trophoblast invasion and placental development.[6, 7]

From this we hypothesise that periconception folic acid use may affect the occurrence of hypertensive pregnancy disorders through improved placental implantation. We therefore investigated the effects of low dose self-reported periconception folic acid use on the intermediates of hypertensive pregnancy disorders, namely uteroplacental vascular resistance and maternal blood pressure. Additionally, we studied the associations between low dose self-reported periconception folic acid intake and the risk of pre-eclampsia and gestational hypertension.

## Materials and Methods

### *Study design*

This study was embedded within The Generation R study, a population-based prospective cohort study from early pregnancy onwards. The cohort includes 9778 women and their children in Rotterdam, The Netherlands. Enrolment was aimed at early pregnancy (planned at gestational age 12 weeks) and all children were born between 2002 and 2006.[8, 9]

### *Folic acid use*

In total 8880 women enrolled prenatally of whom 80% during early pregnancy.[8, 9] At enrolment women were asked by a questionnaire whether they had used folic acid and when use was initiated. Folic acid use was categorised into three groups: 1) preconception start:

defined as start of folic acid at any moment prior to conception; 2) before eight weeks: defined as start of folic acid from the moment that pregnancy was recognised but before the eighth week of gestation; and 3) no use: defined as no folic acid use at all. Self-reported folic acid use was validated by plasma folate and tHcy levels during the first trimester, i.e. before 12 weeks of gestation, in a small random subsample of this study (n = 276). Based on a cut-off value of plasma folate of 19.0 nmol / L this yielded sensitivity and specificity scores of respectively 97% and 56% (Table 1). Less than 15% of the women reported to have used a multivitamin supplement regimen. The doses of folic acid in these multivitamins were approximately 0.4 - 0.5 mg.

**Table 1 Total homocysteine and folate plasma levels according to self-reported folic acid users**

| | Self-reported non-users | | | Self-reported folic acid users | | | |
|---|---|---|---|---|---|---|---|
| **Biomarker** | n | Median | IQR | n | Median | IQR | p value |
| Plasma folate, nmol / L | 82 | 8.7 | 4.3 | 194 | 23.5 | 12.9 | < 0.05 |
| Plasma tHcy, mcmol / L | 82 | 8.4 | 2.9 | 194 | 7.1 | 2.0 | < 0.05 |

Median (interquartile range (IQR) plasma folate and tHcy levels during the first trimester. Mann-Whitney U test was used to test the differences in biomarker concentrations between self-reported folic acid users and non-users

***Uteroplacental vascular resistance***

Ultrasound examinations to assess uteroplacental vascular resistance were performed in mid- (planned at gestational age 20 weeks) and late pregnancy (planned at gestational age 30 weeks). Uteroplacental vascular resistance was assessed by umbilical artery pulsatility index (UmA-PI), uterine artery pulsatility index (UtA-PI), and uterine artery resistance index (UtA-RI). For each measurement three consecutive uniform waveforms were recorded by pulsed Doppler ultrasound and the mean was used for further analyses. Approximately 10% of the women in whom a Doppler ultrasound measurement of the uterine arteries was performed were obese (body mass index [BMI, kg / m2] ≥ 30). Because of significant differences in measurements between obese and non-obese women we restricted all analyses of the associations between folic acid use and UtA-PI and UtA-RI to non-obese women (BMI < 30).

***Maternal blood pressure***

Maternal systolic (SBP) and diastolic (DBP) blood pressures were measured in early, mid-, and late pregnancy using the validated Omron 907® automated digital oscillometric sphygmanometer (OMRON Healthcare Europe B.V. Hoofddorp, The Netherlands). The presence of gestational hypertension or pre-eclampsia was retrieved from medical records. The diagnosis was based on the following criteria: development of a blood pressure ≥ 140/90 mmHg after 20 weeks of gestation in a previously normotensive woman on at least two occasions: 1) without (i.e. gestational hypertension), or 2) with the presence of proteinuria (i.e. pre-eclampsia). Proteinuria was defined as two or more dipstick readings of 2+ or greater, one catheter sample reading of 1+ or greater, or a 24 – hour urine collection containing at least 300 mg of protein.[10]



***Covariates***

Data on sociodemographic, medical history, and behavioural variables were obtained from questionnaires. Information on maternal age, educational level (primary school, secondary school, university or college), ethnicity (Western, non-Western), smoking habits, alcohol consumption, and parity were available.[11, 12] At enrolment height (m) and weight (kg) were measured and BMI was calculated (kg / m2). Medical records completed by midwives and obstetricians were used to obtain information about antenatal care and gestational age at birth (weeks).

***Study population***

Of the prenatally enrolled women those with missing information on folic acid use or who started use after the eighth pregnancy week (n = 2320) were excluded. We also excluded women with chronic hypertension, diabetes mellitus, hypercholesterolaemia, heart disorders, and Systemic Lupus Erythematosus (n = 239); as well as fetal deaths before 20 weeks of gestation or terminations of pregnancy, twin pregnancies, and those who were loss to follow-up (n = 328). In the end 5993 women were eligible.

***Data analysis***

With respect to statistical analyses the Independent Student's t-test, Mann-Whitney U test, and chi-square test were used to test differences in baseline characteristics between the folic acid categories. Additionally, associations of self-reported folic acid use with placental vascular resistance (PI and RI) and maternal blood pressure (SBP and DBP) were assessed using simple linear regression models (crude model). Multiple regression models were then used to control for confounding factors (adjusted model). In these analyses missing values were substituted using the missing indicator method.[13] The consideration of confounding variables was determined a priori and based on earlier literature. These included gestational age at time of measurement, maternal age, height, weight, parity, ethnicity, educational level, smoking habits, alcohol consumption, and antenatal care. To evaluate patterns of blood pressure change between the folic acid categories multiple linear mixed models were used with blood pressure as a repeated outcome measure.[14] First, the best fitting model to predict SBP and DBP as a function of gestational age was built using fractional polynomials.[15] To these models self-reported maternal folic acid use was added as main determinant. To compare the slopes of the curves between the categories an interaction term of folic acid use with gestational age was included. Last, to analyse associations of self-reported folic acid use with the risks of gestational hypertension and pre-eclampsia we used simple and multiple logistic regression models with a similar approach to select confounders.

In all analyses women who reported not to have used folic acid represent the reference group. Statistical analyses were performed using Statistical Package of Social Sciences version 15.0 for Windows (SPSS Inc, Chicago, IL, USA) and the Statistical Analysis System (SAS) for Windows, version 9.1.2 (SAS, Institute Inc. Gary NC, USA).

**Table 2 Baseline maternal characteristics stratified per folic acid category**

| | Folic acid use | | |
|---|---|---|---|
| | No use | Before eight weeks | Preconception start |
| | n = 1770 | n = 1861 | n = 2362 |
| **Maternal age (mean)** | 27.8 (5.3) | 29.8 (5.6)* | 31.6 (4.9)*† |
| **BMI (median)** | 24.6 (6.3) | 23.9 (5.0)* | 23.5 (4.4)* |
| **Western ethnicity (%)** | 28.2 | 68.8* | 84.3*† |
| **Primary school (%)** | 26.8 | 6.7* | 3.0*† |
| **Nulliparous (%)** | 44.1 | 62.9* | 60.9* |
| **Smoking habits any time in pregnancy (%)** | 27.6 | 31.1* | 15.8*† |
| **Alcohol consumption any time in pregnancy (%)** | 26.4 | 52.0* | 50.0* |
| **Midwifery antenatal care (%)** | 91.5 | 93.0 | 92.5 |
| **Mean umbilical artery pulsatility index** | | | |
| Mid-pregnancy | 1.2 (0.19) | 1.2 (0.19) | 1.2 (0.27)* |
| Late pregnancy | 0.99 (0.17) | 0.98 (0.17) | 0.98 (0.19) |
| **Mean uterine artery pulsatility index** | | | |
| Mid-pregnancy | 0.94 (0.31) | 0.89 (0.27)* | 0.88 (0.26)* |
| Late pregnancy | 0.76 (0.21) | 0.74 (0.21)* | 0.73 (0.20)* |
| **Mean uterine artery resistance index** | | | |
| Mid-pregnancy | 0.56 (0.09) | 0.54 (0.9)* | 0.53 (0.09)* |
| Late pregnancy | 0.50 (0.18) | 0.49 (0.09)* | 0.48 (0.14)* |
| **Mean systolic blood pressure (mmHg)** | | | |
| Early pregnancy | 113.5 (12.0) | 115.2 (11.9)* | 116.7 (11.9)*† |
| Mid-pregnancy | 114.7 (11.7) | 116.8 (12.0)* | 117.6 (11.8) *† |
| Late pregnancy | 116.6 (12.2) | 118.4 (11.8)* | 119.2 (11.6)*† |
| **Mean diastolic blood pressure (mmHg)** | | | |
| Early pregnancy | 67.0 (9.4) | 67.8 (9.2)* | 68.6 (9.1)*† |
| Mid-pregnancy | 66.2 (9.1) | 66.9 (9.3)* | 67.4 (9.1)* |
| Late pregnancy | 68.1 (9.4) | 69.0 (9.1)* | 69.4 (8.8)* |
| **Pregnancy outcomes** | | | |
| Median gestational age at birth (weeks) | 39.8 (2.0) | 40.1 (1.9) | 40.1 (1.9) |
| Mean birth weight (grams) | 3335 (563) | 3428 (557)* | 3492 (553)* |
| Mean placental weight (grams) | 626 (149) | 634 (146)* | 643 (149)* |
| Gestational hypertension (%) | 2.7 | 4.7* | 7.0* |
| Pre-eclampsia (%) | 2.2 | 2.2 | 1.9 |

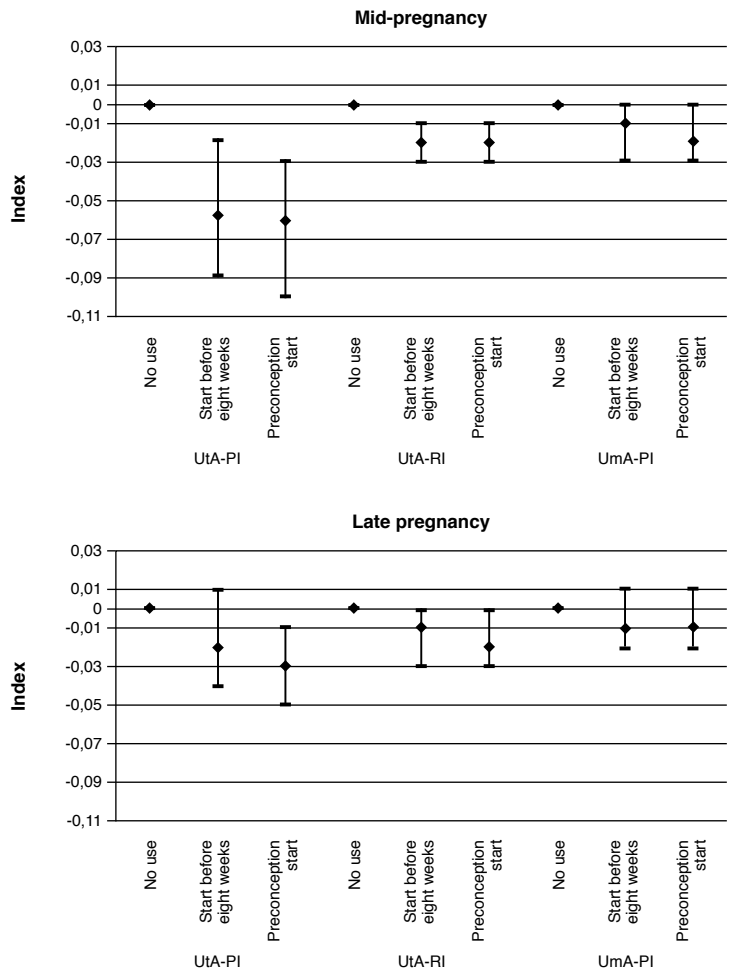
Values represent means (SD), median (interquartile range), or percentages (%) within column. The independent Student's t-test, Mann-Whitney U test, and chi-square test were used to test differences in baseline characteristics between the folic acid categories *p < 0.05 vs. no folic acid use †p < 0.05 vs. start after pregnancy recognition



# Results

Baseline characteristics are shown in Table 2. Of the women 39% reported to have started folic acid use preconceptionally, 31% started folic acid use after pregnancy recognition, and 30% did not use folic acid. Maternal characteristics associated with preconception folic acid use were higher maternal age, lower BMI, higher educational level, Western ethnicity, and being nulliparous.

**Figure 1 Associations between periconception folic acid use and uteroplacental vascular resistance**




Results from multiple regression analyses. Values are regression coefficients (95% Confidence Interval) and reflect differences in umbilical artery and uterine artery pulsatility index (UmA-PI and UtA-PI), or uterine artery resistance index (UtA-RI), compared to no folic acid use, in mid-pregnancy (median 20.7 weeks) and late pregnancy (median 30.4 weeks). All values are adjusted for gestational age at time of measurement, maternal age, height, weight, parity, ethnicity, educational level, smoking habits, alcohol consumption, antenatal care

Associations between self-reported folic acid use and uteroplacental vascular resistance are shown in Figure 1. A lower pulsatility index of the arteria umbilicalis in mid-pregnancy (difference = -0.06, 95% Confidence Interval (CI) [-0.09; -0.02]) and late pregnancy (difference = -0.03, 95% CI [-0.06; -0.01]), and a lower resistance index of the arteria uterina in mid-pregnancy (difference = -0.02, 95% CI [-0.03; -0.01]) and late pregnancy (difference = -0.02, 95% CI [-0.03; -0.01]) were observed in pregnancies that were preconceptionally exposed to folic acid. For pregnancies exposed to folic acid after pregnancy recognition similar tendencies for a lower pulsatility index of the arteria uterina in mid-pregnancy (difference = -0.05, 95% CI [-0.08; -0.02]) and late pregnancy (difference = -0.02, 95% CI [-0.04; 0.01]), and a lower resistance index of the arteria uterina in mid-pregnancy (difference = -0.02, 95% CI [-0.03; -0.01]) and late pregnancy (difference = -0.01, 95% CI [-0.03; 0.00]) weres observed. In addition, self-reported preconception folic acid use was associated with a lower pulsatility index of the arteria umbilicalis in mid-pregnancy (difference = -0.02, 95% CI [-0.04; -0.001]), compared to no folic acid use. However, this trend was not significant anymore in late pregnancy.

**Table 3 Associations between periconception folic acid use and blood pressure**

| | SBP Early pregnancy | | SBP Mid-pregnancy | | SBP Late pregnancy | |
|---|---|---|---|---|---|---|
| **Folic acid use** | Crude* | Adjusted† | Crude* | Adjusted† | Crude* | Adjusted† |
| No use | Reference | Reference | Reference | Reference | Reference | Reference |
| Before eight weeks | 1.8 | 0.8 | 2.1 | 1.4 | 1.8 | 0.7 |
| | [0.8; 2.7] | [-0.1; 1.8] | [1.3; 2.9] | [0.6; 2.3] | [1.0; 2.6] | [-0.2; 1.5] |
| Preconception start | 3.1 | 2.0 | 2.9 | 2.0 | 2.6 | 1.1 |
| | [2.2; 4.0] | [0.7; 2.6] | [2.1; 3.6] | [1.2; 2.9] | [1.8; 3.3] | [0.2; 2.0] |

| | DBP Early pregnancy | | DBP Mid-pregnancy | | DBP Late pregnancy | |
|---|---|---|---|---|---|---|
| **Folic acid use** | Crude* | Adjusted† | Crude* | Adjusted† | Crude* | Adjusted† |
| No use | Reference | Reference | Reference | Reference | Reference | Reference |
| Before eight weeks | 0.6 | 0.8 | 0.6 | 0.9 | 0.9 | 0.7 |
| | [-0.1; 1.3] | [0.1; 1.6] | [0.1; 1.3] | [0.2; 1.5] | [0.3; 1.5] | [0.1; 1.4] |
| Preconception start | 1.4 | 1.2 | 1.2 | 1.2 | 1.3 | 0.9 |
| | [0.7; 2.1] | [0.4; 2.0] | [0.6; 1.8] | [0.6; 1.9] | [0.7; 1.9] | [0.2; 1.6] |

Results from simple and multiple linear regression analyses. Values are regression coefficients (95% Confidence Interval) and reflect differences in systolic and diastolic blood pressure (in mmHg), compared to no folic acid use in early pregnancy (median 13.5 weeks), mid-pregnancy (median 20.7 weeks), and late pregnancy (median 30.4 weeks)
*Crude: adjusted for gestational age at time of measurement
†Adjusted: additionally adjusted for maternal age, height, weight, parity, ethnicity, educational level, smoking habits, alcohol consumption, antenatal care



**Table 4 Associations between periconception folic acid use and hypertensive pregnancy disorders**

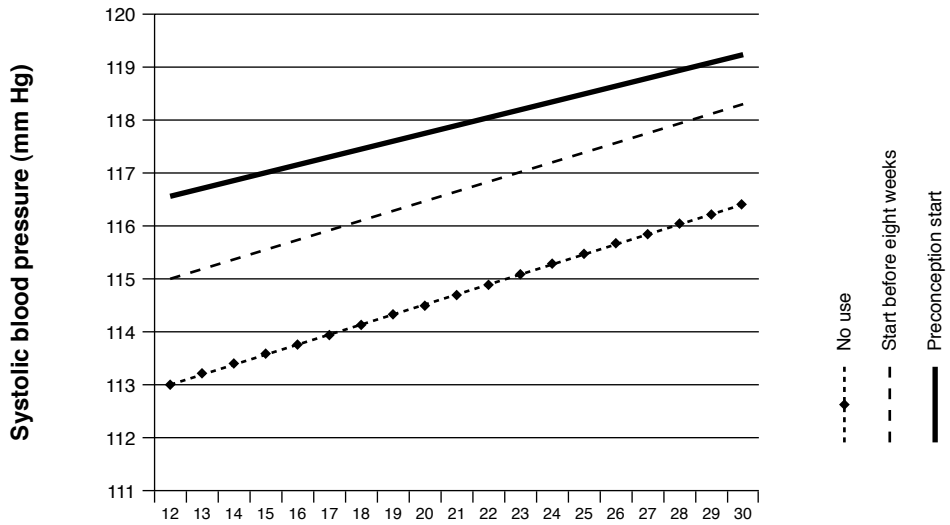
| | Gestational hypertension | | Pre-eclampsia | |
|---|---|---|---|---|
| **Folic acid use** | Crude* | Adjusted† | Crude* | Adjusted† |
| No use | Reference | Reference | Reference | Reference |
| Before eight weeks | 1.8 [1.3; 2.6] | 1.4 [0.9; 2.1] | 1.0 [0.7; 1.6] | 0.9 [0.5; 1.3] |
| Preconception start | 1.5 [1.1; 2.2] | 1.1 [0.7; 1.7] | 0.9 [0.6; 1.4] | 0.8 [0.5; 1.4] |

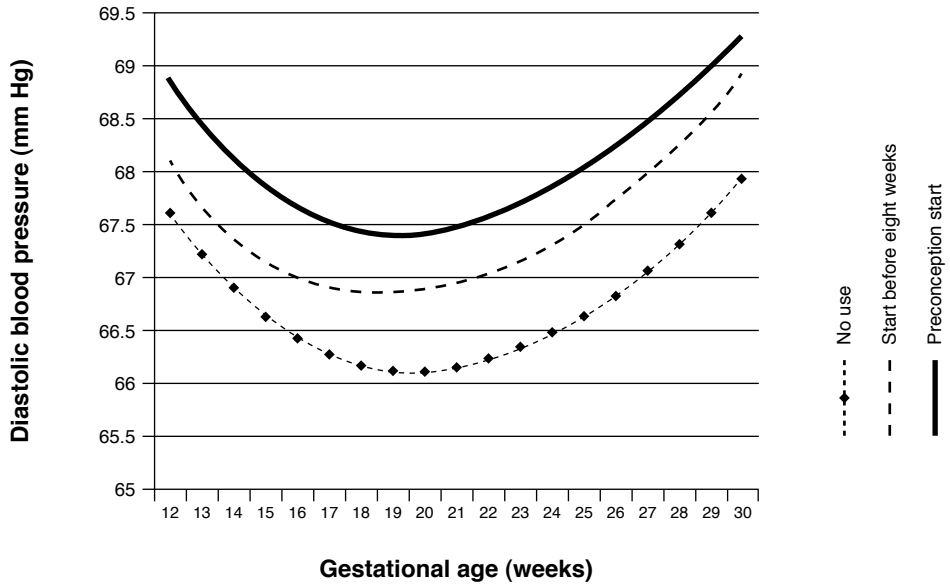
Results from simple and multiple logistic regression analyses. Data are odds ratios (95% Confidence Interval)
*Crude: unadjusted
†Adjusted: adjusted for maternal age, height, weight, parity, ethnicity, educational level, smoking habits, alcohol consumption, antenatal care

Table 3 shows the associations between periconception folic acid use and maternal blood pressure. Preconception start of folic acid was associated with a small increase in systolic blood pressure and diastolic blood pressure throughout pregnancy as compared to women who did not use folic acid. Similar trends towards a higher maternal blood pressure were observed for women who started folic acid after pregnancy recognition. Figure 2 shows the results from the repeated measurement analyses. Overall, systolic and diastolic blood pressures were highest in women who reported to have started using folic acid preconceptionally and lowest in women who reported not to have used folic acid. The differences were significantly different compared to the reference group. In contrast, the patterns of blood pressure change did not significantly differ between the categories.

The associations between self-reported low dose folic acid use and gestational hypertension and pre-eclampsia are shown in Table 4. After adjustment for potential confounders self-reported low dose periconception folic acid use was not associated with the occurrence of gestational hypertension or pre-eclampsia.

**Figure 2 Predicted systolic and diastolic blood pressure patterns plotted against gestational age at time of measurement, stratified by folic acid category**






## Comment

In this observational study we show that self-reported low dose periconception folic acid use is associated with a small but significant reduction in uteroplacental vascular resistance. Additionally, self-reported periconception folic acid use is associated with a slightly higher maternal blood pressure during pregnancy. The marginal effects remain within physiologic ranges and show no associations with the occurrence of hypertensive pregnancy disorders.

This was a large prospective cohort study with a significant number of measurements performed in the mothers which increases the accuracy of our effect estimates. Complete information on folic acid use was missing in approximately 25% of the cohort. Of these women 14% was lower educated and about 46% was of non-Western ethnicity. The effect estimates could be biased if the associations would differ between those with and without complete data. Even though this seems unlikely it cannot be excluded. Second, the use of questionnaires to assess folic acid use encompasses another potential limitation. For this reason, and in line with previous validation studies, we validated the data on self-reported folic acid use with plasma folate levels in a random subsample.[16] Moreover, we aimed to assess folic acid use as early as possible in pregnancy to minimise recall bias. This was done in 80% of our study population. Last, folic acid use is strongly related to various sociodemographic and behavioural variables.[17] Even though we controlled for a large number of confounders residual confounding is always an issue and therefore to be taken into account. For this reason we also performed regression analyses restricted to Dutch Caucasian primiparous women to increase comparability between the folic acid categories. These models did not materially change the effect estimates.

To our knowledge this is the first study to suggest that a relatively 'small' intervention, i.e. low dose folic acid use, during the periconception period is associated with uteroplacental changes that persist until late pregnancy. Even though the effects are small, and the underlying mechanisms remain to be elucidated, the effects could be due to a reduction of plasma tHcy by folic acid.[18] Several studies have shown a relation between elevated tHcy plasma levels and obstetrical complications due to uteroplacental vascular disorders.[6, 19] While the pathogenic mechanisms underlying these tHcy related diseases are unknown it has been shown that tHcy induces cytotrophoblast apoptosis which is crucial for placental development.[20] Furthermore, *in vitro* studies have provided evidence for a protective role of folic acid in tHcy-induced placental disease.[6, 7] In this respect the opposite may be considered namely that an increased supply of folic acid may diminish apoptosis and subsequently improve trophoblast invasion.[7] Another explanation for the observed associations between folic acid use and uteroplacental vascular resistance could be the involvement of folate in protein synthesis including the building blocks of DNA and transfer ribonucleic acid (tRNA).

These functions are implicated in growth processes of actively proliferating and differentiating tissues.[21] Placental implantation is characterised by vascular remodeling, heightened inflammation, oxidative stress, and rapid cell division.[22-24] Previously, Doshi et al.[25] reported that folate can reduce intracellular superoxide levels indicating a direct role of folate as superoxide scavenger. Additionally, Outinen et al.[26] suggested that the presence of folate might induce antioxidant enzymes expression. For this reason a larger supply of folic acid during the periconception period may not only serve as a substrate for increased DNA and protein synthesis but also influence antioxidant defenses thereby affecting placental implantation and vascular remodeling.[22, 27]

In our experience the monitored higher blood pressure levels for women who reported to have been periconceptionally exposed to folic acid have never been observed. The differences in mean blood pressure can be regarded as small and therefore seem without clinical implications. However, they can provide important insight in maternal adaptation mechanisms during pregnancy. In normal pregnancy profound cardiovascular changes take place which allow for a normal pregnancy course. Maternal haemodynamic adaptation during pregnancy is triggered by a primary fall in systemic vascular tone. Subsequently, intravascular volume increases through plasma volume expansion and an increase in red blood cell mass.[28] This process already begins in the fourth pregnancy week. The increase in red cell mass enhances the need for folate. From this we hypothesise that periconception folic acid use is associated with a relative earlier and larger increase in red cell blood mass. This may decrease the relative hypovolaemia associated with early pregnancy with a subsequent slightly higher maternal blood pressure. Moreover, a positive correlation between red cell measures and blood pressure has been shown before.[29, 30] This may explain why we did not only observe a slight increase in blood pressure among folic acid supplemented women but higher haematocrit levels as well (data not shown). Another explanation could be that tissue specific expression of genes involved in placentation by DNA and histon methylation might also be affected by folic acid.[31] This underlying mechanism of gene-nutrient interaction also has to be studied in more detail. Finally, both folic acid use and blood pressure are known to be linked with sociodemographic and health-related behaviours.[17] It can be suggested that blood pressure at baseline was already higher in women reported to have used folic acid. Unfortunately, we do not have access to baseline blood pressure to rule this out.

Only few studies have examined the associations between folic acid use and pre-eclampsia.[22, 32-34] Wen et al.[32] found that use of multivitamins containing folic acid during the early second trimester was associated with increased serum folate, lowered plasma tHcy, and a 63% reduced risk of pre-eclampsia. Similarly, Bodnar et al.[22] and Hernández-Díaz et al.[34] observed that regular use of multivitamins was associated with reduced risks for hypertensive pregnancy disorders. These results are in disparity with ours because they suggest that folic acid plays a protective role in the occurrence of pre-eclampsia. However, as all these studies used multivitamin supplements the differential effects of folic acid itself from that of other components in the regimen cannot be distinguished.[22, 32-34] Only one study assessed folic acid alone and did not observe associations between women who used folic acid as a single preparation and pre-eclampsia.[32] This may imply that other nutrients or a combination of those present in multivitamins may be implicated.[22, 35]

In conclusion, self-reported periconception folic acid use seems to be significantly associated with uteroplacental vascular resistance and maternal blood pressure during pregnancy. The observed effects are within physiologic ranges and are not related with the occurrence of hypertensive pregnancy disorders. Even though there are no direct clinical implications this study is an important step forward in the further understanding of physiologic adaptation mechanisms during pregnancy and in particular of periconception nutritional exposures on early placentation processes. Future studies focused on folate biomarkers are required to replicate the associations.

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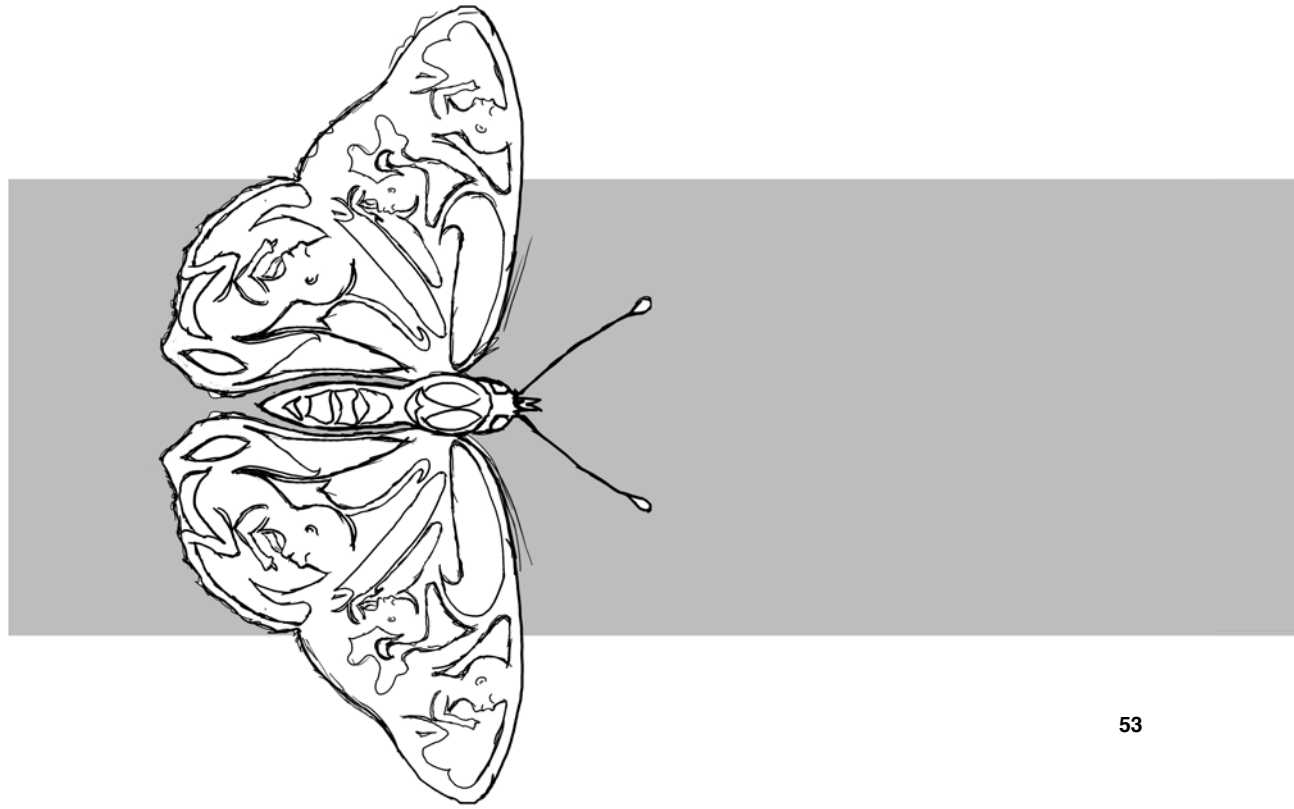
# Chapter 3

# Dietary patterns



# Chapter 3.1

# The Mediterranean diet substantially affects intrauterine growth

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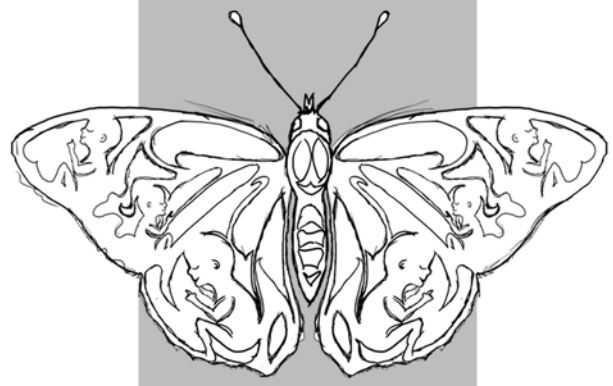


*Submitted for publication*

## Abstract

Developmental adaptations due to nutritional exposures may have permanent health consequences. Studies of diet and fetal growth mainly focused on individual nutrients despite evidence that the pattern of food consumption may be important. The objective of this study was to study associations of dietary patterns in early pregnancy with fetal growth, uteroplacental vascular resistance, placental and birth weight, and being small for gestational age at birth (SGA) in The Generation R Study Rotterdam, The Netherlands. In early pregnancy, 3207 Caucasian pregnant mothers completed a semiquantitative food-frequency questionnaire. The dietary pattern was then generated by logistic regression analysis. The identified dietary pattern, characterised by high intakes of vegetables, vegetable oil, fish, pasta and rice, and low intakes of meat, potatoes, and fatty sauces was designated as 'Mediterranean' dietary pattern. The Mediterranean dietary pattern was positively associated with plasma folate and serum vitamin B12 concentrations and showed an inverse relation with homocysteine and high-sensitive C-reactive protein plasma concentrations (all $p < 0.05$). Low adherence to the Mediterranean dietary pattern was associated with an increased risk of SGA (relative risk 2.78, 95% Confidence Interval (CI) [1.64; 4.76]). Furthermore, various important fetal growth and placental parameters were associated with the dietary pattern revealing a 72 grams lower birth weight (95% CI [-110.8; -33.3]) and a 15 grams lower placental weight (95% CI [-29.8; -0.2]) for women with low adherence to the Mediterranean dietary pattern. In conclusion, low adherence to the Mediterranean dietary pattern impairs intrauterine growth with a lower placental weight and a lower birth weight and an increased risk of SGA.




## Introduction

Fetal growth is an important determinant of future health and development. Increasing evidence suggests that environmental exposures, acting at different stages of fetal development, can cause permanent developmental adaptations that may affect the physiology of various organ systems, leading to fetal growth retardation and / or an increased risk of chronic disease in later life.[1, 2] Maternal nutrition has been recognised as one of the most important extraneous stimuli influencing fetal growth and development.

Until recently, most studies on associations between nutrition and pregnancy outcome focused on individual foods or nutrients. However, nutrition represents a complex set of foods containing nutrients that are strongly correlated. It is still largely unclear to what extent the combinations and balances of nutrients matter.[3]

During the past years, identification of dietary patterns through data-driven methods has increasingly gained interest. First studies have shown significant associations between dietary patterns and congenital anomalies, encompassing neural tube defects and orofacial clefts.[4, 5] In this respect, dietary patterns have also been related to biomarker concentrations in blood that are known to be important intermediates for placental development, embryogenesis, and fetal programming, including folate, vitamin B12, total homocysteine (tHcy), and high-sensitive C-reactive protein (Hs-CRP).[4, 6] Except for one study on birth weight, used as a proxy for fetal growth, we are unaware of studies investigating the relation between dietary patterns in early pregnancy and growth adaptations during fetal life.[7]

Within The Generation R Study in Rotterdam, The Netherlands, a large prenatally recruited birth cohort with detailed fetal growth measurements in pregnancy, we aimed to examine the associations of maternal dietary patterns with being small for gestational age at birth (SGA), uteroplacental vascular resistance, placental weight, fetal growth, and birth weight. The present study was restricted to an ethnic homogenous population, since nutrition generally differs between ethnic groups.[8]

## Materials and Methods

### *Study design*

The present study was embedded in The Generation R Study, an ongoing population-based cohort study that examines early determinants of growth, development, and health from fetal life until young adulthood.[9, 10] Between December 2001 and January 2006 pregnant women living in Rotterdam, The Netherlands were invited. Fetal growth and its main determinants were repeatedly assessed by fetal ultrasonography, physical examinations, biological samples, and detailed questionnaires. For the present study analyses were restricted to prenatally enrolled Dutch women with a spontaneously conceived singleton pregnancy (n = 3218).[11]

The study was conducted in accordance with the guidelines proposed in the World Medical Association Declaration of Helsinki.[12] Approval for the study was obtained from the Medical Ethics Committees of all participating hospitals and every participant provided written informed consent.[9, 10]

***Dietary assessment***

Nutritional intake was assessed at enrolment (median 13.5 weeks of gestation, interquartile range 3.4) using a modified version of the validated semiquantitative food frequency questionnaire (FFQ) of Klipstein-Grobusch et al.[13] This FFQ covers food intake over the prior three months. The FFQ consists of 293 items structured to meal pattern. Questions include consumption frequency, portion size preparation method, and additions. Portion sizes were estimated using household measures and photographs.[14] To calculate average daily nutritional values the Dutch food composition table 2006 was used.[15]

***Fetal and placental parameters***

Fetal ultrasound measurements plus medical records were used to obtain information about the primary outcome variables: fetal growth until birth, placental weight, placental resistance, birth weight and SGA. Ultrasound measurements were used to establish gestational age in early pregnancy (planned at gestational age 12 weeks) and to assess fetal biometry including head circumference, abdominal circumference, femur length, and estimated fetal weight in mid-pregnancy (planned at gestational age 20 weeks ) and late pregnancy (planned at gestational age 30 weeks). Furthermore, longitudinal growth curves and gestational-age-adjusted standard deviation (SD) scores were constructed.[16] The intra- and interobserver reproducibility scores of the ultrasound measurements have been described before.[17] Uteroplacental resistance, measured by colour Doppler, was assessed by the umbilical artery pulsatility index and the uterine artery resistance index in late pregnancy. SGA was defined as a SD-score < -2.0 (< 2.3th percentile) at birth.

***Biomarkers***

In early pregnancy venous blood serum samples and plasma (EDTA) samples were drawn and stored at room temperature before being transported to the regional laboratory for storage at -80 °C.[9] To analyse folate, vitamin B12, tHcy, and Hs-CRP concentrations, EDTA plasma samples (folate, tHcy, Hs-CRP) and serum samples (vitamin B12) were picked and transported to the Department of Clinical Chemistry of the Erasmus MC - University Medical Centre Rotterdam in 2008. After thawing folate, vitamin B12, tHcy, and Hs-CRP concentrations were analysed using a microparticle-enhanced immunoassay on the AxSYM and Architect system (Abbott Diagnostics B.V., Hoofddorp, The Netherlands). The between-run coefficients of variation for plasma folate were 8.9% at 5.6 nmol / L, 2.5% at 16.6 nmol / L, and 1.5% at 33.6 nmol / L; the coefficients of variation for tHcy were 3.1% at 7.6 mcmol / L, 3.1% at 13.7 mcmol / L, and 2.1% at 26.1 mcmol / L, the coefficients of variation for Hs-CRP were 0.9% at 12.8 mg / L, and 1.3% at 39.3 mg / L, and the coefficients of variation for vitamin B12 were 3.6% at 148 pmol / L, 2.7% at 295 pmol / L, and 3.1% at 590 pmol / L. Biomarker concentrations in early pregnancy were available in 78% of the study population. No differences in nutritional intake were observed between women with and without biomarker concentrations (p = 0.76).

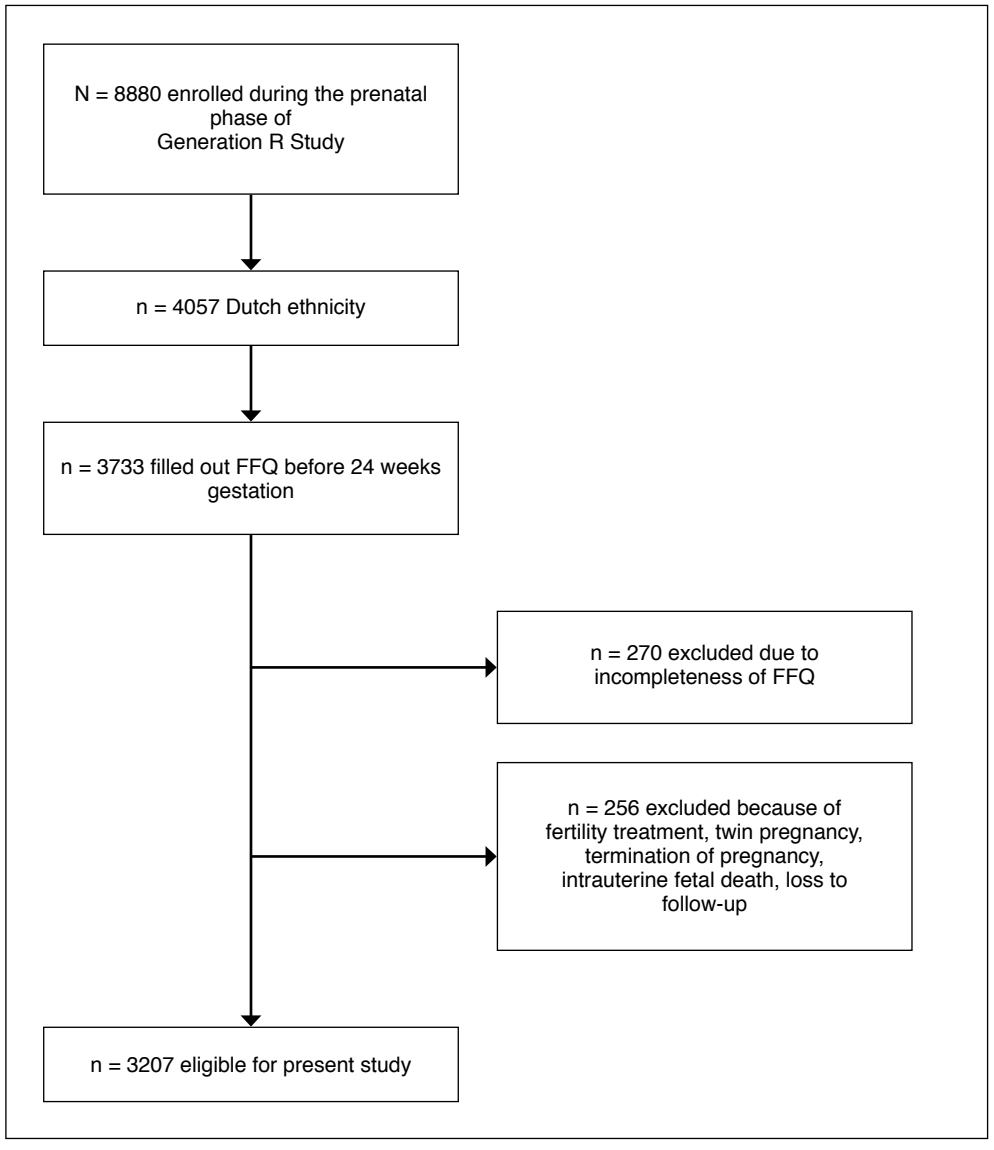
***Covariates***

Data on maternal age, educational level, parity, smoking habits, periconception folic acid use, vomiting during first trimester, and maternal comorbidity (defined as the occurrence of chronic hypertension, and / or heart disease, and / or diabetes, and / or high cholesterol, and / or thyreoid disease, and / or Systemic Lupus Erythematosus) was available from questionnaires. Educational level was assessed by the highest completed education of the mother and classified as 1) low education; 2) middle education; 3) high education.[18] At enrolment maternal height and weight were measured to calculate body mass index (BMI, kg / m2). Information on fertility treatment, fetal gender, and pregnancy outcome was obtained from midwifes, and obstetricians.

***Data analysis***

After exclusion of 11 cases of intrauterine fetal deaths the sample available for final analysis was n = 3207 (Figure 1). Logistic regression analysis was used to predict the occurrence of SGA (yes / no [0 / 1]) as a function of food intake. To arrive at the logistic regression solution the 293 food items were first reduced to 21 predefined food groups.[4, 19] Subsequently, food groups were adjusted for energy intake.[20] Then, logistic regression analysis was performed to identify the dietary pattern predicting SGA. In this study the probability for SGA seemed to express adherence to a Mediterranean-like dietary pattern. Hence, here we call the logistic regression solution: 'adherence to the Mediterranean dietary pattern'. For reasons of interpretability, all women were categorised into tertiles according to their probability score for the dietary pattern, namely: 1) low adherence; 2) medium adherence; 3) high adherence. To test differences in baseline characteristics between the three dietary pattern categories the Analysis of Variance (ANOVA) and chi-square test were used. Likewise, trend tests (linear regression) were used to relate the three dietary pattern groups to the biomarker concentrations and nutrient intakes. We assumed that nutrition affects growth processes underlying SGA in a comparable manner as fetal and placental growth. Therefore, linear regression was used to assess cross-sectional differences between the dietary pattern categories in 1) fetal growth characteristics, and 2) placental parameters. In the multiple regression analyses the inclusion of confounding variables was based on earlier literature, and determined a priori. These were maternal age, parity, educational level, height, weight, smoking habits, folic acid use, vomiting, comorbidity, fetal gender, and gestational age. Potential confounders were selected if the effect estimates changed ≥ 10% in exploratory analyses. By using this approach vomiting and

**Figure 1 Flow chart of study population**




comorbidity were not included into the final multiple analyses. Missing data on the missing covariables BMI (0.4%), educational level (0.5%), parity (0.1%), smoking habits (6.7%), folic acid use (16.7%), vomiting (7.2%), and comorbidity (6.4%) were completed using the Markov Chain Monte Carlo multiple imputation technique. Five completed data sets were created. Subsequently, multiple regression analyses were performed separately on each completed dataset and thereafter combined to one pooled estimate.[21] Lastly, effect modification was tested by multiplying the personal dietary pattern scores with the covariables educational level, parity, smoking, BMI, and periconception folic acid use. If $p < 0.10$ was fullfilled multiple linear regression analyses were performed in strata of that specific determinant. The statistical software package SPSS 17.0 (SPSS Inc, Chicago, IL, USA) was used for data analyses.

## Results

Nutritional intake characteristics are shown in Table 1. The identified Mediterranean dietary pattern comprised of relatively high intakes of pasta, rice, vegetable oils, fish, vegetables, and alcohol, and low intakes of meat, potatoes, and fatty sauces (all $r \geq 0.20$ and $p < 0.01$). Low adherence to the Mediterranean dietary pattern was associated with almost three-fold increased risk for SGA (relative risk (RR) 2.78, 95% Confidence Interval (CI) [1.64; 4.76]).

**Table 1 Spearman's Rank correlation coefficients for the relationship between food intake and adherence to the Mediterranean dietary pattern**

| | Adherence to the Mediterranean dietary pattern | |
|---|---|---|
| **Food group** | **Correlation coefficient** | **p value** |
| Pasta, rice | 0.61 | < 0.01 |
| Bread | 0.33 | < 0.01 |
| Vegetable oil | 0.29 | < 0.01 |
| Fish | 0.26 | < 0.01 |
| Alcoholic drinks | 0.23 | < 0.01 |
| Vegetables | 0.22 | < 0.01 |
| Fruit | 0.18 | < 0.01 |
| Breakfast cereals | 0.09 | < 0.01 |
| Soy and diet products | 0.07 | < 0.01 |
| Margarine | 0.06 | < 0.01 |
| Sweets | 0.0 | NS |
| Butter | -0.02 | NS |
| Dairy products | -0.03 | < 0.05 |
| Non-alcoholic drinks | -0.03 | < 0.01 |
| Soup | -0.03 | NS |
| Starches and wheat | -0.04 | NS |
| Legumes | -0.09 | < 0.01 |
| Eggs | -0.11 | < 0.01 |
| Potatoes | -0.26 | < 0.01 |
| Meat | -0.30 | < 0.01 |
| Sauces and condiments | -0.36 | < 0.01 |

NS: not significant

**Table 2 Baseline characteristics**

| | Mediterranean dietary pattern | | | |
|---|---|---|---|---|
| | Low adherence | Medium adherence | High adherence | p value |
| | n=1070 | n=1068 | n=1069 | |
| **Mean maternal age (years)** | 30.2 (4.8) | 31.5 (4.2) | 32.3 (3.7) | < 0.01 |
| **Median BMI (kg / m2)** | 23.9 (5.2) | 23.3 (4.2) | 22.8 (3.7) | < 0.01 |
| **Educational level (%)** | | | | |
| Low | 6.5 | 2.0 | 0.7 | < 0.01 |
| Medium | 55.0 | 34.1 | 22.7 | |
| High | 37.9 | 63.5 | 76.0 | |
| Missing | 0.6 | 0.5 | 0.6 | |
| **Parity (%)** | | | | |
| 0 | 60.9 | 60.6 | 58.9 | NS |
| ≥ 1 | 39.1 | 39.2 | 41.1 | |
| Missing | 0 | 0.2 | 0 | |
| **Smoking (%)** | | | | |
| Yes, still | 25.1 | 10.6 | 9.4 | < 0.01 |
| Yes, stopped | 8.2 | 8.7 | 8.2 | |
| No | 60.0 | 73.8 | 75.9 | |
| Missing | 6.6 | 6.9 | 8.2 | |
| **Folic acid use (%)** | | | | |
| No | 12.7 | 7.7 | 6.8 | < 0.01 |
| Yes, postconception start | 27.0 | 28.5 | 28.5 | |
| Yes, preconception start | 43.1 | 46.2 | 49.5 | |
| Missing | 17.2 | 17.7 | 15.2 | |
| **Vomiting (%)** | | | | |
| Yes, severe | 16.2 | 13.4 | 9.1 | < 0.01 |
| Yes, moderate | 20.7 | 19.6 | 20.3 | |
| No | 55.7 | 59.7 | 63.6 | |
| Missing | 7.4 | 7.3 | 7.0 | |
| **Comorbidity (%)** | | | | |
| Yes | 7.0 | 4.5 | 4.9 | < 0.05 |
| No | 94.0 | 88.9 | 88.3 | |
| Missing | 6.0 | 6.6 | 6.8 | |
| **Male Gender (%)** | 50.5 | 49.5 | 51.7 | NS |
| **SGA (%)** | 4.7 | 1.8 | 1.7 | < 0.01 |
| **Mean pulsatility index arteria umbilicalis** | 0.99 (0.20) | 0.98 (0.16) | 0.96 (0.15) | < 0.01 |
| **Mean resistance index arteria uterina** | 0.49 (0.18) | 0.47 (0.08) | 0.48 (0.08) | < 0.05 |
| **Mean placental weight (grams)** | 629.5 (143.8) | 648.2 (148.9) | 646.6 (148.3) | < 0.05 |
| **Median gestational age (weeks)** | 40.1 (1.9) | 40.3 (1.9) | 40.3 (1.9) | NS |
| **Mean birth weight (grams)** | 3424.3 (566.1) | 3514.4 (557.9) | 3521.7 (536.7) | < 0.01 |

Values represent percentage (%) within column, mean (SD), or median (interquartile range). ANOVA and chi-square test tested overall differences in baseline characteristics between the dietary pattern categories



Maternal characteristics associated with low adherence to the dietary pattern were younger maternal age, higher BMI, lower educational level, a lower frequency of folic acid use, and continued smoking (Table 2).

Lower folate and vitamin B12 concentrations were observed among women with low adherence to the dietary pattern (Table 3).

**Table 3 Biomarker concentrations and nutrient intakes**

| | Mediterranean dietary pattern | | | | | | |
|---|---|---|---|---|---|---|---|
| | Low adherence | | Medium adherence | | High adherence | | |
| | Median | IQR | Median | IQR | Median | IQR | p trend |
| **Biomarker concentrations** | **n = 846** | | **n = 837** | | **n = 836** | | |
| Folate (nmol/L)* † | 18.2 | 13.9 | 18.8 | 12.7 | 20.0 | 13.3 | <.01 |
| tHcy (mcmol/L)* † | 7.1 | 2.1 | 7.0 | 1.9 | 7.0 | 1.7 | <.01 |
| Vitamin B12 (pmol/L)* † | 168.5 | 96.8 | 178.0 | 101.5 | 180.0 | 103.3 | <.01 |
| Hs-CRP (mg/L)* | 4.8 | 5.8 | 4.1 | 5.8 | 3.6 | 4.3 | <.01 |
| **Energy and macronutrients** | **n = 1070** | | **n = 1068** | | **n = 1069** | | |
| Energy (kJ/day) | 8969.7 | 3311.3 | 8817.0 | 2974.9 | 8968.7 | 2834.2 | NS |
| Fat (% of energy) | 37.0 | 7.5 | 36.5 | 7.3 | 35.8 | 6.8 | <.01 |
| Total fat (g/day)‡ | 80.2 | 16.5 | 79.0 | 15.8 | 77.6 | 15.0 | <.01 |
| Saturated lipids (g/day)‡ | 30.0 | 6.9 | 28.9 | 6.7 | 28.0 | 6.5 | <.01 |
| Mono unsaturated lipids (g/day)‡ | 28.7 | 6.8 | 28.3 | 6.7 | 28.0 | 6.7 | <.01 |
| Poly unsaturated lipids (g/day)‡ | 17.8 | 6.5 | 18.3 | 6.7 | 18.2 | 6.6 | NS |
| Linoleic acid (g/day)‡ | 14.2 | 5.6 | 14.8 | 5.9 | 14.7 | 5.9 | NS |
| Cholesterol (mg/day)‡ | 176.1 | 62.3 | 160.3 | 54.8 | 154.7 | 53.3 | <.01 |
| Ratio unsaturated lipids/ saturated lipids | 1.5 | 0.4 | 1.6 | 0.4 | 1.7 | 0.4 | <.01 |
| Protein (% of energy) | 14.5 | 3.2 | 14.9 | 3.0 | 15.1 | 2.8 | <.01 |
| Total protein (g/day)‡ | 71.8 | 15.4 | 73.1 | 14.0 | 74.6 | 13.1 | <.01 |
| Vegetable protein (g/day)‡ | 25.1 | 6.6 | 28.5 | 5.8 | 30.1 | 5.8 | <.01 |
| Animal protein (g/day)‡ | 45.5 | 14.4 | 44.4 | 14.1 | 44.4 | 14.6 | <.01 |
| Carbohydrate (% of energy) | 48.2 | 8.6 | 48.3 | 7.9 | 48.4 | 7.6 | NS |
| Total carbohydrate (g/day)‡ | 233.9 | 42.1 | 235.0 | 38.8 | 234.5 | 37.1 | NS |
| Carbohydrates monosaccharides (g/day)‡ | 135.9 | 43.6 | 127.5 | 39.5 | 121.2 | 36.1 | <.01 |
| Carbohydrates polymers (g/day)‡ | 95.7 | 23.5 | 104.6 | 21.2 | 111.7 | 21.6 | <.01 |
| Fiber (g/day)‡ | 19.2 | 5.9 | 21.8 | 5.7 | 23.3 | 5.9 | <.01 |

Linear regression tested for trend
*p values for all biomarker concentrations are adjusted for gestational age at venous puncture
†p values for folate, tHcy, and vitamin B12 are additionally adjusted for folic acid use
‡Adjusted for energy intake

Low adherence to the Mediterranean dietary pattern was also associated with higher tHcy and Hs-CRP concentrations. Compared to women with high adherence, women with a low adherence to the Mediterranean dietary pattern had a higher percentage of energy derived from fat and a lower ratio of unsaturated to saturated lipids. These women also consumed relatively lower amounts of vegetable protein, carbohydrate polymers, and fiber.

Table 4 shows the associations between the dietary patterns categories and placental weight and uteroplacental resistance. Placental weight was 15 grams lower in women with low adherence to the Mediterranean dietary pattern compared to placental weight of women with high adherence (95% CI [-29.8; -0.2]). A similar, though not significant, trend towards higher uteroplacental vascular resistance was observed for women with a low adherence to the Mediterranean dietary pattern.

**Table 4 Associations between dietary pattern categories and placental parameters**

| Mediterranean dietary pattern | Placental weight | | Pulsatility index arteria umbilicalis | | Resistance index arteria uterina | |
|---|---|---|---|---|---|---|
| | Crude* | Adjusted† | Crude* | Adjusted† | Crude* | Adjusted† |
| **Low adherence** | -12.2 | -15.0 | 0.02 | 0.01 | 0.01 | 0.01 |
| | [-26.5; 2.1] | [-29.8; -0.2] | [0.01; 0.04] | [-0.00; 0.03] | [0.00; 0.03] | [-0.00; 0.03] |
| **Medium adherence** | 1.1 | -0.78 | 0.02 | -0.01 | -0.00 | 0.02 |
| | [-13.1; 15.2] | [-14.9; 13.3] | [0.00; 0.03] | [0.01; -0.02] | [-0.02; 0.01] | [-0.00; 0.03] |
| **High adherence** | Reference | Reference | Reference | Reference | Reference | Reference |
| **p trend** | NS | < 0.05 | < 0.05 | NS | NS | NS |

Results from simple and multiple linear regression analyses. Values are regression coefficients (95% Confidence Interval) and reflect the difference in grams for placental weight and the difference in uteroplacental resistance in late pregnancy, compared to reference. Uteroplacental resistance analyses are based on respectively 2613 arteria umbilicalis Doppler measurements and 1956 arteria uterina Doppler measurements
*Crude: adjusted for gestational age
†Adjusted: additionally adjusted for maternal age, height, weight, parity, fetal gender, educational level, smoking habits, and folic acid use

The associations between maternal adherence to the Mediterranean dietary pattern and fetal growth characteristics in mid- and late pregnancy are presented in Table 5. Low adherence to the Mediterranean dietary pattern was associated with smaller abdominal circumference in late pregnancy (difference in SD = -0.16, 95% CI [-0.24; -0.07]). A similar, but not significant, trend towards smaller head circumference in late pregnancy was also observed for women with a low adherence to the Mediterranean dietary pattern (difference in SD = -0.08, 95% CI [-0.17; 0.01]).

Figure 2 shows the differences in estimated fetal weight and birth weight between the three dietary pattern categories. Women with low adherence to the Mediterranean dietary pattern



**Table 5 Associations between dietary pattern categories and fetal growth characteristics**

| | Mid-pregnancy | | | | | |
|---|---|---|---|---|---|---|
| **Mediterranean dietary pattern** | **SD head circumference** | | **SD abdominal circumference** | | **SD femur length** | |
| | Crude* | Adjusted† | Crude* | Adjusted† | Crude* | Adjusted† |
| **Low adherence** | -0.02 | 0.01 | -0.08 | -0.05 | 0.11 | 0.07 |
| | [-0.11; 0.07] | [-0.09; 0.10] | [-0.16; 0.01] | [-0.14; 0.04] | [0.02; 0.19] | [-0.02; 0.16] |
| **Medium adherence** | -0.00 | 0.01 | -0.04 | -0.03 | 0.05 | 0.02 |
| | [-0.09; 0.09] | [-0.08; 0.10] | [-0.13; 0.05] | [-0.12; 0.06] | [-0.04; 0.13] | [-0.06; 0.11] |
| **High adherence** | Reference | Reference | Reference | Reference | Reference | Reference |
| **p trend** | NS | NS | < 0.05 | NS | < 0.05 | NS |

| | Late pregnancy | | | | | |
|---|---|---|---|---|---|---|
| **Mediterranean dietary pattern** | **SD head circumference** | | **SD abdominal circumference** | | **SD femur length** | |
| | Crude* | Adjusted† | Crude* | Adjusted† | Crude* | Adjusted† |
| **Low adherence** | -0.11 | -0.08 | -0.16 | -0.16 | 0.01 | 0.01 |
| | [-0.20; -0.02] | [-0.17;0.01] | [-0.24; -0.07] | [-0.25; -0.07] | [-0.08; 0.09] | [-0.08; 0.09] |
| **Medium adherence** | -0.04 | -0.03 | -0.05 | -0.06 | 0.01 | 0.00 |
| | [-0.12; 0.05] | [-0.12; 0.05] | [-0.14; 0.03] | [-0.15; 0.03] | [-0.07; 0.10] | [-0.08; 0.08] |
| **High adherence** | Reference | Reference | Reference | Reference | Reference | Reference |
| **p trend** | < 0.01 | NS | < 0.01 | < 0.01 | NS | NS |

Results from simple and multiple linear regression analyses. Values are regression coefficients (95% Confidence Interval) and reflect the difference in SD-score of each growth charateristic, compared to reference. Analyses are based on respectively 3138 head circumference measurements, 3139 abdominal circumference measurements, and 3136 femur length measurements in mid-pregnancy; and 3111 head circumference measurements, 3136 abdominal circumference measurements, and 3145 femur length measurements in late pregnancy
*Crude: adjusted for gestational age
†Adjusted: additionally adjusted for maternal age, height, weight, parity, fetal gender, educational level, smoking habits, and folic acid use

had significantly smaller fetuses from late-pregnancy onwards (difference in SD at 30 weeks of gestation = -0.10, 95% CI [-0.19; -0.02]). This difference became more pronounced at birth revealing a 72 grams lower birth weight in infants of women with low adherence to the Mediterranean dietary pattern (difference in grams = -72.0, 95% CI [-110.8; -33.3]).

Educational level and smoking modified the effect of use of the Mediterranean dietary pattern on birth weight (both interaction terms $p < 0.10$). Compared to high educated women with high adherence, low adherence to the dietary pattern was associated with a 131 grams lower birth weight (95% CI [-180.9; -81.2]) among middle educated women, and a 160 grams lower birth weight among low educated women (95% CI [-271.4; -50.2]) (Figure 3). Compared to non-smoking women with high adherence to the Mediterranean dietary pattern, smoking during pregnancy combined with low adherence to the Mediterranean dietary pattern was associated with a 214 grams lower birth weight (95% CI [-269.3; -159.6]). In contrast, birth

weight was approximately 66 grams lower in women who continued to smoke during pregnancy with high adherence to the dietary pattern (95% CI [-130.6; -2.5]). No further significant effect modification on the additive scale was observed for parity, BMI, and folic acid use (all inter-action terms $p > 0.10$).

## Comment

This is the first study to examine the relationship between dietary patterns in early pregnancy and fetal and placental growth during intrauterine life. A Mediterranean dietary pattern affects fetal and placental growth with low adherence resulting in a substantially increased risk of having a small for gestational age child at birth.

During past years, the Mediterranean diet has gained considerable attention for its positive health effects.[4, 22] There is no single Mediterranean diet. However, dietary patterns that prevail in the Mediterranean region share common characteristics including high intakes of vegetables and vegetables oil, moderate amounts of fish, poultry, and alcohol, and relatively low consumptions of meat.[23] Adherence to the Mediterranean dietary pattern in our study population was reflected by high amounts of vegetable protein, carbohydrates polymers, fiber, and a favourable ratio of unsaturated to saturated lipids. Increasing concentrations of the biomarkers folate and vitamin B12, and decreasing tHcy concentrations further validated adherence to the dietary pattern. These biomarker concentrations partly represent mother's dietary intake as they also depend on lifestyle, genetic factors, and endocrine- and metabolic functions.

The B-vitamins folate and vitamin B12 serve as substrates and cofactors in several pathways of cellular processes, including cell multiplication, apoptosis, and intracellular signaling.[24] These processes can also be affected by saturated fats, trans fatty acids and cholesterol.[25] For this reason, adherence to a diet characterised by relatively high concentrations of these B-vitamins and a favourable lipid profile is likely to affect intrauterine growth. The results of this study are comparable with our previous findings on folic acid use in relation to infant low birth weight.[26] The results are further supported by one other study showing that use of a diet rich in vegetables, fruit, poultry, and fish was associated with a 25% reduced risk for SGA, as compared to a diet rich in meat, dairy products, snacks, and potatoes.[7] It is noteworthy that this latter study primarily focused on birth weight as proxy for fetal growth. Yet, birth weight is the end-point of different growth patterns determined by multiple constitutional and environmental factors. Studying fetal growth trajectories may better distinguish growth restricted from constitutionally small infants.

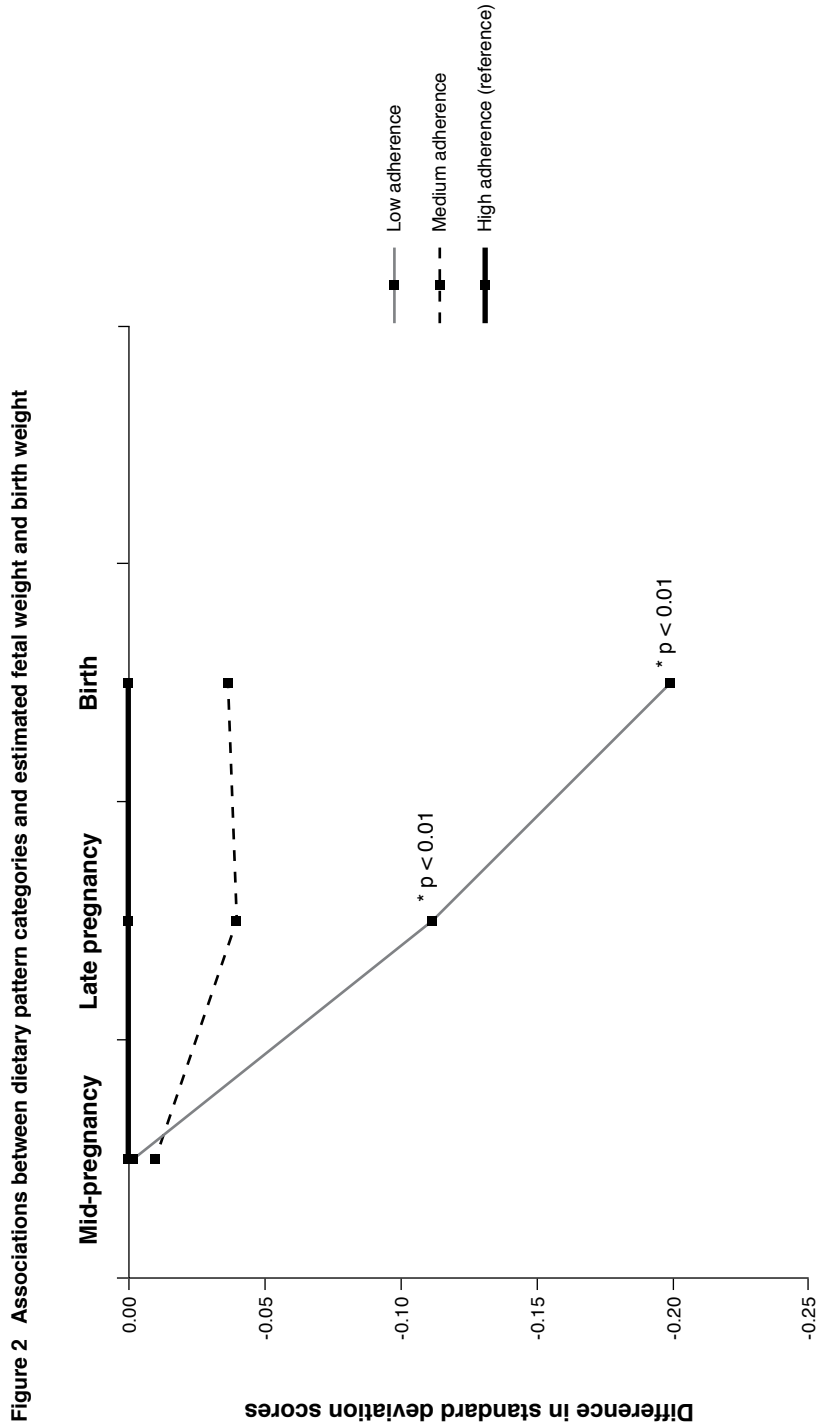
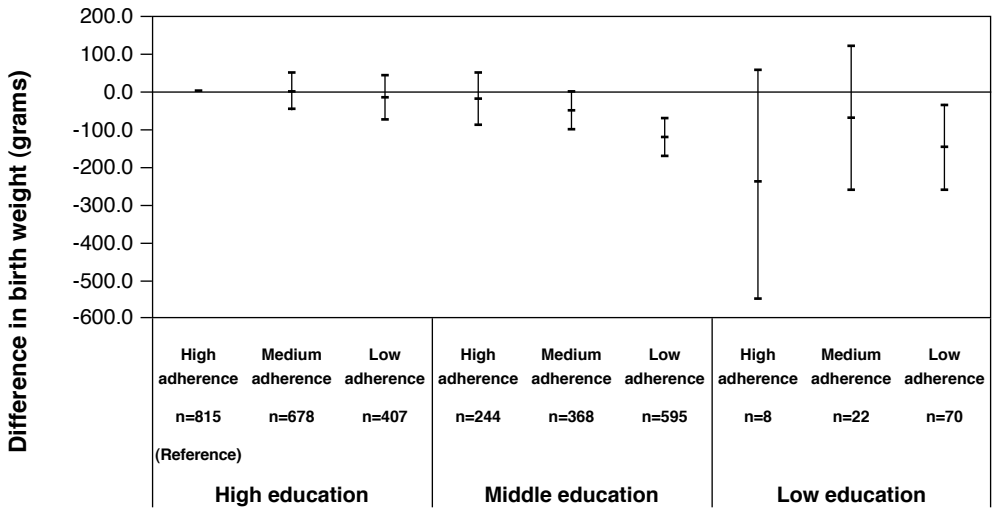
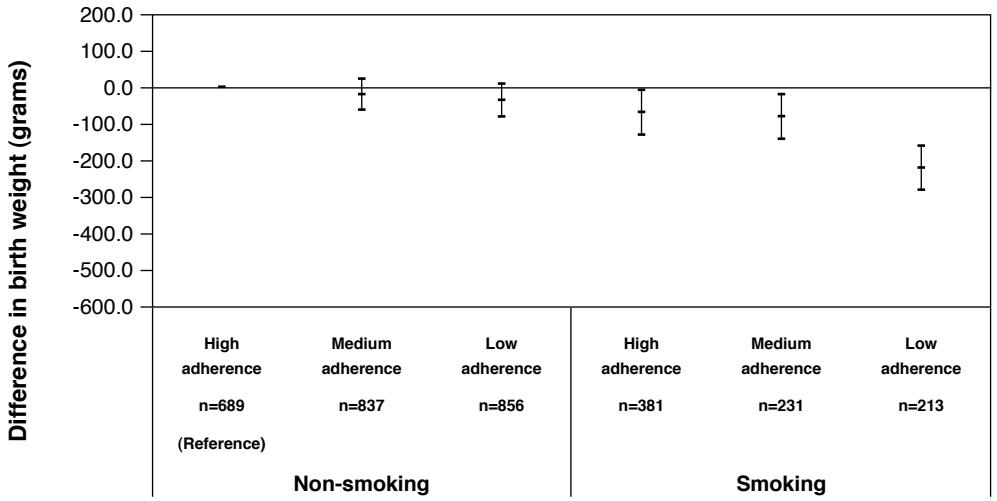



**Figure 2** Associations between dietary pattern categories and estimated fetal weight and birth weight

Results from multiple linear regression analyses. Values reflect the differences in gestational-age-adjusted standard deviation scores of estimated fetal weight / birth weight in mid-pregnancy (n = 3133 measurements), and late pregnancy (n = 3121 measurements), and at birth (n = 3195 measurements) for fetuses / infants of women with low or medium adherence to the Mediterranean dietary pattern, relative to fetuses of women with high adherence (reference). Values are adjusted for maternal age, height, weight, parity, fetal gender, educational level, smoking habits, and folic acid use

**Figure 3 Associations between use of the Mediterranean dietary pattern and birth weight, stratified by educational level and smoking habits**




Results from multiple linear regression analyses stratified per dietary adherence / educational level; dietary adherence / smoking habits category. Values are regression coefficients (95% Confidence Interval) and reflect the difference in birth weight in grams, compared to reference. All values are adjusted for maternal age, height, weight, parity, fetal gender, folic acid use, educational level (only analysis regarding smoking habits), and smoking habits (only analysis regarding educational level)



Both restricted and accelerated fetal growth have been linked with alterations in placental development and function.[27] In this study women with low adherence to the Mediterranean dietary pattern did not only have smaller placentas but also tended towards higher uteroplacental vascular resistance. This seems biologically plausible since early placentation is characterised by vascular remodeling, increased inflammation, oxidative stress, and rapid cell division.[28, 29] In both pregnant and non-pregnant populations these processes have been linked to nutrients associated with the Mediterranean dietary pattern, including folate, n-3 fatty acids, and antioxidant vitamins.[30, 31] Moreover, comparable dietary patterns have been demonstrated to reduce markers of inflammation and endothelial dysfunction, including CRP and E-selectin.[30, 31] Low concentrations of antioxidant vitamins, magnesium and fiber may underlie these biological processes.

The Mediterranean dietary pattern is an important source of methyldonors. Differences in quantitative methylation may affect genes implicated in placental and fetal growth. Our results might suggest that fetal and placental programming can be affected by use of the Mediterranean dietary pattern in early pregnancy.[2] This is supported by recent findings that periconception folic acid use is associated with epigenetic changes in the insulin-like growth factor 2 (IGF2) gene in the child thereby potentially affecting intrauterine programming.[32] This could also apply to our results, suggesting that maternal diet may cause epigenetic modifications in the embryo resulting in altered growth patterns. However, at this moment these underlying mechanisms are just getting started to be explored in humans.

The association between use of the Mediterranean dietary pattern and birth weight differed according to educational level. The relation between socioeconomic status and birth weight has been well established.[33] In general, low educated women practise a less healthy lifestyle.[34] It is conceivable that poor dietary habits of low educated women exacerbated potential harmful effects of other unhealthy behaviours on fetal growth. In this respect we also reported on the modifying effect of smoking in this study sample. Previously, Jaddoe et al.[35] showed that smoking during pregnancy impaired fetal growth. Smoking during pregnancy has also been reported to induce morphological and functional changes in the placenta leading to a reduction of fetal-placental blood flow.[36] We recently observed that smoking modified the effect of folic acid use on first trimester growth.[37] This could suggest a significant role for folate in our observed associations. Both smoking and folate are involved in DNA methylation which may subsequently affect fetal growth.[32] Moreover, Jauniaux et al.[38] described that folate concentrations in serum and coelomic fluid were lower in smokers than non-smokers, which implies that smoking leads to an impaired bioavailability of folate.

The use of dietary pattern analysis has been commonly accepted as it provides essential and complementary insights into overall dietary behaviour associated with disease risk. The major

challenge remains to establish a quantitative method to identify eating patterns. This involves several arbitrary decisions, including the consolidation of food items to food groups and the selected extraction method.[39] Similar to factor analysis, logistic regression analysis is a method to extract directions, i.e. combinations of variables. However, in logistic regression analysis the directions are selected based on their ability to predict a binary response variable.[40] We cross-validated our results in random subgroup. Moreover, the addition or deletion of one or two food groups did not much affect the general pattern of findings.

Nutritional studies are always prone to some bias, including imprecise measurement of nutritional intake. Several studies compared results of dietary pattern analysis using FFQs with those using weighted dietary records, and observed no differences.[41] Moreover, in prospective studies, with the exposure measured before occurrence of the outcome, imprecise measurement of nutritional intake is likely to be random. Another limitation is the potential for residual confounding. Although we attempted to account for this by restricting to an ethnic homogeneous population and by controlling for a large number of confounders, we cannot rule out that residual confounding occured. Lastly, nutritional intake was assessed in early pregnancy because of evidence that the trajectory of fetal growth and development is set at this stage.[1, 2] It could be argued that nutritional intake differs throughout pregnancy. Cucó et al.[42] investigated dietary patterns during different pregnancy periods and observed no significant differences over time.

In conclusion, adherence to the Mediterranean dietary pattern is significantly associated with several features of intrauterine growth resulting in a lower placental weight and a lower birth weight for women with low adherence to the Mediterranean dietary pattern. Further research is warranted to study the effects of dietary patterns and their interference on underlying epigenetic mechanisms and subsequent consequences for postnatal growth and future health.



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# Chapter 3.2

# Major dietary patterns and maternal blood pressure patterns during pregnancy

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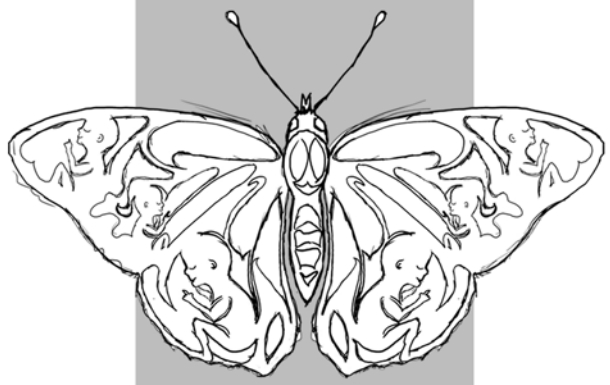


*Submitted for publication*

## Abstract

Maternal diet may influence cardiovascular adaptation to pregnancy. Previous studies focused on single nutrients despite evidence that the combination of nutrients is also important. For this reason we evaluated the associations between dietary patterns and systolic and diastolic blood pressure (SBP, DBP), and the risks of gestational hypertension and pre-eclampsia in 3187 Caucasian pregnant women participating in a prospective cohort study. Participants completed a food-frequency questionnaire in early pregnancy after which the 'Mediterranean' and 'Traditional' dietary patterns were identified using factor analysis. The Mediterranean dietary pattern comprised of high intakes of vegetables, vegetable oils, pasta, rice, fish and legumes, and the Traditional dietary pattern of high intakes of meat and potatoes. A consistent higher SBP was observed throughout pregnancy among mothers with high adherence to the Traditional dietary pattern. Low adherence to the Mediterranean dietary pattern was also associated with higher SBP but only in early and mid-pregnancy. A higher DBP in early, mid- and late pregnancy was observed in both mothers with high adherence to the Traditional dietary pattern and low adherence to the Mediterranean dietary pattern. These effect estimates were most pronounced in mid-pregnancy. Traditional or Mediterranean dietary patterns were not associated with the risks of gestational hypertension and pre-eclampsia. In conclusion, low adherence to a Mediterranean diet and high adherence to a Traditional diet are associated with a higher blood pressure in pregnancy. The effects are within physiologic ranges and seem not to be associated with the risks of gestational hypertenstion or pre-eclampsia.


## Introduction

Important maternal cardiovascular changes occur during normal pregnancy including an increase in maternal blood volume that is preceded by vasodilatation, which results in a drop in blood pressure during the first half of gestation before returning to pre-pregnancy values towards term.[1, 2] In mothers who develop an elevated blood pressure or pre-eclampsia, abnormal cardiovascular adaptation occurs reflected by a different pattern of blood-pressure change.[2, 3] Even though the aetiology of adverse maternal cardiovascular adaptation to pregnancy remains unknown an important role for the endothelium has been suggested.[2] In this respect, various dietary components, such as fatty acids, arginine,vitamin C and E, and folate, have been hypothesised to influence cardiovascular adaptation to pregnancy partly due to their potential effecs on endothelial function.[4]

Many dietary factors are correlated and related to other lifestyle factors. Moreover, the single nutrient approach does not take biological complexity into account resulting from interactions between nutrients.[5] For this reason, recently a shift towards dietary pattern analysis has emerged as a constructive method to explore the relation between diet and disease. First studies have shown significant associations between dietary patterns and reproductive outcomes.[6, 7] In addition, major dietary patterns have been associated with biomarker concentrations in blood related to endothelial function. These include folate, homocysteine (tHcy), and high-sensitive C-reactive protein (Hs-CRP).[6, 8-10]

The objective of this study was to examine the associations of dietary patterns with maternal blood pressure during pregnancy. Additionally, we focused on the occurrence of gestational hypertension and pre-eclampsia.

## Methods

### *Study design*

This study was embedded in The Generation R Study, a prospective cohort study among 8880 pregnant women and their children of various ethnicities in Rotterdam, The Netherlands.[11, 12] The present study was restricted to prenatally enrolled Dutch women with a live born singleton, without a medical history of chronic hypertension, diabetes mellitus, hypercholesterolemia, heart disorders, and Systemic Lupus Erythematosus (n = 3187).[13] Approval was obtained from the Medical Ethics Committee of the Erasmus MC Rotterdam, The Netherlands. All participants provided written informed consent.[11, 12]

### *Nutritional intake*

Participants responses to a self-administered semiquantitative food-frequency questionnaire (FFQ) assessed nutritional intake in the prior three months. The questionnaire was administered

in early pregnancy (median 13.5 weeks of gestation, interquartile range 3.4), and represented a slightly adapted version of the validated FFQ of Klipstein-Grobusch et al.[14] The FFQ consists of 293 items, structured according to meal pattern. Questions include frequency of consumption, portion size, preparation method and additions. Portion sizes were estimated using Dutch household measures and photographs showing different portion sizes.[15] We calculated average daily nutrient intakes by multiplying the frequency of consumption by portion size and nutrient content per gram based on the 2006 Dutch food composition table.[16]

Principal components analysis (PCA) was then used to identify dietary patterns. First the 293 food items were reduced to 20 predefined food groups.[17] Subsequently, food groups were adjusted for energy intake and PCA was performed.[18] To keep rotated factors uncorrelated the solution was rotated using the varimax method.[19] The first two factors, both representing distinct dietary profiles were extracted. A factor consists of a selection of the initial variables each with its own coefficient defining the observed correlation of that variable with the 'latent' constructed factor. As a weighted 'mix' of the initial variables a factor explains a substantial amount of variation in the data set. Participants were assigned a personalised score for the two factors representing a quantification of the individual's diet with the two extracted factors. The factor loadings are presented for each factor separately (Table 1). The association was calculated by Spearman's Rank correlation coefficient. After computation of the personalised scores, for reasons of interpretability, the 3187 women were classified into equal tertiles according to their personal score for the respective dietary patterns (low adherence, medium adherence, high adherence).

**Table 1 Applied criteria for the diagnosis of gestational hypertension and pre-eclampsia[36]**

| |
|---|
| **Criterium gestational hypertension** |
| new onset hypertension<br>(i.e. SBP ≥ 140 mmHg and / or DBP ≥ 90 mmHg after 20 weeks of gestation in a previously normotensive woman on at least two occasions) |
| **Criterium pre-eclampsia** |
| 1) new onset hypertension<br>(i.e. SBP ≥ 140 mmHg and / or DBP ≥ 90 mmHg after 20 weeks of gestation in a previously normotensive woman on at least two occasions)<br>**and**<br>2) proteinuria<br>(i.e. two or more dipstick readings of 2+ or greater, one sample reading of 1+ or greater, or a 24 - hour urine collection containing at least 300 mg of protein) |

### *Blood pressure*

Primary outcome variables were maternal blood pressure (mmHg), gestational hypertension (yes / no), and pre-eclampsia (yes / no). Maternal systolic (SBP) and diastolic (DBP) blood pressure was measured in early pregnancy, mid-pregnancy (median 20.5 weeks of gestation, interquartile range 1.3), and late pregnancy (median 30.4 weeks of gestation, interquartile range 1.1) using the validated Omron 907® automated digital oscillometric sphygmanometer (OMRON Healthcare Europe B.V. Hoofddorp, The Netherlands). The mean value of two blood pressure readings over a 60 seconds interval was documented. The presence of doctor-diagnosed gestational hypertension and pre-eclampsia was retrieved from medical records and was determined based on the criteria of the International Society for the Study of Hypertension in Pregnancy (Table 2).[20, 21]

**Table 2 Factor loadings**

| Food group | Mediterranean dietary pattern Spearman's Rank correlation coefficient | p value | Traditional dietary pattern Spearman's Rank correlation coefficient | p value |
|---|---|---|---|---|
| **Alcoholic drinks** | 0.25 | < 0.01 | 0.12 | NS |
| **Bread** | -0.20 | NS | -0.20 | < 0.01 |
| **Breakfast cereals** | 0.18 | < 0.01 | -0.19 | < 0.01 |
| **Butter** | 0.07 | < 0.01 | -0.01 | NS |
| **Dairy products** | -0.12 | < 0.01 | 0.03 | 0.12 |
| **Eggs** | 0.14 | < 0.01 | -0.03 | NS |
| **Fish** | 0.43 | < 0.01 | -0.25 | < 0.01 |
| **Fruit** | 0.06 | < 0.01 | -0.52 | < 0.01 |
| **Legumes** | 0.40 | < 0.01 | -0.00 | NS |
| **Margarine** | -0.18 | < 0.01 | 0.15 | < 0.01 |
| **Meat** | -0.01 | NS | 0.74 | < 0.01 |
| **Non-alcoholic drinks** | 0.06 | < 0.01 | -0.30 | < 0.01 |
| **Pasta, rice** | 0.68 | < 0.01 | 0.05 | < 0.01 |
| **Potatoes** | -0.03 | NS | 0.62 | < 0.01 |
| **Sauces and condiments** | -0.09 | < 0.01 | 0.04 | < 0.05 |
| **Soup** | 0.16 | < 0.01 | 0.00 | NS |
| **Starches and wheat** | -0.10 | < 0.01 | 0.01 | NS |
| **Sweets** | -0.21 | < 0.01 | -0.12 | < 0.01 |
| **Vegetable oils** | 0.70 | < 0.01 | -0.00 | NS |
| **Vegetables** | 0.76 | < 0.01 | -0.14 | < 0.01 |

PCA was used as an extraction method in which the Spearman's Rank correlation coefficients represent the relative contribution of that food group to the identified dietary pattern. NS: not significant

### *Covariates*

Information regarding maternal age, educational level, parity, smoking habits, folic acid use, and vomiting was available from questionnaires repeatedly applied during pregnancy. At enrolment maternal height and weight were measured to calculate body mass index (BMI, kg / m2).[11, 12] In early pregnancy venous blood serum and plasma samples were drawn and thereafter stored for future purpose at -80 °C.[11]

**Table 3 General characteristics**

| | Mediterranean dietary pattern | | | | Traditional dietary pattern | | | |
|---|---|---|---|---|---|---|---|---|
| | Low adherence n = 1062 | Medium adherence n = 1062 | High adherence n = 1063 | p value | Low adherence n = 1062 | Medium adherence n = 1063 | High adherence n = 1062 | p value |
| **Mean maternal age (years)** | 30.2 (4.6) | 31.6 (4.0) | 32.4 (4.0) | < 0.01 | 32.0 (3.9) | 31.5 (4.2) | 30.7 (4.8) | < 0.01 |
| **Median BMI (kg / m2)** | 24.0 (5.0) | 23.0 (4.2) | 23.0 (3.6) | < 0.01 | 22.8 (3.8) | 23.3 (4.1) | 23.8 (5.1) | < 0.01 |
| **Education (%)** | | | | | | | | |
| Low | 5.0 | 2.7 | 1.6 | < 0.01 | 0.7 | 3.4 | 5.3 | < 0.01 |
| Medium | 53.5 | 32.1 | 23.3 | | 26.4 | 33.7 | 48.9 | |
| High | 40.6 | 64.8 | 74.7 | | 72.3 | 62.3 | 45.5 | |
| Missing | 0.9 | 0.4 | 0.4 | | 0.6 | 0.6 | 0.3 | |
| **Parity (%)** | | | | | | | | |
| 0 | 59.6 | 59.4 | 61.1 | NS | 69.4 | 58.9 | 52.0 | < 0.01 |
| ≥ 1 | 40.1 | 40.5 | 38.7 | | 30.4 | 41.0 | 47.9 | |
| Missing | 0.3 | 0.1 | 0.2 | | 0.2 | 0.1 | 0.1 | |
| **Smoking (%)** | | | | | | | | |
| Yes, still | 19.3 | 13.6 | 11.5 | < 0.01 | 7.5 | 14.2 | 22.6 | < 0.01 |
| Yes, stopped | 7.5 | 7.6 | 9.5 | | 7.5 | 9.0 | 8.1 | |
| No | 65.5 | 70.1 | 71.8 | | 76.5 | 68.0 | 62.9 | |
| Missing | 7.7 | 8.7 | 7.2 | | 8.5 | 8.8 | 6.4 | |
| **Folic acid use (%)** | | | | | | | | |
| No | 11.0 | 7.6 | 7.6 | < 0.01 | 5.7 | 7.7 | 12.8 | < 0.01 |
| Yes, postconception start | 24.9 | 28.8 | 27.5 | | 27.1 | 24.6 | 29.4 | |
| Yes, preconception start | 47.7 | 44.8 | 47.4 | | 51.9 | 48.2 | 40.3 | |
| Missing | 16.4 | 18.8 | 17.5 | | 15.3 | 19.5 | 17.5 | |
| **Vomiting (%)** | | | | | | | | |
| Yes, severe | 17.1 | 12.1 | 8.7 | < 0.01 | 12.0 | 12.3 | 13.6 | < 0.01 |
| Yes, moderate | 19.2 | 19.7 | 20.5 | | 18.0 | 19.8 | 21.5 | |
| No | 55.4 | 59.3 | 62.9 | | 62.0 | 58.2 | 57.8 | |
| Missing | 8.3 | 8.9 | 7.9 | | 8.0 | 9.7 | 7.1 | |
| **Mean SBP (mmHg)** | | | | | | | | |
| Early pregnancy | 118.1 (12.0) | 116.8 (11.9) | 116.2 (11.4) | < 0.01 | 116.1 (11.5) | 117.0 (11.8) | 118.0 (12.0) | < 0.01 |
| Mid-pregnancy | 119.5 (11.9) | 118.2 (11.8) | 117.40 (11.1) | < 0.01 | 117.0 (11.1) | 118.5 (11.3) | 119.6 (12.3) | < 0.01 |
| Late pregnancy | 120.76 (11.6) | 120.5 (11.6) | 119.7 (11.0) | NS | 119.1 (10.9) | 119.8 (11.1) | 122.0 (12.0) | < 0.01 |
| **Mean DBP (mmHg)** | | | | | | | | |
| Early pregnancy | 68.9 (9.3) | 67.9 (92) | 67.5 (8.8) | < 0.01 | 67.6 (8.9) | 68.2 (9.1) | 68.5 (9.3) | NS |
| Mid-pregnancy | 68.4 (9.7) | 66.6 (9.1) | 66.3 (8.7) | < 0.01 | 66.4 (9.1) | 67.3 (9.2) | 67.6 (9.4) | < 0.01 |
| Late pregnancy | 69.9 (9.3) | 69.1 (9.1) | 68.7 (8.9) | < 0.01 | 69.0 (9.1) | 69.1 (8.8) | 69.9 (9.5) | NS |
| **Pre-eclampsia (%)** | 2.1 | 1.8 | 1.6 | NS | 1.8 | 1.3 | 2.4 | NS |
| **Gestational hypertension (%)** | 5.6 | 5.7 | 4.6 | NS | 5.0 | 5.0 | 5.9 | NS |

Values represent percentage (%) within column, mean (SD), or median (interquartile range). ANOVA and chi-square test tested overall differences in baseline characteristics between the dietary pattern categories



Folate, vitamin B12, tHcy, and Hs-CRP concentrations were analysed using an immuno-electrochemoluminence assay on the Architect System (Abbott Diagnostics B.V., Hoofddorp, The Netherlands) at the Department of Clinical Chemistry of the Erasmus MC Rotterdam in 2008. The between-run coefficients of variation for plasma folate were 8.9% at 5.6 nmol / L, 2.5% at 16.6 nmol / L, and 1.5% at 33.6 nmol / L; the coefficients of variation for tHcy were 3.1% at 7.6 mcmol / L, 3.1% at 13.7 mcmol / L, and 2.1% at 26.1 mcmol / L, the coefficients of variation for Hs-CRP were 0.9% at 12.8 mg / L, and 1.3% at 39.3 mg / L, and the coefficients of variation for vitamin B12 were 3.6% at 148 pmol / L, 2.7% at 295 pmol / L, and 3.1% at 590 pmol / L. Biomarker concentrations in early pregnancy were available in 78% of the study population. No differences in nutritional intake were observed between women with and without biomarker concentrations (p = 0.78).

### *Data analysis*

To test differences in baseline characteristics between the dietary pattern categories the Analysis of Variance (ANOVA) and chi-square test were used. Likewise, trend tests (linear regression) were used to relate the dietary pattern groups to the biomarker concentrations and nutrient intakes. Then, we used linear regression to assess cross-sectional differences between the dietary pattern categories in DBP and SBP. In the multiple regression analyses the inclusion of confounding variables was based on earlier literature, and determined a priori.[22, 23] These were maternal age, height, weight, parity, educational level, smoking habits, folic acid use, vomiting, and gestational age at time of measurement. Missing data were completed using multiple imputation (missing: educational level 0.6%, parity 0.2%, smoking 7.5%, folic acid use 17.5%, and vomiting 8.3%). Data were imputed according to the Markov Chain Monte Carlo method assuming no monotone missing pattern. Five imputed data sets were created. Subsequently, multiple regression analyses were performed on each imputated dataset and thereafter combined to one pooled estimate.[24] To further explore blood pressure trajectories between the dietary pattern categories repeated measurement regression models were used with maternal blood pressure as repeated outcome measure. These models take the correlation between repeated measurements of the same subject into account. The best fitting models were constructed using fractional polynomials of gestational age.[25] Lastly, to analyse the associations of the dietary patterns with gestational hypertension and pre-eclampsia, simple and multiple logistic regression models were used. We performed all statistical analyses using the Statistical Package of Social Sciences release 17.0 for Windows (SPSS Inc, Chicago, IL, USA), the Statistical Analysis System version 9.2 (SAS, Institute Inc. Gary NC, USA), and R version 2.9.2 for Windows.

## Results

Characteristics on nutritional intake of the 3187 women are shown in Table 2. The first factor explained 12.8% of nutritional intake' total variance and was labeled as the 'Mediterranean' dietary pattern. It comprised of high intakes of vegetables, vegetable oils, pasta, rice, fish and legumes, moderate intakes of alcohol, and low intakes of sweets (all $r \geq 0.2$ and $p < 0.01$). The second factor explained 8.7% of nutritional intake' total variance. It was labeled as the 'Traditional' dietary pattern because it was characterised by high intakes of meat and potatoes, and low intakes of fruit, non-alcoholic drinks, fish, and bread. Maternal characteristics associated with both low adherence to the Mediterranean dietary pattern and high adherence to the Traditional dietary pattern were younger age, higher BMI, lower educational level, continued smoking during pregnancy, a lower frequency of folic acid use, and more vomiting (Table 3).

Compared to women with high adherence to the Mediterranean dietary pattern, lower plasma folate and serum vitamin B12 concentrations, and higher plasma Hs-CRP concentrations were observed among women with low adherence (Table 4). They also had a lower ratio of unsaturated to saturated fatty acid intake, relatively lower intakes of protein and fiber, and higher intakes of carbohydrates. Women with high adherence to the Traditional dietary pattern had lower plasma folate concentrations, but higher serum vitamin B12 concentrations, as compared to women with low adherence to the Traditional dietary pattern. They also had higher plasma tHcy and Hs-CRP concentrations, a higher proportion of energy derived from fat and a lower ratio of unsaturated to saturated fats. These women also consumed relatively lower amounts of vegetable protein, carbohydrates, and fiber.

A consistent higher systolic blood pressure was observed throughout pregnancy among mothers with high adherence to the Traditional dietary pattern (Table 5). As compared to low adherence, high adherence was associated with a 1.8 mmHg (95% Confidence Interval (CI) [0.7; 2.9]) increase in SBP in early pregnancy, a 2.3 mmHg (95% CI [1.2; 3.3]) increase in mid-pregnancy, and a 2.6 mmHg (95% CI [1.6; 3.6]) increase in late pregnancy. Low adherence to the Mediterranean dietary pattern was associated with comparable effects in early pregnancy and mid-pregnancy (differences 1.1 mmHg (95% CI [0.2; 2.2]) and 1.3 mmHg (95% CI [0.3; 2.3]) respectively). This trend disappeared in late pregnancy (difference 0.2 mmHg, 95% CI [-0.8; 1.2]). Higher diastolic blood pressures were observed during pregnancy in mothers with both high adherence to the Traditional dietary pattern and low adherence to the Mediterranean dietary pattern. These increases were most pronounced during mid-pregnancy (differences 1.3 mmHg, 95% CI [0.5; 2.1]) and 1.6 mmHg, 95 C% [0.8; 2.4], respectively for high adherence to the Traditional, and low adherence to the Mediterranean dietary pattern).

**Table 4 Biomarker concentrations and nutrient intakes**

| | Mediterranean dietary pattern | | | | | | | Traditional dietary pattern | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Low adherence | | Medium adherence | | High adherence | | p trend | Low adherence | | Medium adherence | | High adherence | | p trend |
| | Median | IQR | Median | IQR | Median | IQR | | Median | IQR | Median | IQR | Median | IQR | |
| **Biomarker concentrations** | **n = 846** | | **n = 837** | | **n = 836** | | | **n = 847** | | **n = 837** | | **n = 835** | | |
| Folate (nmol / L)*† | 18.3 | 13.9 | 19.2 | 13.4 | 19.9 | 12.6 | < 0.01 | 21.0 | 12.8 | 19.3 | 13.3 | 16.9 | 13.7 | < 0.01 |
| tHcy (mcmol / L)*† | 7.0 | 2.1 | 7.0 | 1.9 | 7.0 | 1.8 | NS | 7.0 | 1.8 | 6.9 | 2.0 | 7.1 | 2.1 | < 0.01 |
| Vitamin B12 (pmol / L)*† | 167.0 | 99.0 | 178.0 | 99.0 | 182.0 | 96.3 | < 0.01 | 174.0 | 90.0 | 173.0 | 100.0 | 180.0 | 111.5 | < 0.01 |
| Hs-CRP (mg / L)* | 4.6 | 5.7 | 4.0 | 5.4 | 3.8 | 4.5 | < 0.01 | 3.4 | 4.9 | 4.1 | 5.0 | 4.7 | 5.7 | < 0.05 |
| **Energy and macronutrients** | **n = 1062** | | **n = 1062** | | **n = 1063** | | | **n = 1062** | | **n = 1063** | | **n = 1062** | | |
| Energy (kJ/ day) | 8955.9 | 3245.2 | 8914.3 | 2967.2 | 8993.2 | 2815.5 | NS | 9018.9 | 3008.5 | 8887.9 | 2896.3 | 9107.8 | 3113.5 | NS |
| Fat (% of energy) | 35.9 | 7.7 | 36.7 | 7.0 | 36.8 | 6.8 | < 0.01 | 34.9 | 7.7 | 36.8 | 6.6 | 37.6 | 7.1 | < 0.01 |
| Total fat (g / day)‡ | 77.7 | 16.5 | 79.5 | 15.2 | 79.9 | 14.8 | < 0.01 | 75.6 | 16.6 | 79.7 | 14.2 | 81.6 | 15.6 | < 0.01 |
| Saturated lipids (g / day)‡ | 29.0 | 6.9 | 28.9 | 6.7 | 28.6 | 6.6 | < 0.01 | 27.2 | 6.6 | 29.1 | 6.5 | 30.2 | 6.3 | < 0.01 |
| Mono unsaturated lipids (g / day)‡ | 27.0 | 6.4 | 28.4 | 6.2 | 29.9 | 6.9 | < 0.01 | 27.3 | 7.0 | 28.4 | 6.1 | 29.5 | 6.8 | < 0.01 |
| Poly unsaturated lipids (g / day)‡ | 17.9 | 7.0 | 18.3 | 6.3 | 17.9 | 6.4 | NS | 17.8 | 6.6 | 18.2 | 6.3 | 18.2 | 6.9 | < 0.01 |
| Unsaturated / saturated lipids | 1.9 | 0.4 | 2.0 | 0.4 | 2.1 | 0.4 | < 0.01 | 2.0 | 0.5 | 1.9 | 0.4 | 1.9 | 0.4 | < 0.01 |
| Protein (% of energy) | 14.1 | 3.1 | 15.0 | 2.7 | 15.3 | 2.9 | < 0.01 | 15.6 | 2.9 | 14.9 | 2.9 | 15.0 | 3.0 | < 0.01 |
| Total protein (g / day)‡ | 69.6 | 14.9 | 74.0 | 12.9 | 75.7 | 13.5 | < 0.01 | 72.0 | 13.7 | 73.0 | 13.4 | 74.5 | 14.7 | < 0.01 |
| Vegetable protein (g /day)‡ | 26.0 | 7.0 | 28.4 | 6.4 | 29.6 | 6.4 | < 0.01 | 30.7 | 6.4 | 28.0 | 5.7 | 25.6 | 6.4 | < 0.01 |
| Animal protein (g / day)‡ | 43.1 | 15.0 | 45.1 | 13.9 | 45.9 | 14.9 | < 0.01 | 40.9 | 14.1 | 44.9 | 13.5 | 48.0 | 14.3 | < 0.01 |
| Carbohydrate (% of energy) | 49.7 | 9.0 | 48.3 | 7.5 | 47.0 | 7.5 | < 0.01 | 50.1 | 8.3 | 48.0 | 7.4 | 46.8 | 7.9 | < 0.01 |
| Total carbohydrate (g / day)‡ | 241.8 | 43.6 | 234.3 | 37.0 | 228.4 | 37.1 | < 0.01 | 243.7 | 40.6 | 233.3 | 37.3 | 227.4 | 38.8 | < 0.01 |
| Carbohydrates monosaccharides (g / day)‡ | 138.8 | 46.2 | 127.6 | 37.9 | 117.3 | 35.3 | < 0.01 | 135.0 | 40.8 | 126.0 | 38.7 | 120.8 | 42.6 | < 0.01 |
| Carbohydrates polymers (g / day)‡ | 100.0 | 22.5 | 104.8 | 22.5 | 108.7 | 25.0 | < 0.01 | 104.9 | 22.2 | 105.1 | 23.0 | 103.8 | 25.5 | < 0.01 |
| Fiber (g / day)‡ | 19.3 | 5.7 | 21.7 | 5.6 | 23.5 | 6.1 | < 0.01 | 24.0 | 6.3 | 21.2 | 5.4 | 19.4 | 5.9 | < 0.01 |

Linear regression tested for trend. IQR: interquartile range. *p values for biomarker concentrations were adjusted for gestational age at venous puncture. †p values for folate, tHcy and vitamin B12 were additionally adjusted for folic acid use. ‡ adjusted for energy intake

**Table 5 Maternal blood pressure**

| | SBP Early pregnancy | | SBP Mid-pregnancy | | BP Late pregnancy | |
|---|---|---|---|---|---|---|
| | Crude* | Adjusted† | Crude* | Adjusted† | Crude* | Adjusted† |
| **Mediterranean dietary pattern** | | | | | | |
| Low adherence | 1.8 [0.7; 2.9] | 1.1 [0.2; 2.2] | 2.2 [1.2; 3.2] | 1.3 [0.3; 2.3] | 1.0 [0.1; 2.0] | 0.2 [-0.8; 1.2] |
| Medium adherence | 0.5 [-0.6; 1.6] | 0.4 [-0.7; 1.5] | 0.8 [-0.2; 1.8] | 0.7 [-0.3; 1.6] | 0.8 [-0.2; 1.8] | 0.7 [-.3; 1.6] |
| High adherence | Reference | Reference | Reference | Reference | Reference | Reference |
| p trend | < 0.01 | < 0.05 | < 0.01 | < 0.01 | < 0.05 | NS |
| **Traditional dietary pattern** | | | | | | |
| Low adherence | Reference | Reference | Reference | Reference | Reference | Reference |
| Medium adherence | 0.8 [-0.3; 1.9] | 0.8 [-0.2; 1.9] | 1.4 [0.4; 2.4] | 1.3 [0.4; 2.3] | 0.7 [-0.3; 1.7] | 0.7 [-0.3; 1.6] |
| High adherence | 1.9 [0.8; 3.0] | 1.8 [0.7; 2.9] | 2.5 [1.5; 3.5] | 2.3 [1.2; 3.3] | 2.9 [2.0; 3.9] | 2.6 [1.6; 3.6] |
| p trend | < 0.01 | < 0.01 | < 0.01 | < 0.05 | < 0.01 | < 0.01 |

| | DBP Early pregnancy | | DBP Mid-pregnancy | | DBP Late pregnancy | |
|---|---|---|---|---|---|---|
| | Crude* | Adjusted† | Crude* | Adjusted† | Crude* | Adjusted† |
| **Mediterranean dietary pattern** | | | | | | |
| Low adherence | 1.3 [0.5; 2.1] | 0.9 [0.1; 1.8] | 2.2 [1.2; 3.2] | 1.6 [0.8; 2.4] | 1.4 [0.6; 2.2] | 1.0 [0.2; 1.8] |
| Medium adherence | 0.3 [-0.6; 1.1] | 0.3 [-0.6; 1.1] | 0.8 [-0.2; 1.8] | 0.2 [-0.6; 0.9] | 0.5 [-0.3; 1.3] | 0.5 [-0.3; 1.2] |
| High adherence | Reference | Reference | Reference | Reference | Reference | Reference |
| p trend | < 0.01 | < 0.05 | < 0.01 | < 0.01 | < 0.01 | < 0.05 |
| **Traditional dietary pattern** | | | | | | |
| Low adherence | Reference | Reference | Reference | Reference | Reference | Reference |
| Medium adherence | 0.5 [ -0.4; 1.3] | 0.6 [-0.3; 1.4] | 0.1 [-0.6; 0.8] | 0.9 [0.1; 1.7] | -0.2 [-0.9; 0.5] | 0.3 [-0.4; 1.1] |
| High adherence | 0.9 [0.1; 1.7] | 1.0 [0.1; 1.8] | 1.9 [1.3; 2.0] | 1.3 [0.5; 2.1] | 1.1 [0.5; 1.8] | 0.8 [0.1; 1.6] |
| p trend | < 0.05 | < 0.05 | < .01 | < 0.01 | NS | NS |

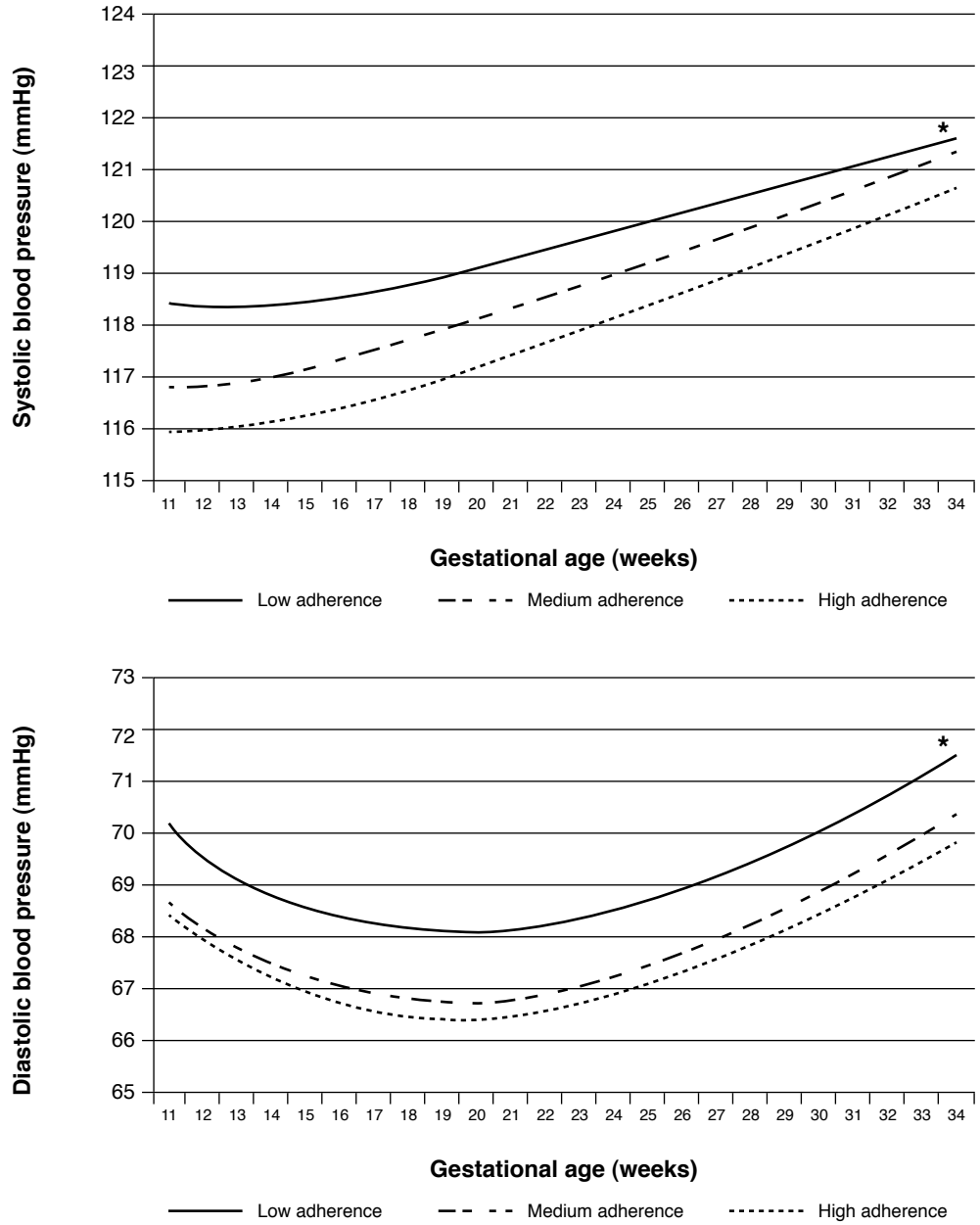
Results from simple and multiple linear regression analyses. Values are regression coefficients (95 % Confidence Interval) and reflect differences in systolic and diastolic blood pressure (in mmHg) compared to reference, in early pregnancy (2706 measurements), mid-pregnancy (3125 measurements), and late pregnancy (3108 measurements)
*Crude: adjusted for gestational age at measurement
†Adjusted: additionally adjusted for maternal age, height, weight, parity, educational level, smoking habits, vomiting and folic acid use

Repeated regression analyses showed similar trends in patterns of blood pressure change. Overall, systolic and diastolic blood pressures were highest among women with low adherence to the Mediterranean dietary pattern (Figure 1) and high adherence to the Traditional dietary pattern (Figure 2).

Neither adherence to the Mediterranean dietary pattern nor adherence to the Traditional dietary pattern was associated with the occurrence of gestational hypertension or pre-eclampsia (Table 6).

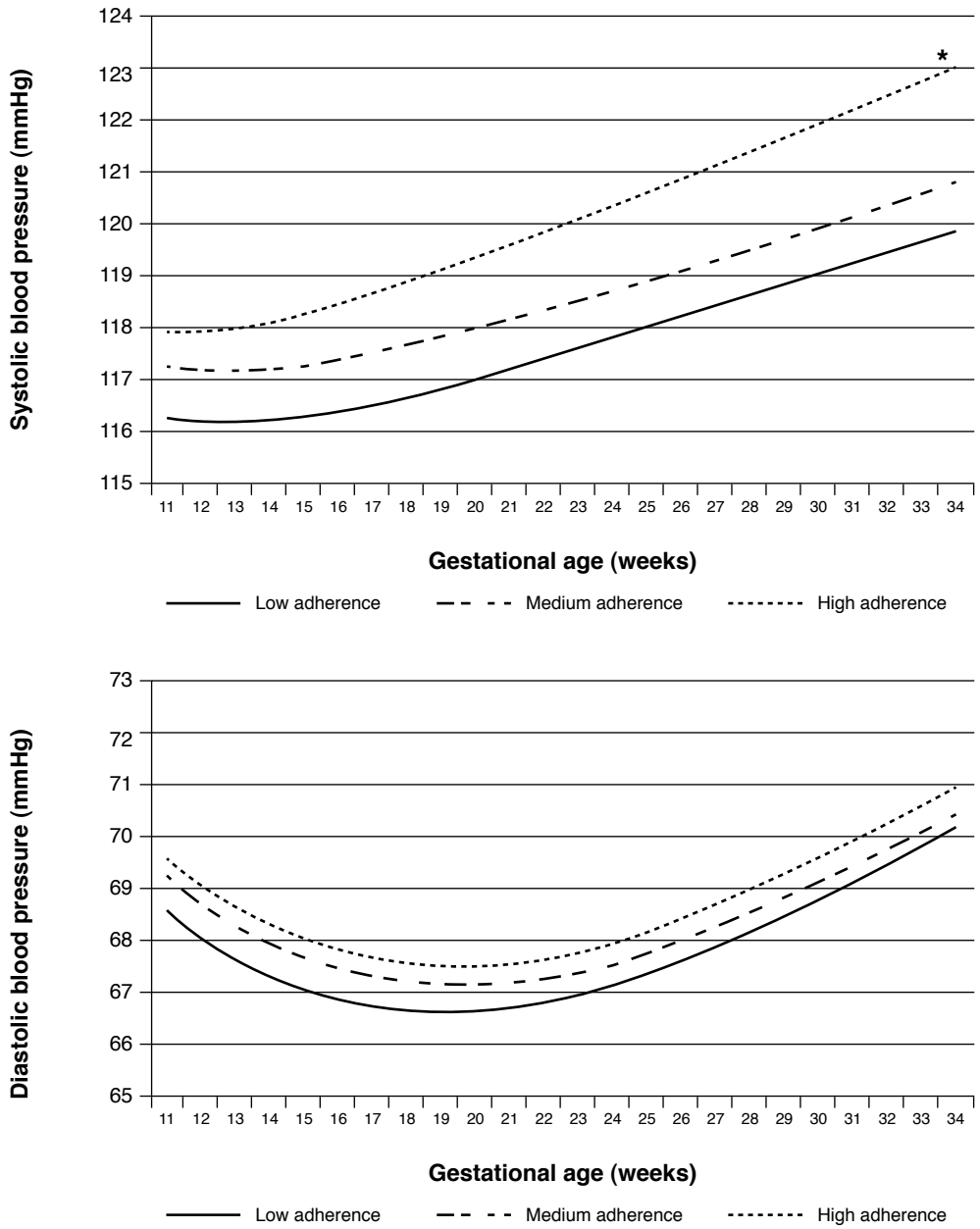


**Figure 1 Blood pressure patterns in Mediterranean dietary pattern categories**




Change in systolic and diastolic blood pressure in mmHg per Mediterranean dietary pattern category with high adherence as reference group based on repeated measurement analysis (systolic blood pressure = ß0 + ß1*dietary pattern + ß2*GA + ß3*GA-2 + ß4*dietary pattern*GA. Diastolic blood pressure = ß0 + ß1*dietary pattern + ß2*GA + ß3*GA.5 + ß4*dietary pattern*GA). GA = gestational age in weeks. *p < 0.05 reflects a significant difference in change in blood pressure per week (ß4) per Mediterranean dietary pattern category, for the different categories compared to reference

**Figure 2 Blood pressure patterns in Traditional dietary pattern categories**




Change in systolic and diastolic blood pressure in mmHg per Traditional dietary pattern category with low adherence as reference group based on repeated measurement analysis. (systolic blood pressure = ß0 + ß1*dietary pattern + ß2*GA + ß3*GA-2 + ß4*dietary pattern*GA. Diastolic blood pressure = ß0 + ß1*dietary pattern + ß2*GA + ß3*GA.5 + ß4*dietary pattern*GA). GA = gestational age in weeks. *$p < 0.05$ reflects a significant difference in change in blood pressure per week (ß4) per Traditional dietary pattern category, for the different categories compared to reference



**Table 6 Hypertensive pregnancy disorders**

| | Gestational hypertension | | | Pre-eclampsia | | |
|---|---|---|---|---|---|---|
| | n cases | Crude* | Adjusted† | n cases | Crude* | Adjusted† |
| **Mediterranean dietary pattern** | | | | | | |
| Low adherence | 58 | 1.2 [0.8; 1.7] | 1.3 [0.9; 1.9] | 22 | 1.3 [0.6; 2.3] | 1.2 [0.6; 2.3] |
| Medium adherence | 59 | 1.2 [0.8; 1.8] | 1.1 [0.8; 1.7] | 19 | 1.2 [0.7; 2.4] | 1.2 [0.6; 2.3] |
| High adherence | 48 | Reference | Reference | 17 | Reference | Reference |
| p trend | | NS | NS | | NS | NS |
| **Traditional dietary pattern** | | | | | | |
| Low adherence | 52 | Reference | Reference | 19 | Reference | Reference |
| Medium adherence | 52 | 0.9 [0.7; 1.5] | 1.0 [0.7; 1.6] | 14 | 0.7 [0.3;1.4] | 0.7 [0.3; 1.4] |
| High adherence | 61 | 1.2 [0.8; 1.7] | 1.3 [0.9; 1.9] | 25 | 1.2 [0.6;2.2] | 1.1 [ 0.6; 2.1] |
| p trend | | NS | NS | | NS | NS |

Results from simple and multiple logistic regression analysis.Values are odds ratios (95% Confidence Interval)
*Crude: unadjusted
†Adjusted: adjusted for maternal age, height, weight, parity, educational level, smoking habits, vomiting and folic acid use

# Comment

We have shown that low adherence to a Mediterranean diet and high adherence to a Traditional diet are associated with a higher maternal blood pressure during pregnancy. These associations are within physiologic ranges. We observed no relation with the occurrence of hypertensive pregnancy disorders.

During recent years, the Mediterranean diet has gained considerable attention for its positive health effects.[26] There is no single Mediterranean diet, though dietary patterns prevailing in the Mediterranean region share common characteristics including an abundance of vegetables, vegetable oil as principal source of fat, moderate amounts of fish and poultry, relatively low consumptions of meat, and moderate alcohol use.[26, 27] Adherence to the identified Mediterranean diet in our study was validated by higher amounts of vegetable protein, carbohydrates polymers, fiber, and a favourable ratio of unsaturated to saturated lipids. Increasing concentrations of the biomarkers plasma folate and serum vitamin B12, further validated adherence to the Mediterranean dietary pattern.

The Traditional diet resembles a typically Northwest European dietary tradition that exists since the 19th century when agriculture and domestic education were widely implemented. Characterised by high amounts of meat and potatoes, the Traditional dietary pattern seems to be fairly reproducible across populations.[27] In our study adherence to this diet was validated by higher amounts of animal protein and saturated lipids, lower amounts of carbohydrates and fiber, lower concentrations of plasma folate, and higher concentrations of serum vitamin B12.

The monitored higher blood pressure levels in early pregnancy, for women with either low adherence to the Mediterranean diet, or high adherence to the Traditional diet, may indicate that blood pressure was already higher before pregnancy. Dietary patterns rich in whole grains, fruit, vegetables, and adequate omega-3 fatty acids, and low in refined grains, and saturated and *trans* fats, have been suggested to offer significant protection against cardiovascular disease.[28] Previously, it was demonstrated that such a diet lowered systolic and diastolic blood pressure levels by 5.5 and 3.0 mmHg.[29] Additionally, van Dam et al.[30] reported on two dietary patterns that were associated with systolic blood pressure levels in Dutch women aged 20 - 65 years. These included a blood pressure lowering diet characterised by higher intakes of vegetables, rice, chicken, fish and wine, and a blood pressure elevating diet, characterised by greater intakes of meat and potatoes, and lesser intakes of low-fat dairy products and fruit.[30] Our findings are in line with these results.

To our knowledge the observed different patterns of blood pressure change have not been observed before. A higher systolic and diastolic blood pressure was observed in mothers with both low adherence to the Mediterranean dietary pattern and high adherence to the Traditional dietary pattern. However, this difference declined with gestational age in women with low adherence to the Mediterranean dietary pattern. With respect to diastolic blood pressure the differences were most pronounced in mid-pregnancy. These observed blood pressure patterns might be explained by effects of the dietary patterns on endothelial function, possibly interfering with normal vascular adjustments (vasodilatation) to pregnancy.[22] In this respect, dietary patterns have been demonstrated to reduce markers of inflammation and endothelial dysfunction, similar to our own results on Hs-CRP concentrations.[9] This may imply that first trimester is a critical period for cardiovascular adaptations related to maternal nutrition and subsequent blood pressure development.[31] Interestingly, our results are different from our previous findings on the association between synthetic folic acid and blood pressure, that showed a small physiological increase in maternal blood pressure among folic acid supplemented women as compared to women who did not use folic acid at all.[8] This may be explained by the stronger and opposite effects of other nutritional compounds than folate. Moreover, in contrast to food folate, folic acid appears also in an oxidized form in the blood which may slightly increase blood pressure by a differential effect on endothelium function.[32] Lastly, concerning the Traditional diet, it can be hypothesised that the lower consumption of fluids in women with high adherence affected the relative state of hypovolemia associated with early pregnancy with subsequent differential haemodynamic adaptation. Future studies are needed to elucidate on these effects and potential mechanisms.

Despite an elevated blood pressure throughout pregnancy and a relatively less steeper mid-pregnancy fall in diastolic blood pressure we observed no associations with the risks of gestational hypertension or pre-eclampsia. Thus, the effects of these dietary patterns may not be strong enough to be associated with the risk of hypertensive pregnancy disorders. Recently, Brantsaeter et al.[33] found that a diet characterised by high intakes of vegetables, and vegetable oils decreased the risk of pre-eclampsia, whereas a dietary pattern characterised by a high consumption of meat, sweet drinks, and snacks increased the risk. These results are in disparity with our study. In their study lack of validation of the diagnosis pre-eclampsia might have influenced the results. As we were limited by a relatively small number of pre-eclamptic cases in this restricted study sample further larger studies with more power are needed.

Dietary pattern analysis is complementary to approaches using individual foods or nutrients which are limited by biologic interactions and colinearity.[5] The large sample size of this study together with its prospective design enabled us to assess associations between maternal diet and blood pressure in close detail. However, our results should be considered in light of some potential limitations. Firstly, nutritional intake was assessed in early pregnancy. It could be argued that nutritional intake differs between different pregnancy periods. However, Cucó et al.[34] investigated dietary patterns during different pregnancy periods and observed no significant differences over time. We assessed diet before occurrence of the outcome which implies that potential misclassification would likely have been non-differential. Another important issue is unmeasured confounding. Although we attempted to account for this by restricting to an ethnic homogeneous population and by controlling for a large number of confounders, we cannot rule out that residual confounding occured. Lastly, assuming our missing data were missing at random we used multiple imputation to deal with missing values since this method is currently believed to be the most credible.[37] Next to multiple imputation we also performed a sensitivity analysis using complete case analysis and the missing-indicator method. These analyses yielded approximately the same results.

The major challenge in dietary pattern analysis remains to establish a quantitative method to identify eating patterns unless a specific dietary pattern has been specified before. Using factor analysis involves several arbitrary decisions including the consolidation of food items into food groups, the number of factors to extract, rotation method, and factor labeling.[35] We conducted sensitivity analyses to examine whether these decisions affected our results. The use of other types of factor analysis (oblique instead of orthogonal rotation) and analyses within a random subgroup (25%) indicated that the identified dietary patterns were robust. Also, the addition or deletion of one or two food groups did not have much effect on the general pattern of findings. Moreover, similar dietary patterns derived by factor analysis have been observed by others.[7, 27, 30, 33]

To conclude, both low adherence to a Mediterranean diet and high adherence to a Traditional diet are associated with physiological higher levels of blood pressure. The differential effects with respect to the patterns of blood pressure change should be further explored.

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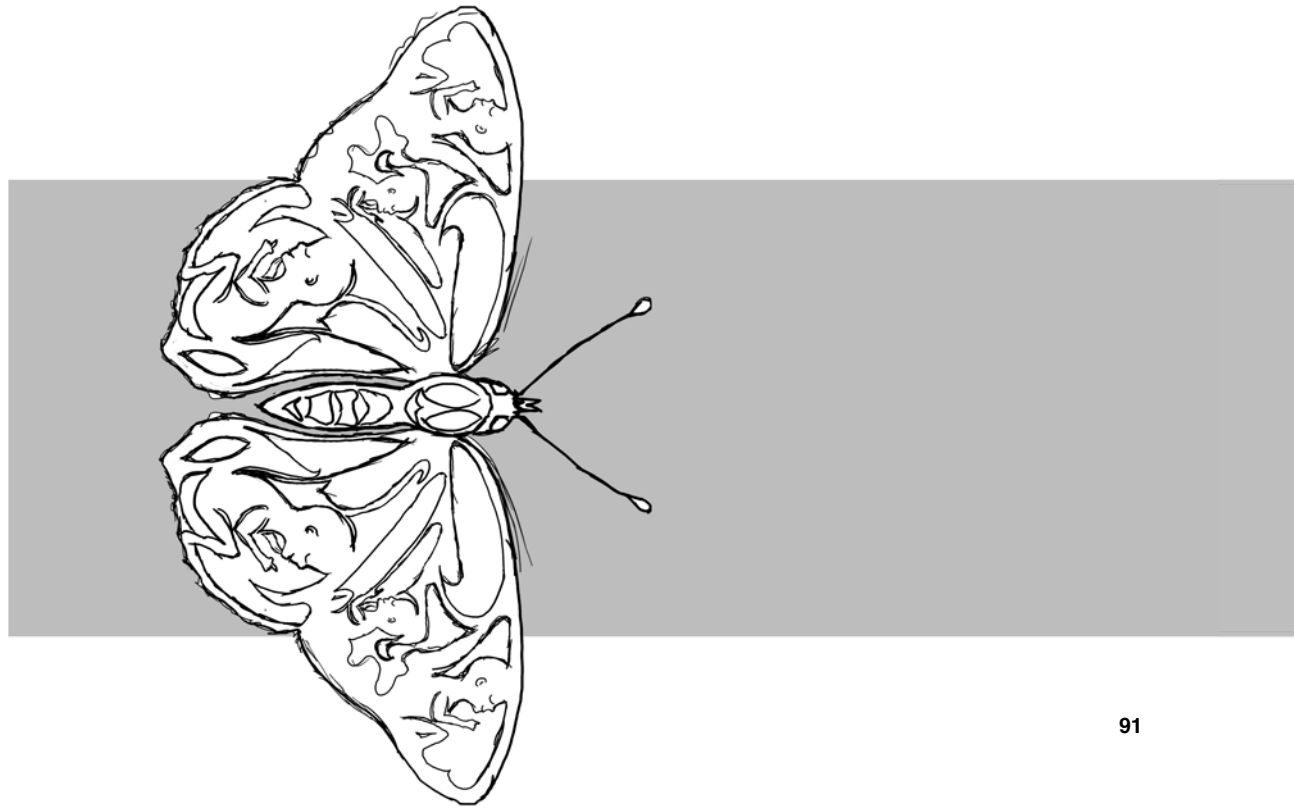
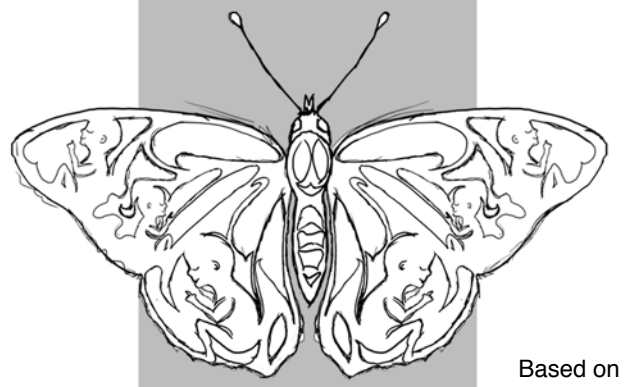
# Chapter 4

# Urban perinatal health



Chapter 4.1

# Determinants of folic acid use during early pregnancy in a multi - ethnic urban population in The Netherlands



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Based on Preventive Medicine 2008; 47: 427 - 432

## Abstract

Recommendations on folic acid use to prevent neural tube defects have been launched in several countries; yet, the adequate use of folic acid according to the current recommendations still seems to be low. This study assesses the prevalence of folic acid use and identifies its determinants in The Generation R Study Cohort in Rotterdam, The Netherlands between 2001 and 2006. Data on 6940 women were available. Information on folic acid use and potential determinants such as sociodemographic status, life style habits, and obstetrical history was obtained from questionnaires, medical records, and physical examinations. Of all women 37% adequately used a folic acid supplement during the preconception period. The strongest risk factors for inadequate folic acid use were unplanned pregnancy (odds ratio (OR) 9.5, 95% Confidence Interval (CI) [7.2; 12.4]), low educational level (OR 2.5, 95% CI [1.8; 3.6]), and non-Western ethnicity, (OR 3.5, 95% CI [2.9; 4.3]). After stratification for ethnicity, unplanned pregnancy remained the most important risk factor for inadequate folic acid use. Other risk factors for inadequate folic acid use were younger age, single marital status, smoking habits, multiparity (all $p < 0.01$), and alcohol consumption ($p < 0.05$). In contrast, history of a previous spontaneous abortion decreased the risk of inadequate folic acid use ($p < 0.01$). To conclude, adequate preconception start of folic acid use is still too low. Implementation of preconception programmes and other public health strategies are strongly needed.


## Introduction

From the early nineties recommendations have been launched that women planning pregnancy should use 0.4 - 0.5 mg folic acid per day from at least one month before until three months after conception, in addition to a healthy diet.[1] These recommendations are largely based on two randomised trials showing that folic acid supplement use reduces the risk of neural tube defects (NTDs) by 72% and 100%.[2, 3] Although folate is an essential micronutrient for cellular growth the underlying mechanisms by which folate prevents NTDs are not clear.[4] However, it is known that pregnant women with a folate deficiency are at an increased risk for several reproductive complications including NTDs.[5]

Despite campaigns to promote folic acid intake and evidence that this B vitamin also has beneficial effects against other reproductive failures, the number of women who take folic acid in the recommended period remains low.[6] A strategy to improve folic acid intake is mandatory food fortification which has not yet been introduced in any European country.[6-9] Therefore, currently the most effective strategy in Europe is to daily use a folic acid supplement.

The Dutch mass media campaign in 1995 together with continuing media attention in the following years to promote folic acid intake resulted in an increase from approximately 19% to 36% adequate folic acid use in The Netherlands. In particular, adequate folic acid use appeared to be insufficient among low educated women.[10] It has furthermore been suggested that language proficiency is an important determinant for folic acid knowledge and subsequently folic acid intake.[11] In order to develop more targeted strategies to improve adequate preconception folic acid use in the future, knowledge about other sociodemographic, behavioural, and pregnancy determinants could be important.

## Materials and Methods

### *Study design*

This study was embedded in The Generation R Study Rotterdam, The Netherlands, a population-based prospective cohort study from early pregnancy onwards. The study was designed to identify determinants of growth, health, and development from fetal life until young adulthood.[12, 13] In total 8880 women enrolled during pregnancy in the study between 2001 and 2006.[12, 13]

### *Folic acid use*

Pregnant women were asked by a questionnaire at enrolment (median 14.4 weeks of gestation, interquartile range 5.0) whether they had used a folic acid supplement and when folic acid intake was started. Based on Dutch recommendations folic acid use was classified into two

categories: 1) adequate folic acid use, defined as preconception start of folic acid use; and 2) inadequate folic acid use, defined as no use of folic acid at all or folic acid use started from pregnancy recognition onwards.[14] Because folic acid use from pregnancy recognition onwards (subadequate use) might still be beneficial we also performed some additional analyses after categorisation of the cohort into three groups (adequate use, subadequate use, no use).[15]

***Determinants***

Information on possible determinants (sociodemographic factors, lifestyle habits, obstetrical history) was obtained from questionnaires. Sociodemographic factors included information regarding maternal age, marital status, educational level, and ethnicity. Ethnic background was derived from the country of birth of the woman herself and her parents. The different ethnic categories were Dutch, Moroccan, Turkish, Antillean and Surinamese. Women with an other ethnic background were grouped as 'other-Western' (European, North American, Oceanean) or 'other non-Western'. (African, Asian, South- and Central American).[16] Educational level was assessed by the highest completed education level and classified into three categories: 1) primary education; 2) secondary education; and 3) university or college.[17] Lifestyle habits included body mass index (BMI; in kg / m2, calculated from length (m) and weight (kg) measured at enrolment), smoking habits, and alcohol consumption. Obstetrical history included information on parity, pregnancy planning, fertility treatment, previous spontaneous abortion, or stillbirth. Pregnancy planning was defined as a confirmatory answer to the question "was the present pregnancy planned?".

***Data analysis***

With respect to statistical analysis we first assessed the prevalence of folic acid use for the whole cohort. Second, we performed simple logistic regression analyses to identify groups at risk and to examine differences in characteristics between adequate and inadequate users. Next, multiple logistic regression models were used to control for potential confounders namely age, educational level, marital status, ethnicity, BMI, smoking habits or alcohol consumption, parity, pregnancy planning, and previous spontaneous abortion. Finally, similar analyses were performed in strata of the largest ethnic groups to examine risk factors related to specific ethnic categories. The size of the effect estimates is given in odds ratios (ORs) with 95% Confidence Intervals (95% CI). Statistical analyses were performed using Statistical Package of Social Sciences version 15.0 for Windows (SPSS Inc, Chicago, IL, USA).



## Results

The response rate of the questionnaire on general characteristics and folic acid use was 78% (n = 6940). Baseline characteristics are shown in Table 1. Thirty-seven percent of these 6940 women started folic acid use preconceptionally (adequate), 35% from pregnancy recognition onwards (subadequate), and 28% did not use folic acid at all (total of 63% inadequate use).

**Table 1 Baseline maternal characteristics**

| | Number of subjects n = 6940 |
|---|---|
| **Sociodemographic factors** | |
| **Folic acid use** | |
| Adequate folic acid use | 2592 (37.3) |
| Inadequate folic acid use | 4348 (62.7) |
| **Maternal age** | |
| < 20 years | 276 (4.0) |
| 20 - 29.9 years | 2989 (43.1) |
| 30 - 35 years | 2633 (37.9) |
| > 35 years | 1042 (15.0) |
| **Marital status** | |
| Married or living together | 5892 (84.9) |
| No partner | 985 (14.2) |
| Missing | 63 (0.9) |
| **Educational level** | |
| Primary school | 766 (11.0) |
| Secondary school | 3139 (45.2) |
| University or college | 2891 (41.7) |
| Missing | 144 (2.1) |
| **Ethnicity** | |
| Dutch | 3457 (49.8) |
| Moroccan | 443 (6.4) |
| Turkish | 618 (8.9) |
| Suriname and Antilles | 859 (12.4) |
| Western otherwise | 826 (11.9) |
| Non-Western otherwise | 705 (10.1) |
| Missing | 32 (0.5) |

Data represent n (%)

(Table 1 continued)

| | Number of subjects n = 6940 |
|---|---|
| **Lifestyle habits** | |
| **BMI** | |
| < 25 | 4243 (61.1) |
| 25 - 30 | 1803 (26.0) |
| > 30 | 857 (12.4) |
| Missing | 37 (0.5) |
| **Smoking habits** | |
| Yes, still | 827 (11.8) |
| Yes, until pregnancy recognition | 821 (11.9) |
| No | 5192 (74.9) |
| Missing | 100 (1.4) |
| **Alcohol consumption** | |
| Yes, still | 1119 (16.1) |
| Yes, until pregnancy recognition | 1840 (26.5) |
| No | 3919 (56.5) |
| Missing | 62 (0.9) |
| **Obstetrical history** | |
| **Parity** | |
| Nullipara | 3938 (56.8) |
| Multipara | 2993 (43.1) |
| Missing | 9 (0.1) |
| **Pregnancy planning** | |
| Yes | 4852 (69.9) |
| No | 1894 (27.3) |
| Missing | 194 (2.8) |
| **Previous spontaneous abortion** | |
| Yes | 1305 (18.8) |
| No | 2781 (40.1) |
| Missing | 2854 (41.1) |

Data represent n (%)

Women who used inadequate folic acid were younger, more often single, and lower educated (Table 2). All non-Dutch groups had increased risks for inadequate folic acid use. Smoking during pregnancy was strongly associated with inadequate folic acid use (OR 2.3, 95% CI [1.8; 3.0]). After controlling for potential confounders, alcohol consumption during pregnancy became a risk factor for inadequate folic acid use (OR 1.5, 95% CI [1.2; 1.8]). BMI was not significantly associated with inadequate folic acid use after adjusting for potential confounders.



**Table 2 Determinants of inadequate folic acid use given in frequency and risks**

| | Folic acid use | | Crude OR (CI 95%) | Adjusted OR (CI 95%)* |
|---|---|---|---|---|
| | Adequate use n = 2592 | Inadequate use n = 4348 | | |
| **Sociodemographic factors** | | | | |
| **Maternal age** | | | | |
| < 20 years | 19 (6.9) | 257 (93.1) | 12.9 [8.1; 20.7] | 1.5 [0.7; 3.2] |
| 20 - 29.9 years | 815 (27.3) | 2174 (72.7) | 2.6 [2.3; 2.9] | 1.4 [1.2; 1.7] |
| 30 - 35 years | 1286 (48.8) | 1347 (51.2) | 1 | 1 |
| > 35 years | 472 (45.3) | 570 (54.7) | 1.2 [1.0; 1.3] | 1.0 [0.8; 1.2] |
| Mean (range) | 31.6 (17.8-46.3) | 28.8 (15.3-44.7) | | |
| **Marital status** | | | | |
| Married or living together | 2477 (42) | 3415 (58) | 1 | 1 |
| No partner | 102 (10.4) | 883 (89.6) | 6.3 [5.1; 7.8] | 2.0 [1.4; 2.7] |
| Missing | 13 (20.6) | 50 (79.4) | | |
| **Educational level** | | | | |
| Primary school | 83 (10.8) | 683 (89.2) | 8.6 [6.8; 11.0] | 2.5 [1.8; 3.6] |
| Secondary school | 952 (30.3) | 2187 (69.7) | 2.4 [2.2; 2.7] | 1.3 [1.1; 1.6] |
| University or college | 1541 (53.3) | 1350 (46.7) | 1 | 1 |
| Missing | 16 (22) | 128 (88) | | |
| **Ethnicity** | | | | |
| Dutch | 1838 (53.2) | 1619 (46.8) | 1 | 1 |
| Moroccan | 55 (12.4) | 388 (87.6) | 7.9 [5.9; 10.5] | 6.6 [4.4; 10.0] |
| Turkish | 98 (15.9) | 520 (84.1) | 5.9 [4.7; 7.4] | 3.8 [2.7; 5.3] |
| Suriname and Antilles | 154 (17.9) | 705 (82.1) | 5.1 [4.3; 6.2] | 2.6 [1.9; 3.5] |
| Western otherwise | 326 (39.5) | 500 (60.5) | 1.7 [1.5; 2.0] | 1.5 [1.2; 2.0] |
| Non-Western otherwise | 117 (16.6) | 588 (83.4) | 5.6 [4.6; 6.9] | 3.6 [2.6; 4.9] |
| Missing | 4 (12.5) | 28 (87.5) | | |
| **Lifestyle habits** | | | | |
| **BMI** | | | | |
| < 25 | 1691 (39.9) | 2552 (60.1) | 1 | 1 |
| 25 - 30 | 645 (35.8) | 1158 (64.2) | 1.2 [1.1; 1.3] | 1.0 [0.8; 1.2] |
| > 30 | 241 (28.1) | 616 (71.9) | 1.7 [1.4; 2.0] | 1.1 [0.9; 1.5] |
| missing | 15 (40.5) | 22 (59.5) | | |
| **Smoking habits** | | | | |
| Yes, still | 161 (19.5) | 666 (80.5) | 2.9 [2.5; 3.5] | 2.3 [1.8; 3.0] |
| Yes, until pregnancy recognition | 237 (28.9) | 584 (71.1) | 1.7 [1.5; 2.1] | 1.6 [1.2; 2.0] |
| No | 2165 (41.7) | 3027 (58.3) | 1 | 1 |
| Missing | 29 (29) | 71 (71) | | |
| **Alcohol consumption** | | | | |
| Yes, still | 500 (44.7) | 619 (55.3) | 0.6 [0.5; 0.7] | 1.5 [1.2; 1.8] |
| Yes, until pregnancy recognition | 771 (41.9) | 1069 (58.1) | 0.7 [0.6; 0.8] | 1.3 [1.0; 1.6] |
| No | 1306 (33.3) | 2613 (66.7) | 1 | 1 |
| Missing | 15 (24.2) | 47 (75.8) | | |

(Table 2 continued)

| | Folic acid use | | Crude OR (CI 95%) | Adjusted OR (CI 95%)* |
|---|---|---|---|---|
| | Adequate use n = 2592 | Inadequate use n = 4348 | | |
| **Obstetrical history** | | | | |
| **Parity** | | | | |
| Primipara | 1576 (40) | 2362 (60) | 1 | 1 |
| Multipara | 1011 (33.8) | 1982 (66.2) | 1.3 [1.2; 1.4] | 1.6 [1.3; 1.9] |
| Missing | 5 (55.6) | 4 (44.4) | | |
| **Pregnancy planning** | | | | |
| No | 101 (5.3) | 1793 (94.7) | 17.7 [14.4; 21.8] | 9.5 [7.2; 12.4] |
| Yes | 2423 (49.9) | 2429 (50.1) | 1 | 1 |
| Missing | 68 (35.1) | 126 (64.9) | | |
| **Previous spontaneous abortion** | | | | |
| Yes | 561 (43) | 744 (57) | 0.6 [0.5; 0.7] | 0.7 [0.7; 0.8] |
| No | 882 (31.7) | 1899 (68.3) | 1 | 1 |
| Missing | 1149 (40.3) | 1705 (59.7) | | |

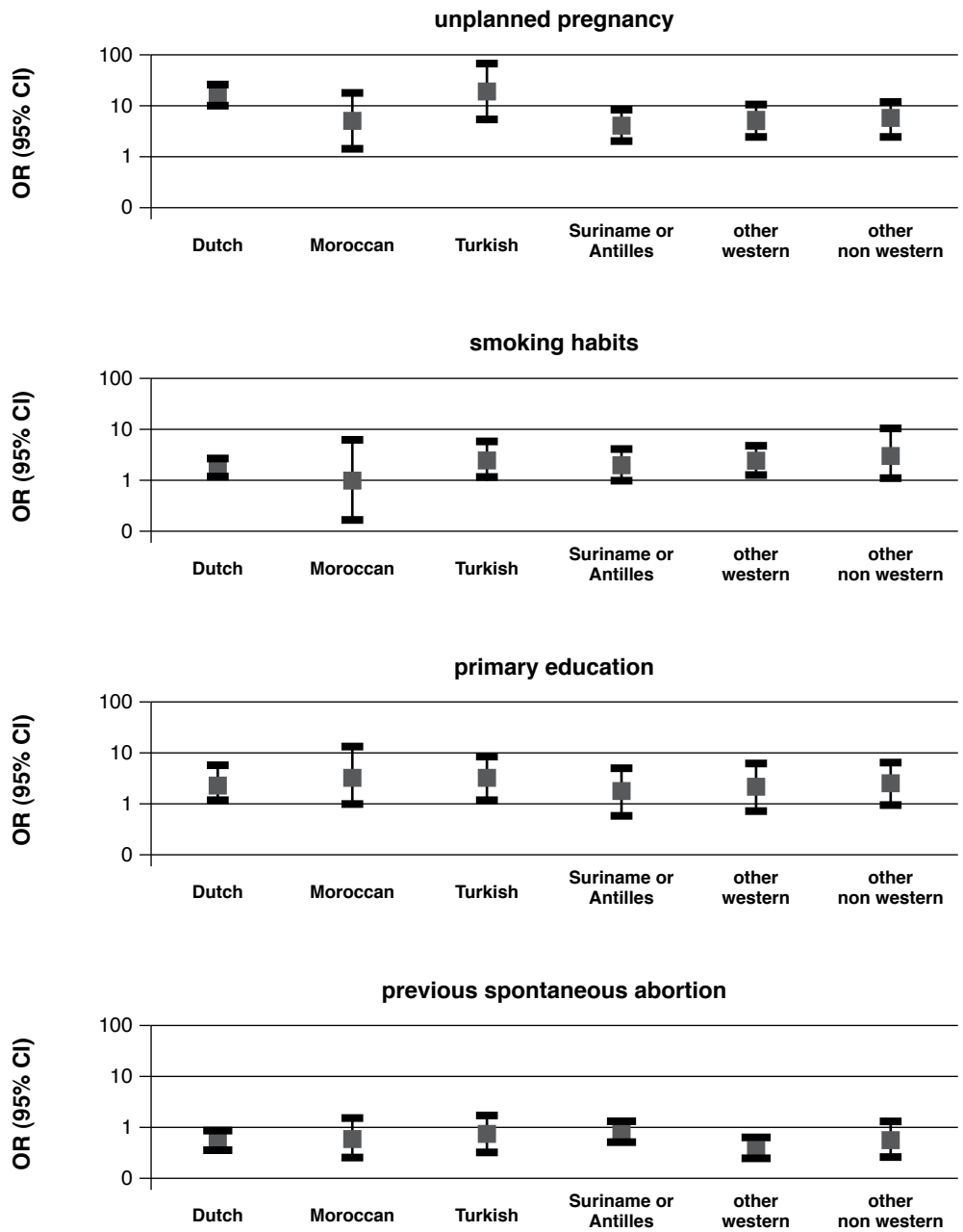
Data are n (% within row), and odds ratios (95% Confidence Interval); * adjusted for maternal age, marital status, educational level, ethnicity, BMI, smoking habits, alcohol consumption, parity, pregnancy planning, and previous spontaneous abortion

Pregnancy was planned in 70% of the women of whom 2% became pregnant after fertility treatment (n = 107 / 4852). Thirty percent of the women who became pregnant after fertility treatment did not start folic acid use preconceptionally. Overall, unplanned pregnancy was the highest risk factor for inadequate folic acid use (OR 17.7, 95% CI [14.4; 21.8]). Multiparity was also positively associated with inadequate folic acid use (OR 1.6, 95% CI [1.3; 1.9]). A previous spontaneous abortion was associated with a decreased risk of inadequate folic acid use (OR 0.7, 95% CI [0.6; 0.8]). The results of the analyses with the cohort categorised into three groups (adequate use, subadequate use, no use) showed same tendencies for all described risk estimates. Adequate and subadequate folic acid users were comparable with respect to socio-demographic, lifestyle, and pregnancy characteristics.

The largest ethnic groups were Dutch (n = 3457), Moroccan (n = 443), Turkish (n = 618), Antillean and Surinamese (n = 859), other-Western (n = 826), and other non-Western (n = 705). Overall, trends for ethnic specific risk factors for inadequate folic acid use were similar as those for the whole group. The largest and most significant effects were found for pregnancy planning, educational level, smoking, and previous spontaneous abortion (Figure 1). Unplanned pregnancy remained the highest risk factor for inadequate folic acid use and the largest effect was observed in the Dutch and Turkish population. For educational level the effect estimates were only significant in the Dutch and Turkish population.



**Figure 1 Ethnic specific risk factors for inadequate folic acid use**




Four main determinants of inadequate folic acid use stratified for ethnicity and adjusted for maternal age, marital status, education, BMI, smoking habits, alcohol consumption, parity, pregnancy planning, and previous spontaneous abortion

Risk estimates for smoking were significant for the Dutch, Turkish, 'other Western', and 'non-Western' population. After stratification for ethnicity previous spontaneous abortion still decreased the risk of inadequate use. However, these effect estimates were only significant among the Dutch and 'other Western' population. As for the other four risk estimates comprising age, marital status, alcohol use, and multiparity similar tendencies were found after stratification. However, most risk estimates were no longer significant. Younger age (20 - 30 years) was only significantly associated with inadequate folic acid use in the Dutch population (OR 1.4, 95% CI [1.1; 1.9]). Single marital status remained only significant for the Dutch and 'other Western' population (OR 2.1, 95% CI [1.2; 3.7] and OR 3.2, 95% CI [1.2; 8.6]). The same effect was found for multi-parity in the Dutch and Turkish population (OR 1.7, 95% CI [1.3, 2.2] and OR 2.1, 95% CI [1.1; 4.2]). Finally, if a woman consumed alcohol during pregnancy the risk of inadequate use of folic acid was significant in the Dutch population (OR 1.7, 95% CI [1.3; 2.2]) and with a decreased risk in the Moroccan population (OR 0.4, 95% CI [0.2; 0.7]).

## Comment

In this multi-ethnic pregnancy cohort between 2001 and 2006, the adequate preconception use of folic acid was as low as 37%. Thus, over the past years this percentage has not significantly changed.[10] The most important factors for inadequate folic acid use appear to be unplanned pregnancy, low socioeconomic status (SES), and non-Dutch ethnicity. Other identified determinants are younger age, single marital status, multiparity, previous spontaneous abortion, smoking, and alcohol consumption. Remarkably, this latter determinant only transformed into a risk factor for inadequate folic acid use after controlling for confounders. Since alcohol use, folic acid use, and SES are highly correlated this may explain this change in the effect estimate.

This was a survey embedded in a large multi-ethnic cohort study in Rotterdam. The population in our study may not be a complete representative of the general Rotterdam population because of an overrepresentation of high educated women in our cohort.[12, 13, 18] Moreover, extrapolation to the whole Dutch population seems inappropriate since this was a multi-ethnic cohort from a metropolitan area. Information on folic acid use was retrospectively obtained by questionnaires in Dutch or English. Although a questionnaire is a valid method for retrospective information collection misclassification or bias through language proficiency should always be considered. For this reason women who did not speak Dutch or English were offered individual help in Arabic, French, Portuguese, or Turkish by translated questionnaires and study staff speaking these languages. However, because response rates of these women were still lower some underestimation of the effect estimates might have occurred.[12, 13] Last, although we have specific information when use was started, data on actual duration of folic acid use are lacking. However, it can be assumed that women taking preconception folic acid are likely to use folic acid throughout the entire advised period. Moreover, preconception intake of folic acid results in a maternal folate depot which lasts for several weeks.

Pregnancy planning appeared to be the most important factor for inadequate folic acid use even among the various ethnic groups which is in accordance with previous studies.[19-21] In our cohort 70% of the pregnancies was planned which is lower than the 85% reported for the general Dutch population.[22] A possible explanation is the large number of immigrants in our cohort and the lower rate of pregnancy planning of this particular group (60.6%).[11, 20, 21] However, we are aware that the question pregnancy planning may be subject to confounding by social desirability.[20, 21] Remarkable is that 30% of the women who became pregnant after fertility treatment did not adequately use folic acid. This could be explained by a lack of information provided by the general practitioner or obstetrician which emphasises the need of postgraduate training, or calls for a change in information routines in the fertility clinics.

Folic acid use is extremely low among low educated women independent of ethnicity. This finding is also in line with other adverse health behaviours such as smoking and alcohol consumption during pregnancy in these women.[19, 23] Since low educated women tend to plan their pregnancy less this is not surprising. However, it could also be assumed that this target group is less conscious about general health.[20, 21] In addition, awareness about folic acid is still low among low educated women.[10] Apparently it is difficult to communicate not only the importance of folic acid use but also the importance of a healthy lifestyle during pregnancy towards low educated women.

Adequate folic acid use was very low among pregnant non-Dutch women. Van Eijsden et al.[11] reported that folic acid use was low in a multi-ethnic metropolitan pregnancy cohort in The Netherlands. Even though our study population is different from theirs because it comprises of different ethnic groups the prevalence of inadequate folic acid use is comparable. It seems that in the Moroccan population alcohol use was associated with adequate folic acid use. A possible explanation can be that Moroccans who drink have adapted to a more Western lifestyle and therefore are more willing to use a folic acid supplement. Smoking and multi-parity appear to be specific risk factors for inadequate folic acid use among the Dutch Turkish population which is in accordance with the findings of Nilsen et al.[19] Furthermore, the prevalence of smoking and multiple births is high in the Dutch Turkish population.[24, 25] It is remarkable that unplanned pregnancy was the only and also lowest specific risk factor significantly associated with inadequate use of folic acid among the Suriname and Antillean population. A possible explanation may be the reluctance in this population to use medication.

Beliefs about the lack of efficacy of folic acid use may have contributed to inadequate folic acid use in these women as well.[26] Among the 'other non-Western' population pregnancy planning and smoking were associated with inadequate folic acid use. However, the numbers in this group are too small to draw strong conclusions.

The task to prevent NTDs by adequate folic acid intake is far from being fulfilled. Our data suggest that the launching of recommendations on folic acid use has not been enough to increase intake. Ten years ago, the United States introduced mandatory fortification of grains with folic acid. Since then a significant decline was seen in the prevalence of NTDs.[7, 27] In addition, it has been suggested that folic acid fortification might have other beneficial effects as well.[28, 29] In The Netherlands mandatory food fortification is again under review.
In the past, one of the reasons against the introduction of mandatory folic acid fortification has been that it masks the diagnosis of megaloblastic anaemia. Nowadays, this is not an argument anymore because vitamin B12 deficiency can easily be diagnosed.[9] However, if mandatory food fortification is going to be introduced in The Netherlands we emphasise the importance of surveillance programmes to monitor potential side effects. [30]

We are aware that there is a gap between awareness and actual adequate folic acid intake especially among lower educated women [10] Information and advise are the most important factors for adequate folic acid use.[21] In the past, mass media contributed significantly to folic acid awareness. However, this was an incidental and anonymous activity which is not enough to change behaviour in the major proportion of women.[31] We assume that "face-to-face" communication embedded in preconception counseling is more likely to be successful in actually changing life style behaviours especially in immigrant women because these women are often missed in providing information about the need of adequate folic acid use.[20, 21, 32, 33] Therefore, all women planning to become pregnant should have access to preconception counseling with special attention for language barriers and health literacy determined by cultural, religious, and social issues.[32, 34, 35] Interestingly, there is an inverse relation between adequate folic acid use and adverse health behaviours such as smoking and alcohol consumption during pregnancy. For this reason, this study supports the further development and improvement of national public health strategies together with programmes of preconception care.

In conclusion, adequate preconception use of folic acid is still low. Unplanned pregnancy, low socioeconomic status, and non-Dutch ethnicity are the main determinants of inadequate folic acid use. There is a relation between folic acid use and adverse lifestyle factors. To change not only awareness but also attitude towards adequate preconception folic acid use a change in total lifestyle is necessary. Preconception health educational programmes should be developed and applied to improve the intake of folic acid.



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## Chapter 4.2

# Individual accumulation of heterogeneous risks explains perinatal inequalities within deprived neighbourhoods in Rotterdam, The Netherlands

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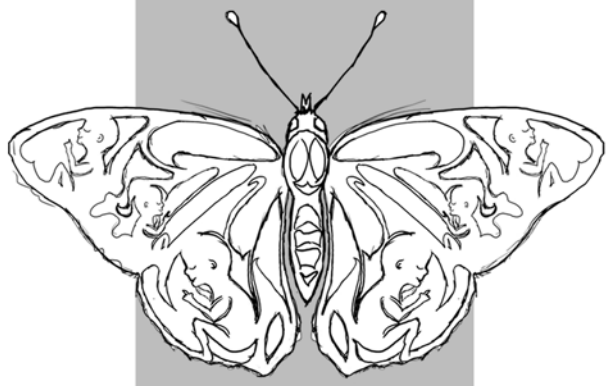

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## Abstract

The persistent poor Dutch' figures on perinatal mortality are of great concern. Recent numbers showed considerable geographic differences in perinatal health with particular increased risks for pregnancies from socioeconomic deprived neighbourhoods. The objective of this study was to examine associations between modifiable perinatal risk factors and adverse perinatal outcomes on a neighbourhood level. The study was embedded in a large population-based prospective cohort study in Rotterdam, The Netherlands. For the present study we used data on 7359 women with a singleton pregnancy ≥ 22 weeks of gestation who were expected to deliver between 2002 and 2006. The Dutch index of deprivation 2007 was used to determine neighbourhood deprivation. Information regarding sociodemographic, lifestyle, obstetrical and health-related risk factors was obtained from questionnaires, physical examinations, ultrasound measurements, biological samples, and medical records. Based on the sum of individual weights that was assigned to each individual risk factor, study participants were categorised into low-, medium-, or high risk factor category. Main outcome measures included perinatal death, intrauterine growth restriction (IUGR), prematurity, congenital malformations, Apgar score < 7 at five minutes after birth, and pre-eclampsia. Compared to pregnancies from a non-deprived neighbourhood, pregnancies from a deprived neighbourhood had almost a twofold increased risk for perinatal death (relative risk (RR) 1.8, 95% Confidence Interval (CI) [1.1; 3.1]). IUGR, prematurity, Apgar < 7 after five minutes, and pre-eclampsia also showed higher prevalences ($p < 0.05$). Residing within a deprived neighbourhood was associated with an accumulation of individual risk factors (35.8% high-risk for women from a deprived neighbourhood versus 15.1% high-risk for women from a non-deprived neighbourhood, $p < 0.05$). This risk accumulation attributed directly to the observed neighbourhood differences in perinatal outcomes. Further exploration of risk factors indicated that women from a deprived neighbourhood had significantly more 'possibly avoidable' risk factors including ≥ 36 working hours, obesity, smoking, recreational drug use, unplanned pregnancy, no folic acid use, late gestational age at booking, maternal psychopathology, comorbidity, and sexual transmittable diseases, as compared to women from a non-deprived neighbourhood. In conclusion, women from a socioeconomically deprived neighbourhood are at an increased risk for adverse pregnancy outcomes. The excess risk can directly be attributed to accumulation of individual risk factors. Differences with regard to possibly modifiable risk factors imply that preventive strategies may prove effective.




## Introduction

The 2009' results from EURO-PERISTAT II demonstrated a relatively high and persistent poor perinatal mortality rate in The Netherlands (9.8 per 1000 total births in 2006).[1] A broader comparison of maternal and neonatal outcomes revealed that the Dutch figures are among the worst in Europe.[1]

Additionally, considerable geographic differences exist with respect to most other perinatal health indicators.[2-4] Perinatal mortality and morbidity are significantly more prevalent in the four largest cities with particular increased risks for pregnancies from socioeconomically deprived neighbourhoods. In some deprived neighbourhoods perinatal mortality appears to be as high as 17 / 1000.[2]

These observed geographic inequalities emphasise the need for improvements. In light of limited opportunities to lower perinatal mortality by therapeutic interventions, increased attention to primary and secondary preventive strategies is warranted. This requires detailed information on modifiable sociodemographic, lifestyle, obstetric, and other health-related risk factors. From comparison of these perinatal risk factors across neighbourhoods novel opportunities may emerge to reduce Dutch perinatal health inequalities.

Whilst extensive regarding perinatal outcomes and interventions, the Dutch perinatal registration databases are limited concerning individual- and geographical risk factors.[2, 3] The Generation R Study is a prospective cohort study from early pregnancy onwards in Rotterdam, the second largest city in The Netherlands.[5, 6] Its detailed data collection enables the examination of the presence of neighbourhood' related effects.

## Materials and Methods

### *Study design*

The study was embedded within The Generation R Study, an ongoing population-based cohort study designed to identify early determinants of growth, development, and future health. [5, 6] The Generation R Study is conducted in Rotterdam, the second largest city in The Netherlands comprising about 585.000 inhabitants.[7] Study participants were pregnant Rotterdam women expected to deliver between April 2002 and January 2006. Of all eligible born children approximately 61% participated. Assessments included questionnaires, physical examinations, fetal ultrasound measurements, and biological samples in early, mid-, and late pregnancy. [5, 6] For the present study we restricted our analyses to prenatally enrolled women with a singleton pregnancy ≥ 22 weeks of gestation (n = 8668). With respect to mothers with multiple pregnancies in The Generation Study Cohort, we randomly excluded one of these pregnancies to avoid bias due to paired data (n = 460). The study was conducted in accordance with the guidelines as proposed in the World Medical Association Declaration of Helsinki and

was approved by the Medical Ethics Committee of the Erasmus Medical Centre Rotterdam. Written consent was obtained from all participants.[5, 6] [8, 9]

***Neighbourhood deprivation***

We categorised women as residing within a deprived neighbourhood (deprived neighbourhood) or outside of a deprived neighbourhood (non-deprived neighbourhood), based on the postcode of their place of living which can be converted appropriately by the published Dutch index of deprivation 2007. We acquired the participants' postcodes from the Centre for Research and Statistics Rotterdam.[7] The deprivation index 2007 is national indicator of neighbourhood deprivation designed by The Netherlands' Department of Housing, Spatial Planning, and the Environment.[10] The deprivation index is based on separate scores based on five domains of deprivation: housing, employment, education, integration, and safety respectively. The index assigned the label 'deprived' to 83 out of 4878 Dutch postcodes, of which 23 postcode-areas were located in Rotterdam.

***Risk factors***

Sociodemographic risk factors were assessed by a survey in early pregnancy. These included maternal age, marital status, consanguinity, and measures of socioeconomic status and ethnicity. Socioeconomic status was defined by educational level, net household income, and employment status.[5, 6, 11, 12] Maternal occupation was classified as employed (paid or self-employment) or unemployed (job-seeking, social security or disability benefit, housewife, student). The number of weekly working hours was also assessed to make a further distinction. Ethnicity was defined by country of birth of the woman herself and her parents. The following classification was applied: Dutch, Moroccan, Turkish, Cape-Verdean, Antillean, Surinamese, other non-Western, and other Western. Because women of Antillean or Surinamese descent are of mixed ethnic origin we further categorised these women into Surinamese Hindustani or Afro-African, Antillean Afro-African, and Antillean/Surinamese-other.[12] Information regarding obstetric characteristics was obtained from the same questionnaire. This included parity, pregnancy planning, folic acid use, obstetric history, and gestational age at booking.[5, 6] With respect to lifestyle factors, height and weight were measured to calculate body mass index at inclusion (BMI; in kg / m2). Information on smoking, alcohol, and recreational drug use was obtained by questionnaires in early, mid-, and late pregnancy.[5, 6] Psychopathology was assessed using the Brief Symptom Inventory (BSI) in mid-pregnancy.[5, 6, 13] The BSI is a validated questionnaire outlined to ascertain the psychological state in the preceding week. For the present study we used both anxiety (ANX) and depression scales (DEP) with a cut-off score ≥ 0.67. Other health characteristics included self-reported history of hypertension, diabetes, heart disorders, hypercholesterolaemia, Systemic Lupus Erythematosus, multiple sclerosis, or thyreoid disease (i.e. comorbidity), as well as sexual transmittable diseases (STD) comprising of chlamydia infection (measured in urine by PCR), hepatitis B (hepatitis B surface antigen measured in blood samples), toxoplasmosis (immunoglobulin G [IgG] and IgM Toxoplasma gonadii antibodies measured in blood samples), and HIV-infection (self-reported).[5, 6, 14]

***Pregnancy outcomes***

Several overlapping sources (obstetric caregivers, ultrasound facilities, Municipal Health Services) provided information about A) location and mode of delivery (the Dutch obstetric system is characterised by its home delivery policy); and B) pregnancy outcomes including intrauterine growth restriction (IUGR), pre-eclampsia, intrauterine fetal death, birth weight, gestational age at birth, congenital malformations, Apgar score five minutes after birth, and early neonatal death.[5, 6, 15, 16] IUGR and low birth weight were defined as a SD-score < -1.28, and prematurity as delivery < 37.0 weeks of gestation.[17] Last, we conveniently defined the compound measure 'Adverse Outcome (Ao)' as the presence of one or more of the following perinatal events: perinatal death, congenital malformations, prematurity, low birth weight, and / or Apgar < 7.

***Data analysis***

Of the eligible women 849 had ≥ 35% missing values. After exclusion of these women the sample available for final analysis was 7359. With respect to the remaining data missing values were imputed using multiple imputation.[18, 19] In the present study for each missing value five draws were performed providing five substituted data which in turn created five completed data sets. Analyses were performed separately on each completed dataset and thereafter combined into one global result.[18-20] Table 1 provides the percentages of missing values per covariate. With respect to data analysis the distribution of risk factors and pregnancy outcomes between deprived and non-deprived neighbourhoods was compared using the Independent Students' t, Mann-Whitney U, and chi-square test. Next, for women living within a deprived neighbourhood as risk factor, we estimated the relative risk (RR) with 95% Confidence Interval (95% CI) for the outcomes hospital delivery, emergency and elective caesarean section, instrumental vaginal delivery, IUGR, low birth weight, prematurity, congenital malformations, Apgar < 7, pre-eclampsia, and intrauterine or neonatal death. Then, weighted risk scores were constructed including all individual risk factors from Table 1.[21, 22] The approach was performed by assigning a 'whole number weight' to the adverse level of each separate risk factor. This weight was derived from literature estimates and exploratory analyses using our own cohort.[22] Based on the sum of the individual weights for each present risk (range 0 - 20) we classified women into: A) low-risk (risk score sum < 3); B) medium-risk (risk score sum 3 - 7); or C) high-risk (risk score sum ≥ 7). Differences in risk scores were tested using Mann-Whitney U test. Subsequently, we calculated the observed risk for the compound measure 'Adverse Outcome' within the three risk score groups (A, B, or C). Logistic regression analysis was applied to relate individual characteristics, including living within a deprived area, to the occurrence of 'Adverse Outcome'. In view of collinearity both a forward and backward strategy was applied

**Table 1 Percentages and relative risks of pregnancy and birth outcomes according to neighbourhood classification**

| | | Deprived neighbourhood | | |
|---|---|---|---|---|
| | Total | Yes | No | RR (95% CI) |
| | n= 7359 | n = 2779 | n = 4580 | |
| **Location of delivery** | | | | |
| Hospital | 82.3 | 85.6 | 80.2 | 1.07 [1.05; 1.09]a |
| At home | 16.4 | 12.9 | 1a8.5 | |
| Missing | 1.3 | 1.5 | 1.2 | |
| **Start delivery** | | | | |
| Elective caesarean section | 4.3 | 3.4 | 4.9 | 1.01 [1.00; 1.02]b |
| Induction of labour | 12.7 | 12.7 | 12.7 | |
| Spontaneous | 76.1 | 76.7 | 75.7 | |
| Missing | 6.9 | 7.2 | 6.7 | |
| **Method of delivery** | | | | |
| Instrumental vaginal delivery | 12.6 | 10.9 | 13.7 | 1.06 [1.03; 1.09]c |
| Emergency caesarean section | 6.7 | 6.7 | 6.6 | |
| Elective caesarean section | 4.8 | 3.8 | 5.4 | |
| Breech | 0.1 | 0.1 | 0.2 | |
| Spontaneous | 66.4 | 69.3 | 64.5 | |
| Missing | 9.4 | 9.2 | 9.6 | |
| **Pregnancy outcomes** | | | | |
| **IUGR mid-pregnancy** | | | | |
| Mean EFW mid-pregnancy (grams, SD) | 381.8 (94.3) | 383.2 (99.0) | 381.0 (91.5) | |
| < p10 | 1.7 | 1.9 | 1.6 | 1.25 [0.88; 1.78] |
| Missing | 6.2 | 7.1 | 5.6 | |
| **IUGR late pregnancy** | | | | |
| Mean EFW late pregnancy (grams, SD) | 1617.4 (262.8) | 1602.4 (267.5) | 1626.0 (259.7) | |
| < p10 | 1.6 | 2.2 | 1.2 | 1.78 [1.25; 2.55] |
| Missing | 4.0 | 4.7 | 3.6 | |
| **Pre-eclampsia** | | | | |
| Yes | 2.4 | 3.0 | 2.1 | 1.46 [1.09; 1.95] |
| Missing | 2.8 | 2.7 | 2.8 | |
| **Intrauterine fetal death** | | | | |
| Yes | 0.5 | 0.7 | 0.3 | 1.94 [1.02; 3.69] |
| **Birth outcomes** | | | | |
| **Low birth weight** | | | | |
| Mean birth weight (grams, SD) | 3416.0 (559.2) | 3355.8 (569.0) | 3451.1 (550.5) | |
| < p10 | 11.9 | 14.5 | 10.4 | 1.41 [1.24; 1.59] |
| Missing | 1.2 | 1.7 | 0.9 | |
| **Premature delivery** | | | | |
| Median gestational age delivery (weeks, range) | 40.1 (22.4 - 43.6) | 40.0 (22.6 - 43.6) | 40.2 (22.4 - 43.4) | |
| < 37.0 weeks | 5.2 | 5.9 | 4.8 | 1.22 [1.00; 1.48] |
| Missing | 0.5 | 0.7 | 0.4 | |
| **Congenital malformations** | | | | |
| Yes | 4.2 | 3.8 | 4.4 | 1.07 [0.86; 1.35] |
| Missing | 34.8 | 43.9 | 29.3 | |
| **Apgar score 5 minutes after birth** | | | | |
| < 7 | 1.1 | 1.5 | 0.9 | 1.57 [1.02; 2.41] |
| Missing | 4.1 | 4.0 | 4.1 | |
| **Neonatal death** | | | | |
| Yes | 0.2 | 0.3 | 0.2 | 1.65 [0.66; 4.20] |

Values represent percentage (%) within column or relative risk (95% Confidence Interval). a RR (95% CI) compared to home delivery (reference). b RR (95% CI) compared to spontaneous start or induction of labour (reference). c RR (95% CI) compared to spontaneous vaginal delivery (reference)



for all five imputed datasets separately (p inclusion = p exclusion = 0.10). If the same association was observed across four or five datasets, this was interpreted as being significant. In that case we report on the median size of that coefficient together with its 95% CI. Lastly, to assess possibilities for preventive strategies, we recoded our risk factors into 'possibly avoidable' and 'possibly non-avoidable', based on the list of Rutstein et al.[23, 24] Avoidability was judged based on the presence of evidence that the occurrence of an adverse perinatal outcome might be prevented by primary or secondary preventive measures embedded in preconception or prenatal care.[25] [26] This approach classified the following risk factors as 'possibly avoidable': number of working hours (occupation), BMI, smoking, alcohol and recreational drug use, pregnancy planning, folic acid use, gestational age at booking, maternal psychopathology, comorbidity, and STDs. Age, ethnicity, education, net income, marital status, consanguinity, parity, complications previous pregnancy, and moving were classified as 'possibly non-avoidable'. Statistical analyses were performed using Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc, Chicago, IL, USA). Multiple imputation was conducted in R version 2.7.2 (2008 – 6 - 23).

## Results

Data were available on 7359 women belonging to 71 area-postcodes. Of these women 37,8% was resident within a deprived neighbourhood (n = 23 area-postcodes) and 62,2% outside of a deprived neighbourhood (n = 48 area-postcodes). Considerable differences in pregnancy outcomes were apparent across deprived and non-deprived neighbourhoods (Table 1). Overall perinatal mortality encompassing intrauterine and neonatal death was 0.7% (55 / 7359). Women residing within a deprived neighbourhood had almost a twofold increase in risk for perinatal death as compared to women residing outside of a deprived neighbourhood (RR 1.8, 95% CI [1.08; 3.11]). This difference was mainly explained by the substantially higher number of intrauterine fetal deaths in deprived neighbourhood pregnancies (20 / 2779 versus 17 / 4580). Nearly all other adverse pregnancy outcomes, including IUGR, pre-eclampsia, low birth weight, prematurity, and Apgar < 7, showed higher prevalences in pregnancies from deprived neighbourhoods. In contrast, the risks for hospital delivery, elective caesarean section, and instrumental vaginal were similar.

**Table 2 Prenatal maternal characteristics according to neighbourhood classification**

| | Total | Deprived neighbourhood | |
|---|---|---|---|
| | | Yes | No |
| | n = 7359 | n = 2779 | n = 4580 |
| **Gestational age enrolment (median, range)** | 14.4 (5.1 - 39.2) | 14.9 (5.0 - 39.2) | 13.9 (6.4 - 38.9)* |
| **Sociodemographic factors** | | | |
| **Maternal age** | | | |
| Mean (SD) | 29.7 (5.3) | 28.1 (5.6) | 30.6 (4.9)* |
| < 20 years | 4.1 | 6.3 | 2.8* |
| 20 – 35 years | 80.9 | 81.6 | 80.4 |
| ≥ 35 years | 15.0 | 12.1 | 16.8 |
| **Ethnicity** | | | |
| Dutch or Western | 60.1 | 39.6 | 72.5* |
| Surinam Hindu | 3.9 | 5.6 | 2.9 |
| Surinam Afro-African | 3.3 | 4.6 | 2.5 |
| Antillean Afro-African | 1.3 | 1.7 | 1.0 |
| Antillean or Surinam other | 2.0 | 2.6 | 1.6 |
| Turkish | 9.3 | 15.8 | 5.4 |
| Moroccan | 6.7 | 11.3 | 3.9 |
| Cape-Verdean | 4.2 | 6.9 | 2.6 |
| non-Western other | 8.8 | 11.3 | 7.3 |
| Missing | 0.4 | 0.6 | 0.3 |
| **Educational level** | | | |
| Primary | 11.5 | 18.1 | 7.4* |
| Missing | 2.0 | 2.8 | 1.5 |
| **Net income** | | | |
| Euro < 1200-, | 15.8 | 25.7 | 9.8* |
| Euro 1200 – 2200-, | 19.8 | 23.4 | 17.6 |
| Euro ≥ 2200-, | 42.4 | 21.2 | 55.2 |
| Missing | 22.0 | 29.7 | 17.4 |
| **Occupation** | | | |
| Non-working | 20.3 | 26.7 | 16.5* |
| < 20 hours / week | 5.3 | 4.5 | 5.7 |
| 20 - 36 hours / week | 24.7 | 18.0 | 28.8 |
| ≥ 36 hours / week | 23.7 | 17.8 | 27.3 |
| Missing | 26.0 | 33.0 | 21.7 |
| **Marital status** | | | |
| Single | 15.0 | 21.6 | 11.0* |
| Missing | 1.0 | 1.1 | 1.0 |
| **Consanguinity** | | | |
| Yes | 3.9 | 6.9 | 2.1* |
| Missing | 5.6 | 6.4 | 5.1 |
| **Moved during pregnancy** | | | |
| Yes | 12.8 | 14.4 | 12.0* |
| **Lifestyle factors** | | | |
| **BMI intake** | | | |
| Mean (SD) | 24.8 (4.6) | 25.3 (4.9) | 24.5 (4.3)* |
| < 20 | 9.0 | 9.1 | 8.9* |
| 20 - 30 | 78.5 | 75.7 | 80.2 |
| ≥ 30 | 12.5 | 15.2 | 10.9 |

Values represent percentage (%) within column, gestational age at inclusion in weeks. *p value < 0.05



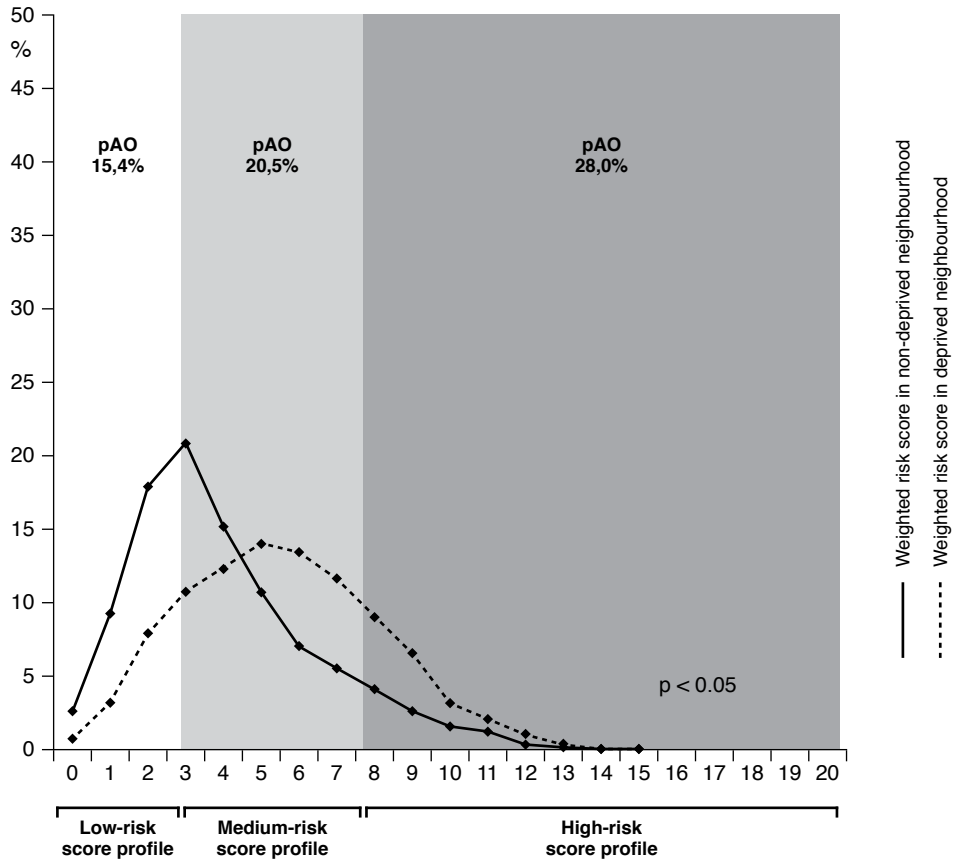
(Table 2 continued)

| | Total | Deprived neighbourhood | |
|---|---|---|---|
| | | Yes | No |
| | n = 7359 | n = 2779 | n = 4580 |
| **Smoking habits** | | | |
| Yes, stopped after pregnancy recognition | 8.0 | 7.1 | 8.5* |
| Yes, continued throughout pregnancy | 16.8 | 20.6 | 14.5 |
| Missing | 5.1 | 4.5 | 5,5 |
| **Alcohol consumption** | | | |
| Yes, but stopped after pregnancy recognition | 12.9 | 10.4 | 14.4* |
| Yes, continued throughout pregnancy | 34.2 | 25.6 | 39.4 |
| Missing | 4.6 | 3.9 | 4.9 |
| **Recreational drug use** | | | |
| Yes, during the periconception period | 2.6 | 3.0 | 2.3* |
| Yes, continued throughout pregnancy | 0.5 | 0.8 | 0.3 |
| Missing | 3.9 | 3.1 | 4.3 |
| **Obstetric characteristics** | | | |
| **Parity** | | | |
| 0 | 57.9 | 57.5 | 58.3* |
| 1 - 3 | 38.4 | 37.6 | 38.9 |
| ≥ 3 | 3.7 | 4.9 | 2.8 |
| **Pregnancy planning** | | | |
| No | 28.1 | 36.9 | 22.8* |
| Missing | 5.3 | 5.7 | 5.0 |
| **Folic acid use** | | | |
| No | 24.8 | 36.2 | 17.8* |
| Missing | 5.3 | 5.1 | 5.4 |
| **Complications previous pregnancy** | | | |
| Yes | 7.8 | 7.7 | 7.9 |
| Missing | 0.1 | 0.1 | 0.2 |
| **Gestational age at booking** | | | |
| Median (range) | 12.1 (4.6 - 33.4) | 12.4 (5.1 - 32.0) | 11.9 (4.6 - 33.4)* |
| < 14 weeks | 41.7 | 45.3 | 44.7* |
| ≥ 14 weeks | 15.0 | 22.1 | 12.1 |
| Missing | 43.3 | 32.6 | 43.2 |
| **Health characteristics** | | | |
| **Depressive complaints** | | | |
| Score DEP median (range) | 0 (0 - 4.0) | 0.17 (0 - 4.0) | 0 (0 - 4.0)* |
| Yes | 8.5 | 11.3 | 6.7* |
| Missing | 20.3 | 25.7 | 16.9 |
| **Anxiety complaints** | | | |
| Score ANX median (range) | 0.17 (0 - 4.0) | 0.17 (0 - 4.0) | 0.17 (0 - 3.7)* |
| Yes | 9.3 | 12.2 | 7.6* |
| Missing | 20.2 | 25.8 | 16.8 |
| **Comorbidity** | | | |
| Yes | 5.9 | 5.5 | 6.2 |
| Missing | 4.2 | 3.4 | 4.7 |
| **Sexual transmittable diseases** | | | |
| Yes | 2.7 | 3.9 | 1.9* |
| Missing | 11.5 | 13.1 | 10.5 |

Values represent percentage (%) within column, gestational age at inclusion in weeks. *p value < 0.05

Maternal characteristics and risk factors are presented in Table 2. Forty percent of the study population was of non-Western descent (n = 2903). The majority of these women resided within a deprived neighbourhood (1660 / 2779). We observed large differences in the prevalence of sociodemographic, lifestyle, obstetrical, and health-related determinants between women living within and outside of a deprived neighbourhood. Deprived neighbourhood' women were younger, had lower measures of socioeconomic status, and suffered more often from obesity, psychopathology, and STDs (all $p < 0.05$). We also observed higher percentages of unplanned pregnancies among these women as well as higher percentages of adverse lifestyle factors including smoking or no folic acid use.

**Figure 1 Distribution of individual weighted risk scores for women residing within and outside of a deprived neighbourhood**




The graph represents the distribution of individual weighted risk scores between deprived and non-deprived neighbourhoods. Differences in weighted risk score profiles were tested using Mann-Whitney U test. The risk for the compound measure 'Adverse Outcome' is represented by 'pAo' given as percentage per risk stratum. Analyses are based on multiple imputed dataset, n = 7359



In Figure 1 the poor total risk scores for women from a deprived neighbourhood are shown (35.8% high-risk score for women from a deprived neighbourhood versus 15.1% high-risk score for women from a non-deprived neighbourhood). As this score is linked to the compound measure 'Adverse Outcome' this explains in part the inferior perinatal (health) outcomes in deprived neighbourhood areas (28.0% risk for an adverse pregnancy outcome within the

**Table 3 Association between individual risk factors and the occurrence of 'Adverse Outcome', results from five imputed datasets and forward and backward regression respectively**

| | Forward approach | | Backward approach | |
|---|---|---|---|---|
| | Significant | Size | Significant | Size |
| **Risk factor** | Association | (Exp B, 95% CI) | Association | (Exp B, 95% CI) |
| Neighbourhood deprivation | - | | - | |
| Maternal age < 20 years | - | | + | 1.34 (1.00; 1.79) |
| Ethnicity Afro-African | - | | + | 1.36 (1.11; 1.65) |
| Ethnicity non-Western other | + | 1.20 (1.05; 1.37) | + | 1.25 (1.08; 1.44) |
| Net income Euro < 1200-, | + | 1.22 (1.05; 1.42) | + | 1.20 (1.02; 1.40) |
| Occupation non-working | - | | - | |
| Occupation ≥ 36 hours / week | - | | - | |
| Marital status single | - | | - | |
| BMI intake < 20 | + | 1.34 (1.11; 1.61) | + | 1.36 (1.13; 1.63) |
| Smoking habits during pregnancy | + | 1.17 (1.03; 1.33) | + | 1.21 (1.06; 1.37) |
| Recreational drug use | - | | - | |
| Nullipara | + | 1.84 (1.61; 2.10) | + | 1.85 (1.62; 2.11) |
| Unplanned pregnancy | - | | - | |
| No folic acid use | ± | | ± | |
| Complications previous pregnancy | + | 1.33 (1.04; 1.68) | + | 1.32 (1.04; 1.67) |
| Gestational age booking ≥ 14 weeks | ± | | ± | |
| Depressive or Anxiety complaints | - | | - | |
| Comorbidity | - | | ± | |
| Sexual transmittable diseases | - | | - | |
| Interaction: single marital status * ethnicity Afro-African | + | 1.39 (1.19; 1.62) | - | |
| Interaction: single marital status * age < 20 years | - | | + | 1.35 (1.12; 1.61) |
| Interaction: single marital status * unplanned pregnancy | - | | - | |
| Interaction: net income Euro < 1200-, * folic acid | - | | - | |

Results from multiple logistic regression analysis relating individual risk factors to the occurrence of 'Adverse Outcome'. Both forward and backward approaches are shown (in- / exclusion cut-off p value < 0.10). The analyses were performed separately on each multiple imputed dataset (5 * n = 7359). Here, we show those covariables that significantly contributed to the outcome measure 'Adverse Outcome' in at least 4 (out of 5) multiple imputed datasets (+). In that case the median size of that coefficient (exp B) together with its 95% CI is presented. – not included in model at all, or only in 1 multiple imputed dataset; ± significant contributor in 2 / 3 multiple imputed datasets; + significant contributor in 4 / 5 multiple imputed datasets

high-risk score stratum compared to a 15.4% risk for an adverse pregnancy outcome within the low-risk score stratum). Logistic regression (with 'Adverse Outcome' as outcome) consistently showed strong effects for the risk factors non-Western and Afro-African ethnicity, low income, BMI < 20, smoking, nulliparity, complications in previous pregnancy, and two interaction-terms (low age*single; and Afro-African ethnicity*single) (Table 3). We observed minor differences across the five datasets as well as in the forward/backward approaches. In none of these analyses, living within a deprived neighbourhood emerged as significant independent risk factor. Additional prediction of 'living within a deprived neighbourhood' from the same risk set provided the same solution (equal risk factors) as the analysis predicting 'Adverse Outcome'. Apparently, in the present study the effect of neighbourhood deprivation on perinatal outcome was explained by a differential prevalence of individual risk factors (so called intermediate factors).

Lastly, Figure 2 shows that women residing within a deprived neighbourhood have significantly more 'possibly avoidable' risk factors as compared to women from of a non-deprived neighbourhood.

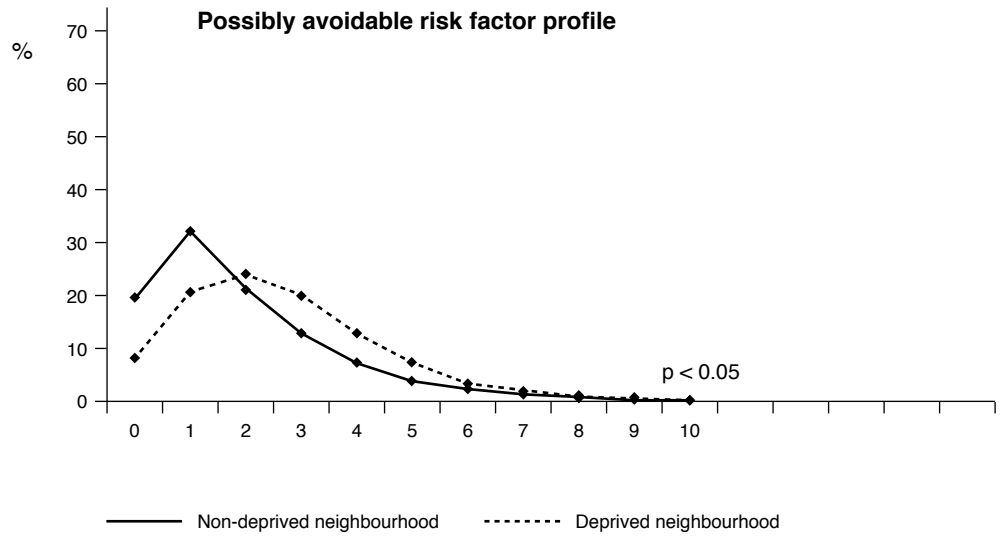
## Comment

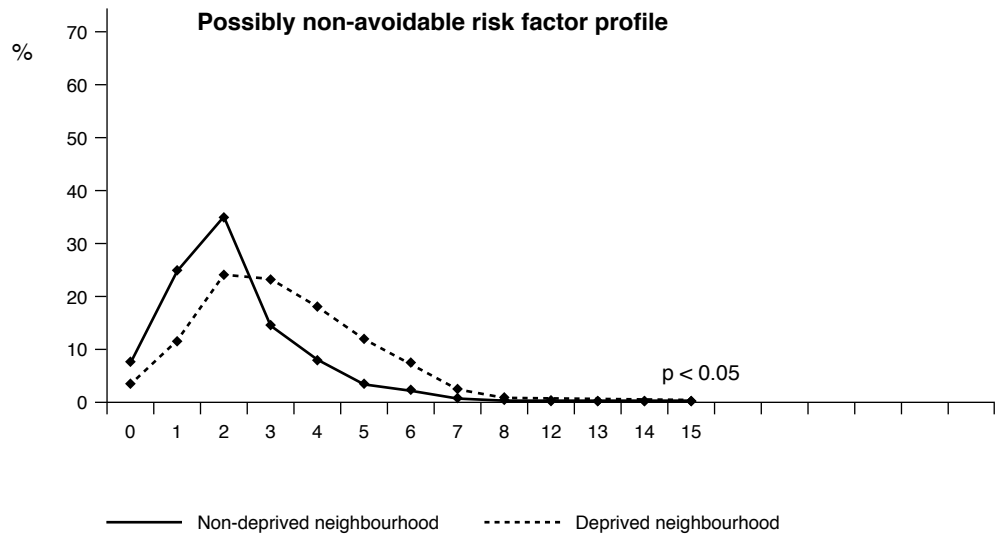
To our knowledge, this is the first study to examine associations between modifiable perinatal risk factors and adverse perinatal outcomes on the neighbourhood level to enable the narrowing of perinatal inequalities in disadvantaged urban areas. Women from a socioeconomically deprived neighbourhood are at a substantially increased risk for adverse pregnancy outcomes, with a twofold increased risk for perinatal death. This excess risk can directly be attributed to an accumulation of sociodemographic, lifestyle, obstetric, and health-related risk factors present within deprived neighbourhoods. The higher proportion of modifiable risk factors in disadvantaged areas provides opportunities for primary and secondary prevention.

This was a survey embedded in a large population-based prospective cohort study with an extensive data collection on both perinatal risk factors and outcomes. The use of structural variables to characterise neighbourhood deprivation rather than using aggregates of individual-level variables adds to its strength.[10, 27] Nevertheless, some limitations need to be discussed. First, selective participation has been demonstrated for socioeconomically deprived areas.[28] Though participation rates for The Generation R Study are relatively high and the ethnic distribution differs only moderately from that of the eligible population in the study area, The Generation R Study is characterised by a rather highly educated and healthy study population compared to available city data.[5, 6] This selective non-response towards higher socioeconomic status may have influenced the associations.[29] Moreover, we had to exclude ten percent



**Figure 2 'Possibly avoidable' and 'possibly non-avoidable' weighted risk scores according to neighbourhood classification**




The graphs represent the distributions of 'possibly avoidable' and 'possibly non-avoidable' risk factors between deprived and non-deprived neighbourhoods. Differences in risk factors were tested using Mann-Whitney U test. Analyses are based on multiple imputed dataset, n = 7359

of the study population because of high levels of missing data with subsequent considerable multiple imputations. Since these participants were predominantly lower educated and/or of non-western ethnicity this may have biased our results. Last, we obtained most information on perinatal risk factors by questionnaires in Dutch and English. If required individual support in Arabic, French, Portuguese, or Turkish was available. Despite these efforts, misclassification through language proficiency should always be considered.

Our findings on perinatal outcome inequalities within deprived neighbourhoods strengthen previously reported studies from Europe and the United States.[2, 4, 30-37] The majority of these studies focussed only on preterm birth and low birth weight, as important risk factors for infant mortality and morbidity.[31-34] We added pre-eclampsia, congenital anomalies, and suboptimal start at birth (Apgar) since the latter two contribute to perinatal mortality, whereas pre-eclampsia is a major driver for both perinatal and maternal mortality.[1, 38] To our knowledge to date no other study on neighbourhood deprivation and perinatal outcome has been conducted in such detail. An important finding that has not been reported before was the 50% increased risk of pre-eclampsia for women from a deprived neighbourhood, despite the inevitably higher prevalence of 'protective' smoking. Neighbourhood deprivation and cardiovascular disease are known to be strongly linked.[39] Since pre-eclampsia has been shown to be related to cardiovascular disease, a higher prevalence of the latter may have contributed to these results.[40] Interestingly, the risk for congenital malformations for women from a deprived neighbourhood was not increased. Previously, de Graaf et al.[2] reported similar results. They suggested that the higher uptake of prenatal screening in urban areas, as compared to rural areas, might be causative.[2] Future studies are needed to investigate this finding in closer detail.

In our study accumulation of risk factors largely explained perinatal inequalities according to place of living. Although the exact underlying mechanisms through which neighbourhood deprivation influences both perinatal risk factors and outcomes are unknown, several pathways have been proposed which share that geographical and individual characteristics are closely linked.[27, 41] One model proposes that neighbourhood-characteristics affect reproductive outcome by modelling of a wide range of individual-level economic opportunities and risk behaviours.[27, 42, 43] In this model access to education and training programs is determined by place of living. Better education yields higher socioeconomic status which in turn, by multiple pathways, benefits perinatal outcomes.[42] On the other hand, locally shared social characteristics may influence unhealthy risk behaviours through common cultural norms and beliefs, which in turn are associated with perinatal outcomes (i.e. smoking habits, sexual behaviour).[27, 43] Essentially all perinatal risk factors investigated in our study have been previously reported to be associated with both adverse neighbourhood conditions and reproductive health.[27, 42-46]



During recent years increasing attention has focused on preconception care as a means of optimising women's health and knowledge before planning and conceiving pregnancy in order to reduce the risk of adverse health effects for the woman, fetus, or neonate.[47-49] Several countries have succesfully introduced structured preconception health care programmes.[47-57] One of the most effective programmes, The Hungarian Periconceptional Service, reported reductions in congenital defects and ectopic pregnancy rates, and higher birth weights. Moreover, urogenital infections were detected and treated, and screening for diabetes and cardiovascular disease led to referral of 3% of women to special clinics.[57] The observed difference in modifiable perinatal risk factors between deprived and non-deprived neighbourhood' women provides opportunities for improvement. In this respect we consider the active provision of information as vital to reduce perinatal inequalities since most women appear to be largely ignorant on the consequences of an adverse lifestyle or of the presence of a medical condition during pregnancy, and in particular during the periconception period.[47, 58] A precondition for success of intensified care is free access to, and due start of preconception and prenatal care. Most women from a deprived neighbourhood entered antenatal care at such a late stage that it can be concluded that opportunities to improve the health of these women and their offspring were completely missed.[59] Immigrant women and women of very low socioeconomic class require special attention because they are often not reached in the provision of information about the consequences of an adverse lifestyle or medical condition during pregnancy due to language, cultural, and/or social barriers.[60] Without extra attention, intensification of prenatal care will have a differential effect according to the place of living.[27, 37, 41]

The inequalities in the prevalence of aetiological factors urge for detailed analysis of the provision of care, in particular the presence of substandard care relative to the risk exposure. In The Netherlands, recently concerns were raised on the quality of delivered perinatal care.[61-65] Substandard factors in maternity care were more likely to occur in immigrant women than in indigeneous women.[64, 65] The similar prevalence of instrumental vaginal delivery and caesarean sections across deprived neighbourhoods may suggest under-treatment in view of the much higher prevalence of conditions like IUGR and pre-eclampsia. Such research should apply multiple methods: epidemiological analysis of national data, qualitative in-depth research into professional mechanisms, and perinatal audit methods such as the one currently being implemented.[63] [66]

In conclusion, women from a socioeconomically deprived neighbourhood are at almost double risk for adverse pregnancy outcomes, which can be largely attributed to accumulation of individual risk factors present within underprivileged urban areas. Targeted preconception health care programmes should be implemented in order to lower the geographic inequalities.

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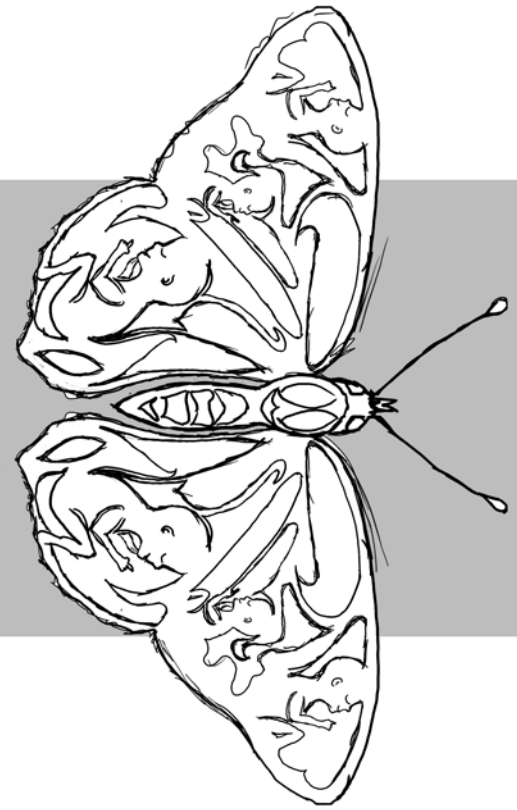
# Chapter 5

# Future perspectives



The reward for work well done is
the opportunity to do more

## Introduction

The aim of the present thesis, entitled "Folic acid, Dietary Patterns and Perinatal Health", was to elucidate the role of maternal nutrition during early pregnancy on pregnancy course and outcome, and to gain more knowledge about perinatal and maternal health risk factors for women living in a large urban city.

All studies described in this thesis were embedded in The Generation R Study, a prospective cohort study from early pregnancy onwards in Rotterdam, The Netherlands.[1-3] It aims to gain more insight into nutritional, environmental, and social determinants of growth, development, and health in a contemporary population based multi-ethnic cohort of urban children from fetal life until young adulthood. Eventually, these results from The Generation R Study will contribute to the development of strategies for optimising health and healthcare for both pregnant women and their children.

In this thesis we focused on folic acid, which is currently the most investigated micronutrient in reproductive medicine (Chapter 2), dietary patterns, an innovative new method to elucidate relationships between diet and disease (Chapter 3), and lastly on individual risk factors in relation to urban perinatal and maternal health in order to identify specific groups at risk (Chapter 4). In addition to the known protective effects regarding congenital anomalies, we showed that use of 0.4 – 0.5 mg folic acid per day during the periconception period has a large impact on pregnancy course and outcome (Chapter 2).[4] Periconception folic acid use is significantly associated with increased fetal growth, resulting in a slightly higher placental and birth weight, and a substantially decreased risk of having a growth restricted child (Chapter 2.1). Furthermore, periconception folic acid use is significantly associated with uteroplacental vascular resistance and maternal blood pressure during pregnancy (Chapter 2.2). These studies imply that a relatively 'small' intervention during the periconception period is associated with various features of fetal and placental growth and development that persist throughout pregnancy. The possible important clinical implications for the future health of both mother and child warrant further investigation.

We also demonstrated the importance of maternal diet during the early pregnancy period on perinatal and maternal health (Chapter 3). The Mediterranean dietary pattern, characterised by high intakes of vegetables, vegetables oil as principal source of fat, moderate amounts of fish and poultry, relatively low consumptions of meat, and moderate alcohol use, i.e. wine, is notorious for its beneficial effects regarding longevity and health.[5, 6] In contrast, The Traditional dietary pattern, characterised by high intakes of potatoes and meat, is known for its unhealthy cholesterol and systolic blood pressure increasing effects.[6, 7] We showed that low adherence to the Mediterranean dietary pattern in early pregnancy impairs intrauterine growth resulting in a lower placental and birth weight with a substantially increased risk of having a child being small for gestational age at birth (Chapter 3.1). Furthermore, both low adherence to the Mediterranean dietary pattern and high adherence to the Traditional dietary

pattern in early pregnancy are associated with an increased maternal blood pressure as well as a differential pattern of blood pressure change during pregnancy (Chapter 3.2). The latter indicates that diet affects the maternal cardiovascular system during pregnancy.

Hence, an adequate maternal nutrition status, through the consumption of a healthy diet and periconceptionally supplemented with folic acid, is of undisputable importance for optimal pregnancy course and outcome. In this respect, we demonstrated that the adequate preconception start of folic acid use is still as low as 37%, in particular in unplanned pregnancies, low educated women, and women from non-Dutch ethnicity. It seems that a strong relation exists between folic acid use and adverse lifestyle factors (Chapter 4.1). In addition, we showed high prevalences with respect to other unhealthy lifestyle behaviours, such as obesity, smoking, recreational drug use, unsafe sexual behaviours, and late start of antenatal booking in relation to adverse pregnancy outcomes, particularly in women from a deprived neighbourhood (Chapter 4.2). The extent of inequalities in adverse perinatal outcomes for women living within an urban deprived neighbourhood can be quantitatively related to an accumulation of sociodemographic, lifestyle, obstetric, and health-related risks factors. Thus, in addition to the importance of optimal maternal nutrition, this thesis stresses the significance of a healthy environment in general in order to improve the health of both pregnant women and their newborn children.

## Future research

***Early embryonic growth and placental function***

About a quarter of a century ago the view of the relationship between fetal growth and birth weight was summarised as follows: "In the first half of pregnancy genetic control is dominant and gives rise to relatively narrow limits of variability of fetal growth patterns. The bulk of fetal weight gain only takes place in the second half of pregnancy".[8] This led to the impression that maternal nutrition is a key factor in determining fetal growth and birth weight, but only in late pregnancy.[9] However, studies focusing on the use of dietary preparations in the third, but also second, trimester as a means of reducing the risk of delivering a low birth weight infant showed divergent effects.[9-11] These findings led to the hypothesis that various late pregnancy complications have their origin, at least in part, during the early pregnancy period.[12-15]

In the present thesis we have shown the importance of maternal nutrition and lifestyle in early pregnancy in relation to later pregnancy course and outcome. Over the past years, various studies have shown associations between embryonic and early fetal growth and the risk of adverse perinatal outcomes. First trimester growth restriction has been associated with increased risks of intrauterine growth retardation, preterm delivery, and still birth.[16-19] This was first observed by Smith et al.[19] who reported that embryos and fetuses with a crown rump length (CRL) smaller than expected showed an increased risk for low birth weight, fetal growth restriction, and preterm birth.[19] These findings were recently confirmed by Mook-Kanamori et al.[16] in the Generation R Study. In addition, they showed that a smaller fetal size between 10 and 15 weeks of gestation was associated with more rapid growth in infancy suggesting compensating ex-utero catch-up growth. Moreover, independent associations between several maternal risk factors, including smoking and no periconception folic acid use, and early fetal growth were demonstrated.[16] This may imply that first trimester CRL is an intermediate in the associations between maternal lifestyle habits and growth variation in later pregnancy, and possibly health and disease risk in later life.[16] In this respect, further studies should focus on the role of the maternal diet.

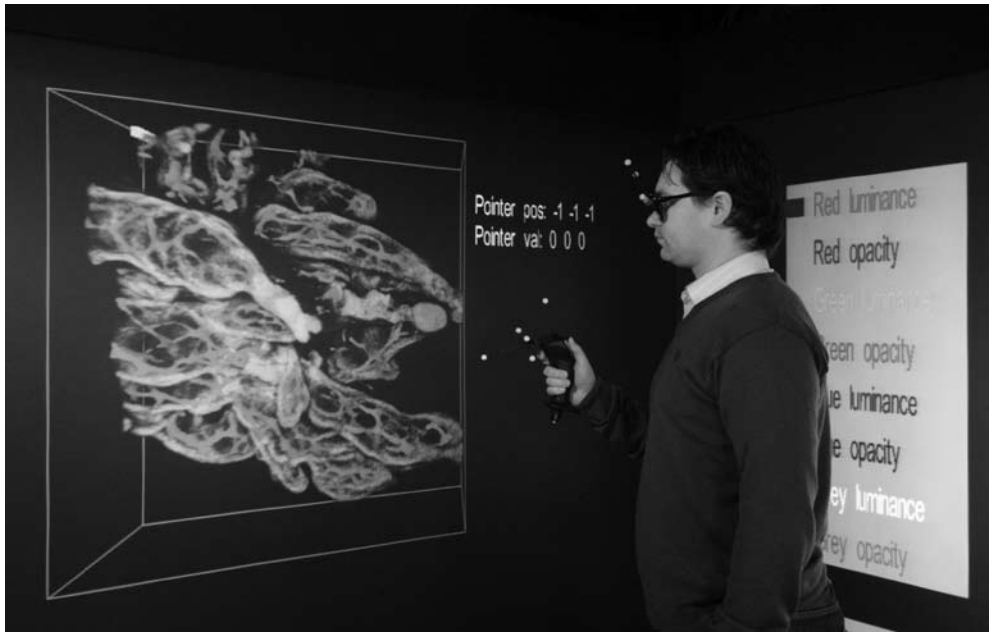
The key issue is to elucidate underlying mechanisms through which early fetal growth can be affected by maternal nutrition and lifestyles. Since throphoblast invasion of the decidua and myometrium is a specific feature of the first trimester in humans, in the past, it was suggested that embryonic nutrition in early pregnancy was affected by placental transport from maternal blood.[9] However, the opportunity for haemotrophic nutrition of the embryo during the first trimester is limited due to the absence of intervillous blood flow before 12 weeks of gestation.[20-23] Given this knowledge, it has been demonstrated by Burton et al.[24] that nutrition in early pregnancy is, at least in part, provided by the secrete of decidual glands (histiotrophic nutrition). Additionally, they proposed an important role for the yolk sac in nutrient exchange before vascularisation of the chorionic villi.[24] Further research may focus on biochemical analyses of the composition of the intervillous fluid during the first trimester, in for example (spontaneous) abortion tissue, to confirm and further explore this pathway of histiotrophic nutrition.

Concerning the measurement and visualisation of early embryonic growth and placental function, an impressive new technique has recently emerged that enables the study of first trimester villous vascularisation, as well as the detailed measurement of yolk sac volume, and early embryonic growth and development.[25, 26] The Virtual Reality system "The I - Space" installed at the Erasmus MC Rotterdam, The Netherlands, allows for visualisation of three dimensional (3D) datasets as enlarged 3D holograms floating in front of the viewer (Figure 1). Measuring fetal size by means of CRL alone may not adequately reflect embryonic growth and development. Combining length and volume measurements with viewing developmental features using virtual reality techniques will greatly improve knowledge of normal and abnormal embryonic growth, development and morphology.[25, 26]

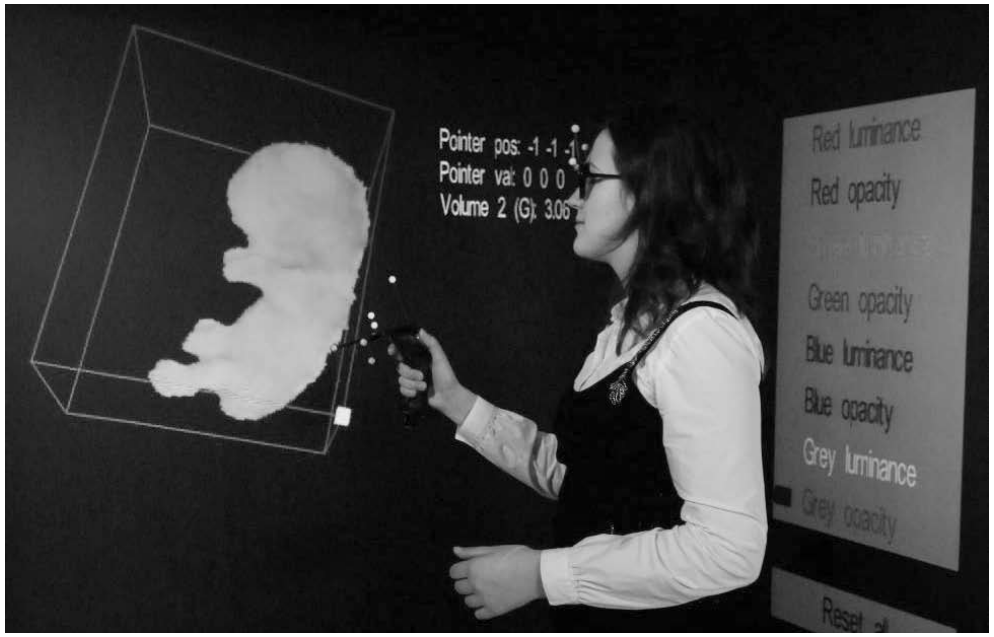
***Epigenetics***

It may also be that maternal nutrition and lifestyle habits affect embryonic and (early) fetal growth by other mechanisms rather than through and altered nutritional supply and / or (early pregnancy) placental function. Epidemiologic and animal studies indicate that prenatal and early postnatal nutrition can influence adult susceptibility to chronic diseases.

**Figure 1 I – Space**




A) Operator using the I – Space to examine trophoblast villous vascularisation at 6 weeks of gestation




B) Operator using the I – Space to examine an embryo of 9 weeks of gestation. The volume of the embryo is segmented



One proposed mechanism is that of epigenetics, causing heritable changes in gene expression that are not mediated by deoxyribonucleic acid (DNA) sequence alterations.[27-31] Epigenetic mechanisms are susceptible to nutrition and environmental influences which are thought to be of particular influence during early development. Currently, the best understood epigenetic mechanisms encompass the methylation of cytosines in cystosine-guanine (CpG) dinucleotides and the modification of histones that package DNA.[32] Methyl donors including folate are required to establish and maintain DNA methylation. Evidence for this mechanism comes from experiments in yellow *Avy* agouti mice showing that supplementing the diet of pregnant dams with methyl donors results in silencing of the agouti gene due to DNA methylation. This in turn results in offspring with a (different) brown coat colour, and a lower tendency for obesity, cancer, and diabetes.[33] Recently, several reports showed that periconception environmental conditions are also associated with persistent changes of the human epigenome.[34, 35] Maternal periconception folic acid use has been associated with a 4.5% increased methylation of the maternally imprinted insulin - like growth factor 2 (IGF2) gene in the child.[34] Furthermore, IGF2 methylation shows a significant (inverse) correlation with birth weight as marker of intrauterine growth. This finding was further illustrated by the Dutch Hunger Winter Study showing that periconception exposure to the famine reduced IGF2 methylation on average by 5.2% in individuals six decades thereafter.[35] From this knowledge it can be hypothesised that the observed differential placental and fetal growth patterns as described in this thesis may, in part, be explained by epigenetic modifications related to maternal nutrition and lifestyle habits. Further research should focus on this potential mechanism and its consequences.

***Risk profiles***

If complications in late pregnancy can already be predicted in the first three months of pregnancy based on ultrasound measurements and / or individual maternal characteristics, such as smoking, no folic acid use, maternal age, haematocrit levels, and preferably also based on specific biochemical measurements related to, for example, placentation (i.e. pregnancy-associated plasma protein A, soluble fms-like tyrosine kinase-1, placental growth factor, plasminogen activator inhibitor-2), this will provide important tools to improve pregnancy outcome.[9, 36] In this thesis we have shown strong relationships between various sociodemographic, lifestyle, obstetric, and health-related risk factors. For example, low educational level and smoking were important predictors of inadequate folic acid use. Furthermore, differential effects of the Mediterranean dietary pattern on fetal growth were observed among smoking women. Likewise, we showed that accumulation of various individual risk factors directly contributed to perinatal and maternal health inequalities for deprived neighbourhood women. Traditionally, epidemiological studies focused on pregnancy outcome in relation to a single risk factor. However, some of these factors may be strongly correlated, such as for example educational level and lifestyle habits. Therefore the single risk factor approach may be insufficient to take

complicated interactions into account.[37-39] Similarly, the effect of a single risk factor may be too small to detect, but the cumulative effect of multiple risk factors included into a risk profile may be sufficiently detectable.[39] This is also known as the "Causal Pie Model" of Rothman, that states that a given disease can be caused by more than one causal mechanism, in which every mechanism involves the joint action of a multitude of component causes.[40] Moreover, Rothmans model states that individual factors do not act in isolation from each other but often interact to increase (or decrease) the risk of disease.[40] For this reason, knowledge about specific risk profiles in relation to pregnancy outcomes may be important in the development of intervention programmes tailored at the individual.[41] Additional research is recommended to identify those women at high risk for poor pregnancy outcome. In this context, besides individual maternal characteristics, genetic traits related to pregnancy outcome, though not assessed in this thesis, should also be investigated and included into a multiple risk factor model, since it is known that genetic and environmental factors interact.

## Clinical implications

The relatively high prevalences of unhealthy nutrition and lifestyle behaviours during pregnancy, in these women from The Generation R Study, highlight the need for improvement. Since in this thesis many of the studied pregnancy outcomes were associated with maternal nutrition and lifestyle, intervention programmes may be needed. In this respect, it was previously shown, following the life course perspective theory, that pregnancy represents one of those life events with a possible long-term impact on health and health-related behaviours (window of opportunity).[42, 43] Promotion of a healthy diet and lifestyle should therefore be implemented in future antenatal care to optimise pregnancy course and outcome as well as future health. Moreover, as preconception nutrition is also crucial for an optimal onset and subsequent development of pregnancy, efforts to increase awareness of a healthy diet and lifestyle should be strengthened not only during pregnancy but also before.[44]

A strategy for success may be an individual approach tailored to personal needs, preferences, and psychological characteristics. In this context, various types of awareness and motivation groups should be distinguished. For example, women who are motivated because of their own interest are more autonomously aware of an unhealthy lifestyle or medical condition than women who feel pressured by their social environment.[43, 45] Moreover, immigrant women and women of very low socioeconomic class are often not reached at all in the provision of information about the consequences of an adverse lifestyle or medical condition during pregnancy due to language, cultural, and / or social barriers.[46] This stresses the importance of targeted and interactive preconception lifestyle guidance rather than a "one size fits all" approach in order to improve pregnancy course and the future health of women and their offspring.



In order to achieve this, technology-enhanced intervention programmes may appear feasible. During past years, rapid growth of the Internet has created innovative opportunities for Web-based health education and behaviour change applications.[47, 48] Moreover, mobile phones and the short message service (SMS) are also increasingly, and successfully, being used in intervention programmes aimed to change lifestyle behaviour.[49, 50] These new technologies allow for the provision of timely information to consumers as well as individual tailored intervention at distance. Moreover, it has been shown that the effectiveness of such an intervention further increases if supplemental support through personalised counselling is provided.[47] This is in line with very recent, and promising, findings from the preconception counselling program "Achieving Healthy Pregnancy" of the Erasmus MC Rotterdam, The Netherlands, implicating that health behaviour changes can be initiated and sustained with the assistance of new technologies together with personalised counselling during the preconception and antenatal period.[51]

## Final conclusions

The present thesis demonstrates the importance of maternal nutrition and a healthy lifestyle during the early pregnancy period. Periconception use of folic acid as well as adherence to a diet rich in vegetables, fish and vegetable oils, has a positive effect on perinatal and maternal health. Relatively high prevalences of adverse lifestyle factors, such as inadequate periconception folic acid use, obesity, smoking, recreational drug use, unsafe sexual behaviours, and late antenatal booking, in relation to adverse pregnancy outcomes, particularly for women from a deprived neighbourhood, emphasise the need to change awareness and attitude among couples in the community, as well among midwives, general practitioners, and obstetricians. Targeted preconception health educational programmes should be developed and applied in order to improve the health of both pregnant women and their children.

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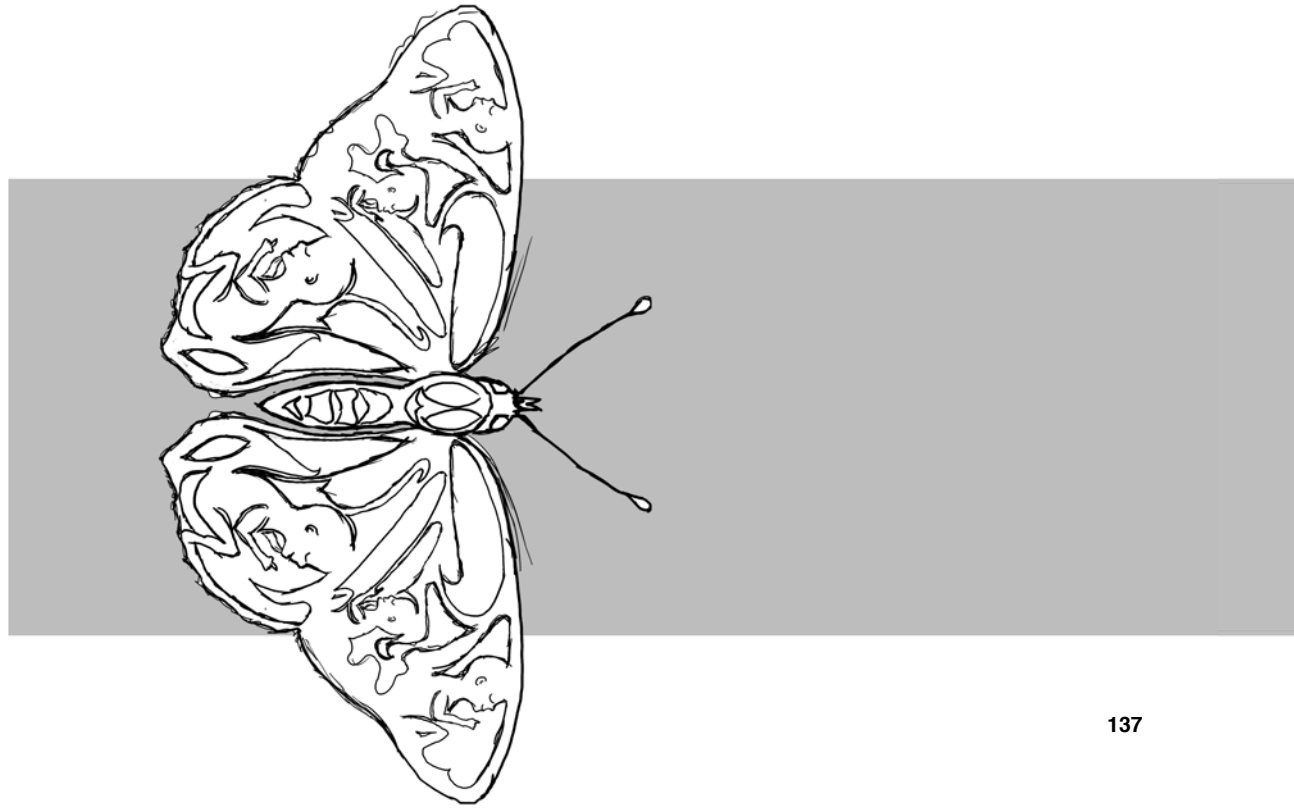
# Chapter 6

# Summary / Samenvatting



## English summary

Maternal nutrition and folic acid use are among the most important environmental factors influencing pregnancy course and outcome. The periconception period and first weeks of gestation are of particular interest since some major pregnancy disorders seem to originate during this period. Most nutritional studies on nutritional intake and folic acid use, however, have been conducted during mid- and late pregnancy. As a consequence, relatively little is known about maternal nutrition during the critical early pregnancy period. Next to nutrition growing attention has recently focused on the impact of neighbourhood environment on perinatal and maternal health. It has been suggested that perinatal and maternal health inequalities are the result of differential accumulation of lifestyle, constitutional and medical factors within large cities. This may have important policy implications given the possibility of designing preventive strategies. However, data on the effects of neighbourhood environment as well as perinatal risk factors are scarce.

Given this knowledge, the aim of this thesis as described in Chapter 1 was to elucidate the role of maternal nutrition and folic acid use during early pregnancy on pregnancy course and outcome. The focus was on folic acid, the most investigated micronutrient in reproductive medicine, and dietary patterns, an innovative and constructive determinant to elucidate relationships between nutrition and disease. Second, we aimed to gain more knowledge about perinatal and maternal risk factors for women living within a large urban city.

All studies were embedded in The Generation R Study, a large prospective cohort study in Rotterdam, The Netherlands. Between 2001 and 2006, 8880 pregnant women were included. Information regarding nutritional, sociodemographic, lifestyle, obstetrical, and health-related risk factors, and pregnancy course and outcome was obtained from questionnaires, physical examinations, ultrasound measurements, biological samples, and medical records.

The first part of this thesis focused on the associations between folic acid use, fetal growth, and maternal haemodynamic adaptation mechanisms during pregnancy. In Chapter 2.1 we examined whether low dose periconception folic acid use affects fetal growth. We showed that, compared to no use, periconception folic acid use was positively associated with both fetal and placental growth, resulting in a slightly higher birth weight and placental weight. Periconception folic acid use was also associated with reduced risks of low birth weight compared to women who did not use folic acid. All effects were most pronounced in women who preconceptionally started using folic acid. In Chapter 2.2 we hypothesised that folic acid use influences early placentation and thereby the occurrence of hypertensive pregnancy disorders. To test this hypothesis we examined the associations between periconception folic acid use and uteroplacental vascular resistance, maternal blood pressure, and the risks of gestational hypertension and pre-eclampsia. Compared to women who did not use folic acid at all, periconception folic acid users had lower pulsatility and resistance indices of the umbilical and uterine arteries, and a higher systolic and diastolic blood pressure. The risk of gestational

hypertension and pre-eclampsia did not differ between the folic acid categories. Even though the conclusions from this latter study did not provide us with direct clinical implications, the study was important for the further understanding of physiologic maternal adaptation mechanisms during pregnancy.

The second part of this thesis focused on dietary patterns in early pregnancy and the associations with fetal and placental growth, and pre-eclampsia. In Chapter 3.1 we evaluated the effects of dietary patterns on intrauterine and placental growth, and low birth weight. This study was restricted to Caucasian women since dietary habits generally differ between ethnic groups. The mothers completed a food-frequency questionnaire, after which dietary patterns were generated by logistic regression analysis. The identified dietary pattern was characterised by high intakes of vegetables, vegetable oil, fish, and pasta and rice, and low intakes of meat, potatoes, and fatty sauces, and was labeled 'the Mediterranean dietary pattern'. Low adherence to this dietary pattern was associated with an increased risk of having a growth restricted child. Almost all fetal growth and placental parameters were also associated with the dietary pattern revealing a lower birth weight and lower placental weight for women with low adherence to the Mediterranean dietary pattern. In Chapter 3.2 we assessed the associations between major dietary patterns in early pregnancy, maternal systolic and diastolic blood pressure, and pre-eclampsia. This study was also restricted to Caucasian mothers. In this study two dietary patterns were generated using factor analysis: a 'Mediterranean', and a 'Traditional' dietary pattern. The Mediterranean dietary pattern again comprised of high intakes of vegetables, vegetable oils, pasta and rice, fish and legumes, moderate intakes of alcohol, and low intakes of sweets. The Traditional dietary pattern was characterised by high intakes of meat and potatoes, and low intakes of fruit, non-alcoholic drinks, fish, and bread. Both low adherence to the Mediterranean diet and high adherence to the Traditional diet were associated with a slightly higher blood pressure during pregnancy. These effect estimates were most pronounced in mid-pregnancy but were not associated with the risk of pre-eclampsia.

In the third part of this thesis we studied individual risk factors in relation to urban perinatal health. In Chapter 4.1 we assessed the prevalence of adequate folic acid use according to current Dutch recommendations in a multi-ethnic urban population between 2001 and 2006, and investigated possible determinants of adequate folic acid use. For this study data on 6940 urban women were available. Only 37% of these women adequately used folic acid during the preconception period. The strongest risk factors for inadequate folic acid use were unplanned pregnancy, low educational level, and non-Dutch ethnicity. Other important risk factors were younger age, single marital status, smoking, and multiparity. In Chapter 4.2 we examined the associations between modifiable perinatal risk factors and adverse pregnancy outcomes on a neighbourhood level among 7359 pregnant women. Compared to pregnancies from a non-deprived neighbourhood, pregnancies from a deprived neighbourhood had almost a twofold increased risk for perinatal death. Intrauterine growth restriction, prematurity, Apgar < 7 at five minutes after birth, and pre-eclampsia also showed higher prevalences. Residing within a deprived neighbourhood was associated with an accumulation of individual risk factors which attributed directly to the observed neighbourhood differences in perinatal outcomes. Further exploration of these risk factors revealed that women from a deprived neighbourhood had significantly more modifiable risk factors including ≥ 36 working hours, obesity, smoking, recreational drug use, unplanned pregnancy, no folic acid use, late gestational age at booking (≥ 14 weeks of gestation), maternal psychopathology, comorbidity, and sexual transmittable diseases, as compared to women from a non-deprived neighbourhood. Given the results from Chapter 4.1 and Chapter 4.2 we would like to emphasise the need for targeted preconception health educational programs and other public health strategies.

In the last part of this thesis, encompassing Chapter 5, we reflected on the main findings in our studies in view of clinical implications and suggestions for further research. In short: regarding maternal nutritional exposures in early pregnancy and perinatal and maternal health, our findings support the importance of optimal maternal nutrition during the early pregnancy period. Use of folic acid as well as adherence to a diet rich in vegetables, fish and vegetable oils, seems to have positive effects on fetal and placental growth and development, as well as maternal health. Future studies should focus on the influence of maternal nutrition on early embryonic development and placental function, as well as possible mechanisms through which nutritional exposures and folic acid use affect fetal and placental growth. The relatively high prevalence of adverse lifestyle factors in relation to adverse pregnancy outcomes, particularly for women from a socioeconomically deprived neighbourhood emphasises the need to change awareness and attitude. Targeted preconception health educational programmes should be developed and applied in order to improve the health of both pregnant women and their children.

## Nederlandse samenvatting

In Nederland worden steeds meer vrouwen met zwangerschapscomplicaties, zoals een laag geboorte gewicht bij het kind (intra-uteriene groeivertraging) en zwangerschapsvergiftiging bij de moeder (pre-eclampsie), opgenomen in het ziekenhuis. Dit percentage is schrikbarend hoog. Nederland heeft bijna het hoogste percentage zwangerschapscomplicaties en baby-sterfte in Europa.

Het doormaken van een zwangerschap gecompliceerd door intra-uteriene groeivertraging of pre-eclampsie is een ingrijpende gebeurtenis en heeft voor bijna alle vrouwen een enorme impact op hun leven. Daarnaast is uit verschillende onderzoeken gebleken dat zowel kinderen met groeivertraging als moeders die pre-eclampsie hebben doorgemaakt tijdens de zwangerschap, een sterk verhoogde kans hebben om in de toekomst hart en vaatziekten, zoals hoge bloeddruk, hartinfarct of beroerte, te ontwikkelen.

Een gezonde voeding van de moeder en het gebruik van foliumzuur is belangrijk voor een goed verloop van de zwangerschap. De periode rondom de bevruchting en de eerste zwangerschapsweken zijn hierbij vooral belangrijk omdat steeds meer studies aantonen dat veel ernstige zwangerschapscomplicaties al tijdens deze vroege periode ontstaan. De meeste studies die de invloed van voeding en foliumzuur op de zwangerschap bestudeerden, richtten zich echter vooral op het tweede en derde trimester. Hierdoor is er relatief weinig bekend over de invloed van voeding en foliumzuur op de gezondheid van de baby en de moeder tijdens de zwangerschap, ook wel perinatale en maternale gezondheid genoemd.

Behalve een gezonde voeding van de moeder zijn er ook andere belangrijke omgevingsfactoren die de perinatale en maternale gezondheid kunnen beïnvloeden. Zo is er de laatste tijd steeds meer aandacht voor de invloed van de woon- en leefomgeving op het verloop van de zwangerschap. Zo zouden moeders uit een grote stad een sterk verhoogde kans hebben op zwangerschapsomplicaties. Mogelijk zou een opeenstapeling van verschillende risicofactoren binnen de grote stad hieraan ten grondslag liggen.

Het doel van dit promotieonderzoek was om te bestuderen wat de invloed van foliumzuur tabletten, voedingspatronen en omgevingsfactoren rondom de bevruchting en in de eerste zwangerschapsweken is op de perinatale en maternale gezondheid. Hierbij is onder meer gekeken naar de groei en ontwikkeling van het kind, de gezondheid van de moeder tijdens de zwangerschap en enkele belangrijke zwangerschapscomplicaties zoals bijvoorbeeld intra-uteriene groeivertraging, vroeggeboorte en pre-eclampsie.

Dit alles is onderzocht binnen het Generation R Onderzoek. Generation R is een grootschalig populatie-gebaseerd prospectief cohort onderzoek, waarin bijna 10.000 kinderen vanaf de vroege zwangerschap tot de jonge volwassenheid gevolgd worden. In dit geboorte cohort worden de groei, ontwikkeling en gezondheid van Rotterdamse kinderen bestudeerd. In relatie tot groei en ziekte worden zowel factoren die te maken hebben met voeding, leefstijl en omgeving als biologische factoren bekeken. Alle kinderen zijn tussen 2001 en 2006 geboren.

Gegevens over voeding en leefstijlfactoren zijn verzameld met behulp van vragenlijsten. Tijdens de zwangerschap werden de aanstaande moeders onderzocht bij een zwangerschapsduur van ongeveer 12, 20 en 30 weken. Op die drie meetpunten werden de groei en ontwikkeling van de baby en de gezondheid van de moeder gevolgd aan de hand van echo's en lichamelijk onderzoek. Daarnaast zijn van alle zwangerschappen gegevens over de geboorte, inclusief mogelijke complicaties, bekend.

Het eerste deel van dit proefschrift beschrijft het onderzoek naar de invloed van het gebruik van foliumzuur rondom de bevruchting en in de eerste zwangerschapsweken op de groei en ontwikkeling van het vaatbed van de moederkoek, de groei en ontwikkeling van het kind en de gezondheid (onder andere bloeddruk) van de moeder tijdens de zwangerschap. Uit hoofstuk 2.1 en 2.2 wordt duidelijk dat het slikken van foliumzuur tijdens de periode rondom bevruchting en eerste weken van de zwangerschap zowel de groei en functie van de moederkoek en daarmee ook de groei van het ongeboren kind bevorderd. Het slikken van foliumzuur is geassocieerd met permanente veranderingen in het vaatbed van de moederkoek en veranderingen in het bloeddrukverloop van de moeder tijdens de zwangerschap. Daarnaast is het geboortegewicht van baby's van moeders die foliumzuur gebruiken hoger en is het risico op intra-uteriene groeivertraging sterk verlaagd bij deze kinderen.

In deel twee van dit proefschrift wordt gekeken wat de invloed van voeding in het eerste trimester is op de groei en onwikkeling van het vaatbed van de moederkoek, de groei, ontwikkeling van het kind en de gezondheid van de moeder tijdens de zwangerschap. Hoofdstuk 3.1 toont aan dat het gebruik van een voedingspatroon gekenmerkt door veel vlees, aardappelen en vette saus en weinig groente, fruit en vis, in het eerste trimester, een sterk verhoogde kans geeft op intra-uteriene groeivertraging bij de geboorte. In hoofstuk 3.2 wordt duidelijk dat een dergelijk voedingspatroon ook een negatief effect heeft op de bloeddruk van de moeder tijdens de zwangerschap. Het zogenaamde Mediterraanse voedingspatroon, met veel groente, fruit, vis en plantaardige olie, heeft echter zeer gunstige effecten op zowel de groei van het kind als ook de bloeddruk van de moeder in de zwangerschap. Daarnaast werd ook aangetoond dat de nadelige effecten van roken tijdens de zwangerschap op de groei van het ongeboren kind extra worden versterkt door het volgen van een voedingspatroon dat arm is aan foliumzuur en vitamine B12.

Het derde deel van dit proefschrift beschrijft de relatie tussen bepaalde risicofactoren en grootstedelijke perinatale en maternale gezondheid. In hoofdstuk 4.1 wordt duidelijk dat het adequate gebruik van foliumzuur erg laag is in een grote stad als Rotterdam. Slechts 37% van de aanstaande moeders gebruikt foliumzuur tijdens de voorgeschreven periode. De meest belangrijke risicofactoren hiervoor lijken te zijn: een ongeplande zwangerschap, een laag opleidingsniveau, en de niet-Nederlandse ethniciteit. Ook jonge alleenstaande moeders, moeders die blijven roken tijdens de zwangerschap en moeders die zwanger zijn van hun tweede, derde of vierde kind slikken minder vaak foliumzuur tabletten zoals geadviseerd wordt. In hoofdstuk 4.2 hebben we meer specifiek, dat wil zeggen op buurtniveau, gekeken naar de relatie tussen bepaalde risicofactoren en perinatale en maternale gezondheid. Het blijkt dat er een sterk verband is tussen het wonen in een achterstandswijk, babysterfte en andere ongunstige zwangerschapsuitkomsten zoals intrauteriene groeivertraging, vroeggeboorte, slechte start van het kind net na de geboorte (de Apgar score) en zwangerschapsvergiftiging bij de moeder. De oorzaak hiervan lijkt een opeenstapeling van verschillende risicofactoren te zijn binnen deze achterstandwijken. Voorbeelden van deze risicofactoren zijn, armoede, een lage opleiding, laag inkomen en werkloosheid, maar ook de vele alleenstaande moeders, ongeplande zwangerschappen, roken, geen foliumzuur slikken, ernstig overgewicht en te laat gestarte verloskundige zorg.

De resultaten uit mijn proefschrift onderschrijven het belang van een gezonde voeding, omgeving en leefstijl in het begin van de zwangerschap. Mijn onderzoek laat zien dat relatief veel vrouwen een ongezonde leefstijl en voedingsgewoonten hebben met alle mogelijke negatieve gevolgen van dien. Concluderend wijst mijn onderzoek dan ook uit dat het noodzakelijk is de verloskundige zorg in de vroege zwangerschap in Nederland anders in te richten. Dat wil zeggen actief meer informatie geven over gezonde voeding en leefstijl in het begin van de zwangerschap, om zo optimale omstandigheden voor alle ongeboren kinderen en hun moeders te creëren en zodoende veiligere zwangerschappen en een betere gezondheid in de toekomst te bewerkstelligen.

## Chapter 7

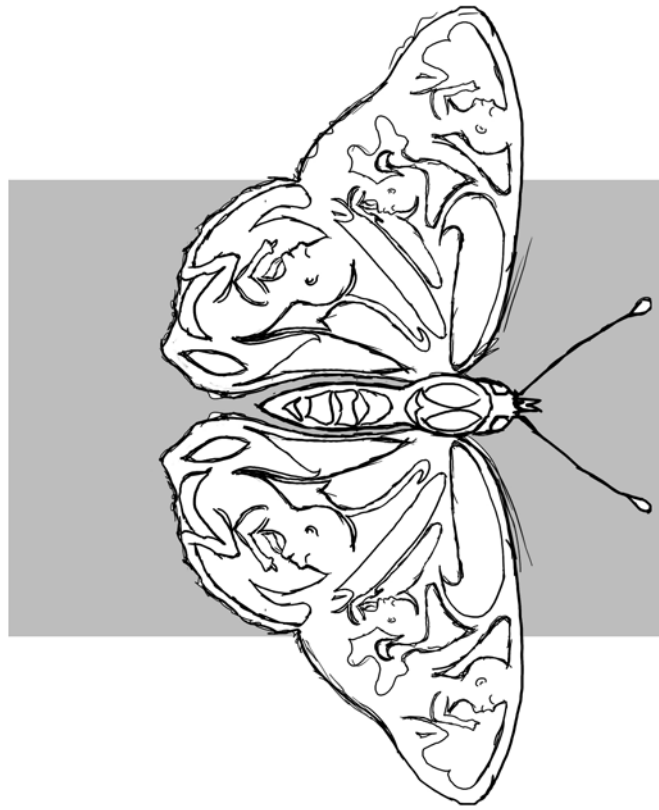
**List of abbreviations**

**Authors and affiliations**

**About the author**

**Publications and awards**

**PhD portfolio**



# List of abbreviations

| | |
|---|---|
| AC | abdominal circumference |
| Ao | adverse outcome |
| ANOVA | Analysis of Variance |
| ANX | anxiety |
| BMI | body mass index |
| BPD | biparietal diameter |
| BP | blood pressure |
| BSI | Brief Symptom Inventory |
| CI | Confidence Interval |
| CpG | cytosine-guanine |
| CRP | C - reactive protein |
| DNA | deoxyribonucleic acid |
| DEP | depression |
| DBP | diastolic blood pressure |
| EFW | estimated fetal weight |
| FL | femur length |
| FFQ | food frequency questionnaire |
| GA | gestational age |
| HC | head circumference |
| Hs | high sensitive |
| IG | immunoglobulin |
| IGF2 | insulin-like growth factor 2 |
| IQR | interquartile range |
| IUGR | intrauterine growth restriction |
| IVF | in vitro fertilization |
| ICSI | intracytoplasmatic sperm injection |
| LBW | low birth weight |
| OR | odds ratio |
| EDTA | plasma |
| PCA | principal component analysis |
| PI | pulsatility index |
| β | regression coefficient |
| RI | resistance index |
| RR | relative risk |
| STD | sexual transmittable disease |
| SMS | short message service |
| SGA | small for gestational age |
| SES | socioeconomic status |
| SD | standard deviation |
| SDS | standard deviation score |
| SAS | Statistical Analysis System |
| SPSS | Statistical Package Social Sciences |
| SLE | Systemic Lupus Erythematosus |
| SBP | systolic blood pressure |
| 3D | three dimensional |
| tHcy | total homocysteine |
| tRNA | transfer ribonucleic acid |
| UmA | umbilical artery |
| UtA | uterine artery |
| Vit | vitamin |
| Wks | weeks |
| Yrs | years |

## Authors and affiliations

From the Generation R Study Group, Erasmus MC, Rotterdam, The Netherlands
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# About the author

Sarah Timmermans is de dochter van Piet Timmermans en Jeanette Timmermans-Lioe a Tjam. Zij werd geboren op 12 januari 1979, twee minuten na haar tweelingbroer Joris Timmermans. Zij heeft verder nog één oudere zus: Anne Timmermans en één jongere broer: Lucas Timmermans. Na het afronden van haar gymnasium op het Stedelijk Gymnasium Nijmegen in 1997, begon zij aan de studie Beleid & Management Gezondheidszorg aan de Erasmus Universiteit Rotterdam. In 1998 is zij gestart met haar studie Geneeskunde aan de Erasmus Universiteit, welke zij in 2006 succesvol heeft afgerond. Tijdens haar studie Geneeskunde is haar interesse voor de wetenschap gewekt en heeft zij binnen de afdelingen Inwendige Geneeskunde (afdeling Infectieziekten) en Verloskunde en Vrouwenziekten van het Erasmus Medisch Centrum Rotterdam wetenschappelijk onderzoek gedaan. In het Vall d'Hebron Ziekenhuis in Barcelona, Spanje heeft zij haar wetenschappelijke interesse verder ontplooid, door onderzoek te doen op de afdeling Neurochirurgie. Haar voorliefde voor Spanje en de Spaanse taal heeft er toe geleid dat Sarah Spaans heeft gestudeerd aan de Universiteiten van Salamanca, Granada en Sevilla in Spanje. Daarnaast heeft Sarah in het Bamalete Lutheran Ziekenhuis in Ramotswa, Botswana, tijdens haar keuze co-schap klinische ervaring opgedaan, onder andere in de verloskunde.

Nadat Sarah haar artsentitel heeft behaald in 2006, is ze als ANIOS Gynaecologie in het Bronovo Ziekenhuis te Den Haag onder supervisie van Dr. C.A.G. Holleboom (opleider) gaan werken. In dit ziekenhuis heeft zij haar vriend Floris Schalekamp ontmoet, met wie zij deze zomer is gaan samenwonen. In juni 2007 is zij begonnen met haar promotie onderzoek binnen de afdeling Verloskunde en Vrouwenziekten van het Erasmus Medisch Centrum Rotterdam, in samenwerking met de Rotterdamse onderzoeksgroep Generation R (promotoren Prof.dr. E.A.P. Steegers en Prof. dr. R.P.M. Steegers-Theunissen, copromotor Dr. V.W.V. Jaddoe). Dit promotie onderzoek heeft geresulteerd in het proefschrift dat nu voor u ligt. Tijdens haar promotie onderzoek heeft Sarah met goed gevolg een Masteropleiding aan het 'Netherlands Institute of Health Sciences' (NIHES) gevolgd. In augustus 2010 behaalt zij dan ook haar Master of Science in de Klinische Epidemiologie.

Gedurende de afgelopen jaren zijn meerdere van Sarah's onderzoeken met prijzen onderscheiden. In 2003 door de Nederlandse Vereniging van AIDS Behandelaren (Young Investigators Award), in 2008 door de 'Society for the Study of Hypertension in Pregnancy' (Young Investigator Travel Award). In datzelfde jaar is ook de presentatie van één van haar onderzoeken bekroond door de 'Society for Gynaecologic Investigation' (President's Presenter Award). De afdeling Verloskunde en Vrouwenziekte van het Erasmus MC Rotterdam, samen met de Rotterdamse Gynaecologen Opleidings Cluster, heeft haar in 2010 de Jury Wladimiroff Onderzoeksprijs toegekend.

In juni 2010 is zij begonnen met de opleiding Verloskunde en Gynaecologie in het Amphia Ziekenhuis te Breda (plaatsvervangend opleider Dr. D.N.M. Papatsonis).

In de toekomst hoopt Sarah een gynaecoloog te worden die de kliniek met wetenschappelijk onderzoek blijft combineren.

*Door Femke Mollema en Fatima Hammiche, paranimfen*

## Manuscripts and awards related to this thesis

Chapter 2.1

**Timmermans S,** Jaddoe VWV, Hofman A, Steegers-Theunissen RPM, Steegers EAP. Periconception folic acid supplementation, fetal growth and the risks of low birth weight and preterm birth: the Generation R Study. - British Journal of Nutrition 2009; 102: 777-785

** Society for Gynecologic Investigation (SGI): President's Presenter Award 2008 **

Chapter 2.2

**Timmermans S,** Jaddoe VWV, Silva LM, Hofman A, Raat H, Steegers-Theunissen RPM, Steegers EAP. Folic acid affects uteroplacental vascular resistance: the Generation R Study. - Nutrition, Metabolism, and Cardiovascular Diseases 2009; Oct 9.Epub ahead of print

** International Society for the Study of Hypertension in Pregnancy (ISSHP): Young Investigator Travel Award 2008 **

Chapter 3.1

**Timmermans S,** Steegers-Theunissen RPM, Vujkovic M, den Breeijen H, Russcher H, Lindemans J, Mackenbach JP, Hofman A, Lesaffre EMEH, Jaddoe VWV, Steegers EAP. The Mediterranean dietary pattern substantially affects intrauterine growth. - Submitted for publication

** Verloskunde en Vrouwenziekten (V&V) Erasmus MC Rotterdam en de Rotterdamse Gynaecologen OpleidingsCluster (RCOG): Juriy Wladimiroff Onderzoeksprijs 2010 **

Chapter 3.2

**Timmermans S,** Steegers-Theunissen RPM, Vujkovic M, Bakker R, den Breeijen H, Raat H,, Russcher H, Lindemans J, Hofman A, Jaddoe VWV, Steegers EAP. Major dietary patterns and maternal blood pressure patterns during pregnancy. The Generation R Study. - Submitted for publication

Chapter 4.1

**Timmermans S,** Jaddoe VWV, Mackenbach JP, Hofman A, Steegers-Theunissen RPM, Steegers EAP. Determinants of folic acid use in early pregnancy in a multi-ethnic urban population in The Netherlands: the Generation R Study. - Preventive Medicine 2008; 47: 427-432

Chapter 4.2

**Timmermans S,** Bonsel GJ, Steegers-Theunissen RPM, Mackenbach JP, Steyerberg EW, Raat H, Verbrugh HA, Tiemeier HW, Hofman A, Birnie E, Looman CW, Jaddoe VWV, Steegers EAP. Individual accumulation of heterogeneous risks explains perinatal inequalities within deprived neighbourhoods in Rotterdam, The Netherlands. - Submitted for publication



## Other publications and awards

N.Bergen, **Timmermans S,** Hofman A, Steegers-Theunissen RPM, Russcher H, Lindemans J, Jaddoe VWV, Steegers EAP. First trimester homocysteine and folate levels are associated with increased adverse pregnancy outcomes. - Submitted for publication

Heppe DHM, Steegers EAP, **Timmermans S,** den Breeijen H, Tiemeier HW , Hofman A, Jaddoe VWV. Fish consumption during pregnancy is not associated with fetal growth or the risks of neonatal complications. The Generation R Study. - Submitted for publication

**Timmermans S,** van Hof AC, Duvekot JJ. Conservative management of abnormal placentation. - Obstetrical and Gynecological Survey. 2007; 62: 529-539

Boer K, Nellen JF, Patel D, **Timmermans S,** Tempelman C, Wibaut M, Sluman MA, van der Ende ME, Godfried MH. The AmRo-study: pregnancy outcome in HIV-1-infected pregnant women under effective HAART and a policy of vaginal delivery. - BJOG 2007; 114: 148-155

**Timmermans S,** Tempelman C, Godfried MH, Nellen JFJB, Dieleman JP, Sprenger HGJ, Schneider MEE, Wolf F, Boer K, van der Ende ME; for the Dutch HMF Study Group. Nelfinavir and Nevirapine side-effects during pregnancy. - AIDS 2005; 19: 795-799

Tempelman C, **Timmermans S,** Godfried MH, Dieleman JP, Boer K, van der Ende ME. Highly active antiretroviral therapy (HAART) in HIV-positive pregnant women in The Netherlands, 1997-2003: safe, effective and with few side effects. - Nederlands Tijdschrift Geneeskunde 2004; 148: 2021-2025

** Nederlandse Vereniging van AIDS behandelaren (NVAB): Bristol-Meyers Squibb Young Investigators Award 2003 **

## Summary of PhD training and teaching activities

| | |
|---|---|
| Name PhD student: | Sarah Timmermans |
| Erasmus MC Department: | Obstetrics and Gynaecology |
| Research School: | NIHES |
| PhD period: | June 2007- April 2010 |
| Promotoren: | Prof. dr. E.A.P. Steegers |
| | Prof. dr. R.P.M. Steegers-Theunissen |
| Copromotor: | Dr. V.W.V. Jaddoe |

**General academic skills**

- Methodologie van patient gebonden onderzoek en voorbereiding subsidie aanvragen, Erasmus MC Rotterdam
- Instellingsgebonden regelgeving and stralingshygiëne niveau 5R, Erasmus MC Rotterdam

**Research skills**

- Msc Clincial Epidemiology, NIHES

**In-depth courses**

- Planning and Evaluation of screening, NIHES
- Prognostic Research, NIHES / UMC Utrecht
- Maternal and Child Health, NIHES
- Ethnicity, Health and Health Care, NIHES

**National and International conferences, seminars, and workshops**

- NWO Bessensap. VWN Masterclass. Museon Den Haag (2010)
- RCOG onderzoeksdag / Wladimiroff Symposium. Erasmus MC Rotterdam (2010)
- Wetenschapsdag gynaecologie en urologie. Erasmus MC Rotterdam (2009)
- Symposium Nederlandse werkgroep Preeclampsie. UMC Groningen (2009)
- Nederlandse Vereniging Obstetrie en Gynaecologie. Gynaecongres Arnhem (2009)
- Nederlandse Vereniging voor Obstetrie en Gynaecologie. Gynaecongres Utrecht (2009)
- Symposium "New imaging and developmental concepts in early pregnancy". Erasmus MC Rotterdam (2009)
- Symposium "Opleiden en Research". Bronovo Ziekenhuis Den Haag (2009)
- SGI 56th Annual Scientific Meeting Glasgow, Scotland (2009)
- RCOG onderzoeksdag / Wladimiroff Symposium. Erasmus MC Rotterdam (2009)
- MRC CAITE symposium / ALSPAC Study Bristol, United Kingdom (2008)
- Wetenschapsdag gynaecologie en urologie. Erasmus MC Rotterdam (2008)
- Nedwep en DSPM, Eerste gecombineerde bijeenkomst, Utrecht (2008)
- ISSHP 16th World Congress. Washington, USA (2008)
- Symposium De Jonge Zwangerschap. Erasmus MC Rotterdam (2008)
- Generation R Symposium. Imaging and early brain development. Erasmus MC Rotterdam (2008)
- ABCD Study Symposium. Een gezonde start voor een gezond leven. Vumc, Amsterdam (2008)
- Nederlandse Vereniging voor Obstetrie en Verloskunde. Gynaecongres Haarlem (2008)
- EARNEST - the Early Nutrition Programming Project Meeting. Granada, Spanje (2008)
- SGI 55th Annual Scientific Meeting San Diego. USA (2008)
- RCOG onderzoeksdag / Wladimiroff Symposium. Erasmus MC Rotterdam (2008)
- Nederlandse Vereniging Toxicologie voorjaarsvergadering. Erasmus MC Rotterdam (2008)
- Epigenetic epidemiology: lecture Rob Waterland. LUMC (2008)
- Wetenschapsmiddag. Erasmus MC Rotterdam (2008)
- Wetenschapsdag gynaecologie en urologie. Erasmus MC Rotterdam (2007)
- Generation R Study Symposium. Fetal Growth and Development, Erasmus MC Rotterdam (2007)

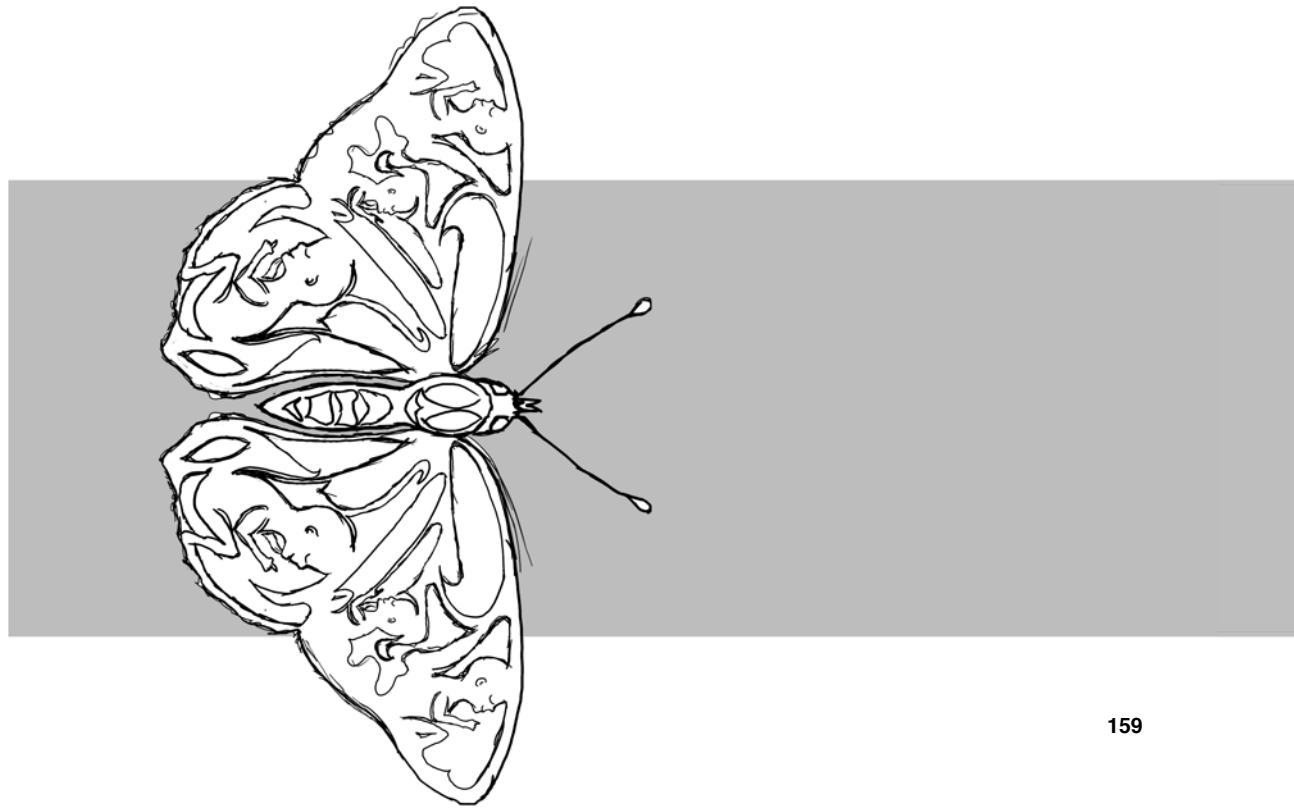
**Grant applications, MEC request, reviewing papers**

- Review paper for Metabolism
- Review paper for British Journal Nutrition
- Review paper for American Journal Obstetrics and Gynecology
- Review paper for Journal of Reproductive Immunology
- Review paper for Journal of Applied Physiology
- MEC request: protocol Validation of a Food Frequency Questionnaire
- Hartstichting: Vascular-related pregnancy disorders and maternal risk of cardiovascular disease in later life
- ZonMW: Maternal dietary patterns, homocysteine and methylation in early pregnancy in association with pregnancy and birth outcomes

**Lecturing, supervising practicals**

- Supervising pratical, Course Basic Introduction Course to SPSS, Molmed
- IMC Weekend School (Children from deprived area Delfshaven Rotterdam aged 9-11), gast lecturer

# Afterword



Wie in 2007 had gezegd dat ik drie jaar later zou promoveren, een Master opleiding zou afronden, dat ik zou samenwonen en ook nog in opleiding zou zijn tot gynaecoloog, zou ik voor gek hebben verklaard. Papa en mama hebben altijd tegen ons gezegd: “door volharding en inspanning kom je er wel”. Wel, zie mij nu hier staan. Ik wil dan ook graag als eerste mijn ouders bedanken voor alle steun en liefde die zij mij altijd geven.

Beste Eric. Drie jaar geleden heb je mij dit onderzoek aangeboden wat geresulteerd heeft in dit proefschrift waar ik ontzettend trots en blij mee ben. Je hebt mij altijd vrij gelaten in mijn keuze en daar waar nodig bijgestuurd. Sommige keuzes waren voor jou soms wel erg vernieuwend (hip noemde jij het ?!). Ik ben van mening dat een beetje spanning geen kwaad kan op zijn tijd. Sterker nog, ik kan me herinneren dat jij juist stimuleerde om het spannend te houden. Over de invulling hiervan valt uiteraard te discussiëren. Daarom zit er in dit proefschrift speciaal voor jou nog een extra (12e) geheime stelling verborgen. Ik daag je uit om 15 september samen te toosten op de oplossing. Ontzettend bedankt.

Beste Vincent en Régine, ik heb me rijk mogen prijzen met maar liefst twee (co) promotoren. Een luxe, die soms ook wel lastig was. Van beiden heb ik veel mogen leren, waarvoor mijn dank! Ik zal de vele besprekingen met “Steegers en co” niet snel vergeten. Régine, jij hebt in april jouw oratie gehad, waardoor ik als eerste met jou als promotor mag verdedigen. Iets waar ik trots op ben. Graag wil ik in dit nawoord ook de hoogleraren professor Burdorf, professor Bindels en professor Katan bedanken voor het plaatsnemen in de leescommissie.

Een promotie is geen promotie zonder paranimfen. Lieve Femke en Fatima: de “F-side”. Trouwere supporters zou ik niet kunnen wensen. Fatima, hbiba djellie, barakkalah ou fik. Ntina Sa7ibti d’basa7. L3am limazji radit koun el7afla djellek. Fem, in 2009 had ik de eer om aan jouw zijde te staan tijdens jouw verdediging. De keus was snel gemaakt wie mijn paranimf zou zijn, want jij kent letterlijk alle “ins” en “outs” van mijn promotie tijd. Ik ben er trots op dat jij je altijd, ondanks alle weerstanden, overal doorheen knokt. Jij bent een waarde vriendin en collega en ik ben heel blij dat jij op 15 september aan mijn zijde staat.

Collega onderzoekers, lieve Marijana, Annelous, Dineke, Nienke, Robbert, Dennis, Miranda, Rachel, Claudia en Elise. Alle wijze raad, DE-cappuccino’s, borrels en etentjes hebben ervoor gezorgd dat jullie, naast collega’s, echte vrienden zijn geworden. Laten we er een mooi feestje van maken! Ik wil ook graag mijn nieuwe collega’s uit het Amphia bedanken voor het fijne welkom. Het was spannend om zo vanuit het onderzoek de patiënten zorg weer in te gaan. Jullie openheid en (Brabantse) gastvrijheid hebben ervoor gezorgd dat ik met veel plezier ben begonnen aan deze nieuwe fase.

Ineke van de Ende, Hans Duvekot en Cas Holleboom. Jullie drieën hebben mijn interesse voor het onderzoek en de gynaecologie gewekt en versterkt. Jullie enthousiasme is aanstekelijk. Betere coaches had ik niet kunnen en willen wensen. Patricia en Jolanda. Jullie hebben een hoop logistiek werk een stuk aangenamer gemaakt. Daarnaast kon ik altijd even kletsen als ik daar behoefte aan had. Marja: ik heb je leren kennen toen je nog voor professor Wilson werkte en wij de kamer deelden. Ik waardeer je adviezen en gezelligheid nog steeds. Lieve Claire (Tempelman), sinds ons geweldige multicenter onderzoek naar HIV positieve zwangere vrouwen, heb ik er een goede vriendin en collega bij gekregen. De herinneringen aan die tijd (korte broekjes) zijn goud waard!



Claire Gasseling, Jelle Mollema en alle sponsoren, door jullie ziet mijn proefschrift er nog mooier uit dan dat ik mezelf ooit had kunnen voorstellen. Dank hiervoor!

Mijn lieve clubgenootjes van Noblesse inclusief mijn “instant paranimfen” Jis en Madeleine en mijn lieve huisgenootjes van Huize Lamberta. Jullie enthousiasme voor mijn onderzoek en jullie reactie als ik mijn geaccepteerde manuscripten via de mail doorstuurde (“Ziet er goed uit Saar! Ik ben afgehaakt bij de derde zin, maar het komt er op neer dat foliumzuur goed is, toch?”) waardeer ik enorm. Lieve Babbels, Fem en ik hebben het stokje aan jou overgedragen. Met liefde stimuleer ik iedereen om voor de WOMB patiënten te includeren. De weg lijkt soms nog lang, maar geloof me als ik zeg dat de eindstreep sneller in zicht komt dan je denkt. Lieve pink ladies: al sinds de jaren ´80 samen en nog steeds zoveel plezier. Dit jaar weer oud en vertrouwd Barca om bij te komen?! Ik zeg ja!

Het is heel bijzonder dat van de vier Timmermans - Lioe a Tjam kinderen er één in 2009 gepromoveerd is, twee in 2010 promoveren en (waarschijnlijk) de benjamin ook nog! Anne, collega in het vak en een geweldige zus. Helaas kan je er niet bij zijn omdat je voor je fellow in Vancouver zit. Weliswaar ver weg, maar ik weet dat je net zo blij (en ook zenuwachtig) zult zijn als ik voor jou vorig jaar. Joris, twee minuten ouder! Ik bewonder de passie waarmee jij je ambitie naleeft. Wat zou het toch mooi zijn als die Marslanding op jou naam komt te staan in de toekomst. Lucas, nog niet eens afgestudeerd en nu al presentaties in het buitenland! Ik ben er van overtuigd dat jij in stijl de hekkensluiter van alle promoties gaat worden.

Lieve Floris, Malcolm Forbes zei ooit: “knowing when to keep your mouth shut is invariably more important than opening it at the right time”. Helaas ben ik hier niet zo sterk in dus in plaats van alleen dank je wel, wil ik toch een paar zinnen citeren uit een liedje waarbij ik altijd aan jou moet denken. *“Kom lief, sta eens even stil. Er is iets wat ik vertellen wil. Ik vond nooit het juiste moment om te zeggen wat jij voor mij bent. Ik wil je danken voor je steun, ik weet dat ik soms op je leun. Maar kan ik het even niet aan, weet ik dat je er voor mij zult staan. En ik ben al blij je stem te horen, zonder jou ben ik verloren…. (Nick en Simon)”.* Ik ben heel blij dat jij in mijn leven bent gekomen.

Als laatste wil ik de 9778 deelnemers van Het Generation R Onderzoek bedanken. Zonder uw trouwe deelname was dit proefschrift nooit tot stand gekomen. Hartelijk dank voor alle inzet.