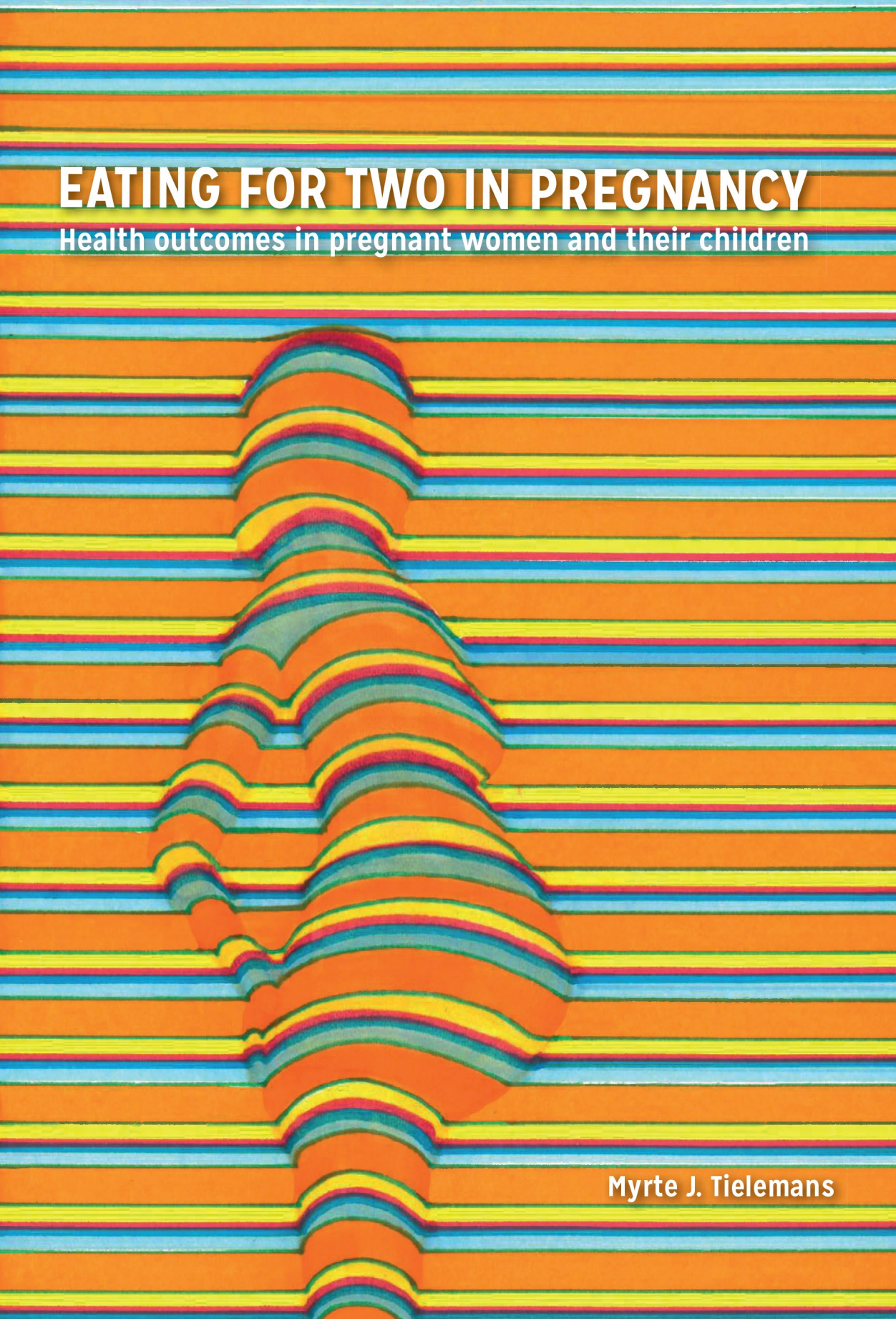
EATING FOR TWO IN PREGNANCY

Health outcomes in pregnant women and their children



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Chapter 3

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Tielemans MJ, Voortman T*, Schoufour JD*, Steenweg-de Graaff J, Jaddoe VWV, Franco OH, Steegers EAP, Tiemeier H, Kiefte-de Jong JC. Circulating fatty acid patterns, gestational weight gain and hypertensive complications in pregnancy: The Generation R Study. *Submitted for publication*

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Chapter 4

Tielemans MJ, Steegers EAP*, Voortman T*, Jaddoe VWV, Rivadeneira F, Franco OH, Kiefte-de Jong JC. Protein intake during pregnancy and offspring body composition at 6 years: The Generation R Study. *In revision (European Journal of Nutrition)*

Leermakers ETM, **Tielemans MJ**, van den Broek M, Jaddoe VWV, Franco OH, Kiefte-de Jong JC. Maternal dietary patterns during pregnancy and offspring cardiometabolic health at age 6 years: The Generation R Study. *Clinical Nutrition, 2016 Jan [Epub ahead of print]*

** Denotes equal contribution*

Chapter 1

General introduction

The old saying 'eating for two during pregnancy' implies that a woman should consume twice as much while being pregnant. However, the question has been raised whether this should be dismissed despite additional micronutrient requirements during pregnancy,[1] because it may contribute to a higher prevalence of adverse pregnancy and birth outcomes.[2]

Maternal pregnancy outcomes such as hypertensive complications (e.g., pre-eclampsia, pregnancy-induced hypertension) as well as adverse birth outcomes including foetal growth restriction and preterm delivery are prevalent worldwide and are an important cause of maternal and perinatal mortality and morbidity.[3-6] The aetiology of these outcomes in pregnancy and at birth has not been completely elucidated, but research suggests that causes of these outcomes are multifactorial. Risk factors to develop pre-eclampsia include for example genetic predisposition, ethnicity, first pregnancy, history of chronic disorders, obesity, gestational weight gain and some dietary factors.[7] Those risk factors that can be modified before and during pregnancy are of particular interest for prevention strategies and may include lifestyle modifications (e.g., changes in dietary intake, physical activity levels, smoking cessation), limiting gestational weight gain, and micronutrient supplementation. A well-known example of a preventive strategy to improve birth outcomes that has been very effective is maternal folic acid supplementation; folic acid supplementation before and during the first trimester of pregnancy reduces the risk of neural tube defects with 69%.[8] Lifestyle modifications during pregnancy may improve maternal health during pregnancy and birth outcomes but it may also modify child health later in life. Although several dietary interventions during pregnancy have shown to improve maternal health outcomes (e.g., reduction in the prevalence of gestational diabetes) and birth outcomes (e.g., reduction of stillbirths and low birth weight newborns),[9-12] long-term consequences of different dietary interventions on maternal and child health have not been studied in detail. Also, not all aspects of maternal dietary intake during pregnancy in association with pregnancy, birth and child health outcomes have been studied yet or previous studies have reported conflicting findings.[13,14] Therefore, it remains to be established which dietary components during pregnancy would result in most optimal pregnancy, birth and child health outcomes.

Health care professionals advise women to start a healthy lifestyle already before conception and to continue this throughout pregnancy. Pregnant women are aware that a healthy lifestyle (e.g., meeting dietary recommendations) may have health benefits for the baby and that it may limit gestational weight gain.[15] Yet, they are less aware of the health benefits it may have for the mother.[15] Moreover, recommendations may be unclear or contradictory and many pregnant women do not adhere to the lifestyle recommendations, and pregnant women are often even unaware that their lifestyle is suboptimal.[16,17] However, women may be more motivated to modify unhealthy behaviours during pregnancy. Hence, the pre-conception period and pregnancy may therefore provide a window of opportunity to introduce healthy lifestyle behaviours.[18,19]

Nutrition during pregnancy

Numerous studies have evaluated the associations between maternal nutrition during pregnancy with maternal health outcomes in pregnancy such as gestational weight gain, change in blood pressure and the occurrence of hypertensive complications. Although the

amount of weight that is gained during pregnancy varies considerably among women,[20] gestational weight gain (GWG) has also been associated with adverse pregnancy and birth outcomes.[2,21] On the one hand, GWG that is too little (i.e., inadequate GWG) has been associated with preterm birth and low-birth weight[22] and on the other hand, GWG that is too excessive has been linked to gestational diabetes, large-for-gestational-age newborns, caesarean delivery and postpartum weight retention.[21] Several studies have suggested that high energy intake during pregnancy is associated with high GWG.[23] Yet, the roles of dietary composition and dietary patterns in GWG still need to be established, because modification of gestational weight gain via maternal nutrition may improve maternal health outcomes in pregnancy and birth outcomes. Maternal dietary intake including dietary patterns and intake of fruit and vegetables has been associated with the course of blood pressure during pregnancy,[24] and with the occurrence of pre-eclampsia.[25] Whether other components of diet, such as algorithms of dietary acid load[26] of maternal fatty acid patterns, are associated with the occurrence of hypertensive complications during pregnancy needs further study.

Moderate intakes of fish during pregnancy as well as supplementation of one of its components namely n-3 polyunsaturated fatty acids (PUFAs) have been associated favourable birth outcomes including lower prevalence of preterm birth and higher birthweight.[27] The occurrence of these birth outcomes has been associated with circulating angiogenic factors in women during pregnancy.[28] However, it is not known to what extend maternal intake of fish or PUFA blood levels in pregnancy may influence maternal angiogenic factors.

Maternal diet during pregnancy can influence foetal growth and development and gestational age at birth.[10,11,29] In addition, maternal diet may influence foetal growth patterns already in early pregnancy.[30] Children with adverse birth outcomes (e.g., small-for-gestational-age, large-for-gestational-age, preterm birth) have often suboptimal subsequent growth.[31,32] Micro-nutrient supplementation might reduce the occurrence of some adverse birth outcomes including low birth weight,[33] but current studies on for example the association between maternal vitamin B12 in pregnancy and birth outcomes have been contradictory.[34-36]

Dietary intake during pregnancy may have, in addition to short-term, also long-term effects on the health of the offspring, and this association can be independent of birthweight.[37] For example, offspring prenatally exposed to a very low energy intake during the Dutch famine developed more often coronary heart disease and obesity later in life than not-exposed offspring.[38] However, more moderate differences in dietary intake and nutritional status during pregnancy have not been consistently associated with cardiometabolic health in the offspring and deserves further study.[39,40] Although cardiovascular disease is in general clinically evident during adulthood, cardiometabolic risk factors may track from childhood to adulthood and increase the likelihood to develop cardiovascular disease and obesity later in life.[41] Therefore, it is important to elucidate modifiable factors during foetal life that may have an effect on cardiometabolic health in children.

Objectives

The aim of this thesis was to gain more insight into the role of maternal nutrition during pregnancy on health outcomes of women and their children. The three main objectives were:

1. To study the effects of maternal dietary intake during pregnancy on maternal health, more specifically on gestational weight gain, blood pressure and the occurrence of hypertensive complications
2. To study the associations of circulating nutritional biomarkers in pregnancy with maternal health (e.g., gestational weight gain and the occurrence of hypertensive complications) and birth outcomes including birth weight and length of gestation
3. To study the associations of maternal dietary intake during pregnancy with offspring cardiometabolic health and body composition 6 years after birth

Main determinants

In this thesis we assessed the associations of several maternal nutritional markers with pregnancy, birth and child health outcomes (**Table 1.1**). Maternal dietary intake was predominantly self-reported in early pregnancy using food-frequency questionnaires.[42] Using these questionnaires, we could assess dietary intake of energy-providing nutrients (i.e., macronutrients), dietary algorithms (e.g., dietary acid load), food groups (e.g., fish) and dietary patterns. We evaluated dietary patterns that were based on recommendations (i.e., *a priori* dietary patterns such as the Dutch Healthy Diet-index) as well as dietary patterns that were population specific (i.e., *a posteriori* dietary patterns such as the 'Margarine, sugar and snacks' pattern). Additionally, we assessed the associations between the nutritional biomarkers plasma fatty acids and vitamin B12 levels with pregnancy and birth outcomes.

Table 1.1. Overview of main determinants and health outcomes of this thesis

Nutritional exposure	Maternal outcomes	Birth outcomes	Child outcomes
Nutrients			
Macronutrients	**Chapter 2.1** *gestational weight gain*		**Chapter 4.1** *body composition*
Algorithms of dietary acid load	**Chapter 2.3** *blood pressure & hypertensive complications*		
Food groups	**Chapter 3.1** *angiogenic factors*		
Overall diet	**Chapter 2.2** *gestational weight gain*		**Chapter 4.2** *cardiometabolic factors*
Nutritional biomarkers	**Chapter 3.1** *angiogenic factors* **Chapter 3.2** *gestational weight gain & hypertensive complications*	**Chapter 3.3** *birth weight & length of gestation*	

Study design

Systematic reviews

The studies presented in Chapter 2.1 (Macronutrient intake and gestational weight gain) and Chapter 3.3 (Vitamin B12 and birth outcomes) are systematic reviews of the literature. An extensive search of the literature was performed in several databases to identify relevant articles. These articles were screened for eligibility by two independent reviewers. Using a predesigned format, all relevant information was extracted. In both systematic reviews, a comprehensive overview of all included studies was given. The systematic review described in Chapter 3.3 was extended with an individual participant data meta-analysis. Thereby, the authors of all eligible articles were approached to provide original data, because the results presented in the articles could not be compared due to large heterogeneity. All results were reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement.[43]

The Generation R Study

Six studies presented in this thesis are embedded in the Generation R Study, an ongoing prospective population-based cohort from foetal life until young adulthood.[42,44] The main aim of the Generation R Study was to identify early environmental and biological causes of growth, development and health during early life, childhood and adulthood in an urban multi-ethnic population.[42] Women were invited to participate when living in the area of Rotterdam, the second largest city of the Netherlands, and having an expected delivery date between April 2002 and January 2006. In total, 9778 pregnant women provided informed consent. For the studies on dietary intake during pregnancy (Chapters 2 and 4), we restricted our population to women of Dutch ancestry.

The role of nutrition during pregnancy within the Generation R Study has been evaluated using extensive questionnaires on dietary intake and using blood biomarkers that were measured during the first and second trimester of pregnancy. Pregnancy and birth outcomes were evaluated using hands-on measurements in pregnancy, and additional information was collected from hospital and midwife records. Child outcomes (i.e., body composition and cardiometabolic factors) were collected at the Generation R research centre when the children had reached the age of six years.

Thesis outline

In **Chapter 2**, we describe the associations of maternal dietary intake with pregnancy outcomes and complications. **Chapter 2.1** gives a comprehensive overview of studies that evaluated macronutrient composition in relation to weight gain during pregnancy. **Chapter 2.2** focuses on the associations of *a priori* and *a posteriori* dietary patterns with gestational weight gain. **Chapter 2.3** describes the associations of dietary acid load with blood pressure development and the occurrence of hypertensive complications in pregnancy.

Chapter 3 covers maternal nutritional biomarkers during pregnancy in relation with maternal health outcomes and birth outcomes. In **Chapter 3.1**, we investigate the

association of maternal fish consumption during early pregnancy and mid-pregnancy plasma fatty acids with concentrations of angiogenic factors in maternal blood during pregnancy and in cord blood. **Chapter 3.2** describes the identification of plasma fatty acid patterns and their associations with gestational weight gain and hypertensive complications in pregnancy. In **Chapter 3.3,** we evaluate the associations between maternal vitamin B12 levels in pregnancy with birth outcomes, including preterm birth and low birth weight in an individual participant data meta-analysis.

In **Chapter 4**, we focus on the role of maternal dietary intake during pregnancy on health outcomes of the offspring six years after birth. More specifically, **Chapter 4.1** describes the association of maternal protein intake on body composition of the offspring at the age of six years. **Chapter 4.2** covers the associations between maternal *a priori* and *a posteriori* dietary patterns during pregnancy and offspring cardiometabolic health at the age of 6 years.

The last chapter, **Chapter 5**, provides an overview of the main findings presented in this thesis. Additionally, we discuss several methodological considerations and we give recommendations for future research. Finally, we discuss the potential implications for public health.

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Chapter 2

Maternal diet & Maternal pregnancy outcomes

Chapter 2.1

Macronutrient composition and gestational weight gain: a systematic review

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Abstract

Background: Abnormal gestational weight gain is associated with unfavourable pregnancy outcomes. Several risk factors have been identified, but the effect of macronutrient intake during pregnancy on gestational weight gain has not been systematically evaluated in both high-income countries and low- and middle-income countries.

Objective: We conducted a systematic review of the literature in 8 different databases (until 12 August 2015) to assess whether energy intake and macronutrient intake (i.e., protein, fat, and carbohydrate) during pregnancy were associated with gestational weight gain (following PRISMA guidelines).

Results: Of 7623 identified references, we included 56 articles (46 observational studies and 10 trials, 28 of which were in high-income countries and 28 of which were in low- and middle-income countries). Eleven of the included articles were of high quality (20%). Results of 5 intervention and 7 high-quality observational studies suggested that higher energy intake during pregnancy is associated with higher gestational weight gain (*n*=52). Results from observational studies were inconsistent for protein intake (*n*=29) and carbohydrate intake (*n*=18). Maternal fat intake (*n*=25) might be associated with gestational weight gain as suggested by observational studies, although the direction of this association might depend on specific types of fat (e.g., saturated fat). Macronutrient intake was not consistently associated with the prevalence of inadequate or excessive gestational weight gain. Associations were comparable for high-income countries and low- and middle-income countries.

Conclusions: The current literature provides evidence that energy intake is associated with gestational weight gain, but the roles of individual macronutrients are inconsistent. However, there is a need for higher-quality research because the majority of these studies were of low quality.

Introduction

Gestational weight gain (GWG) varies considerably among women worldwide,[1,2] and abnormal weight gain (i.e., too little or too much) has been associated with unfavourable pregnancy and birth outcomes. Too little GWG[3] increases risks of low birth weight and preterm birth;[4] the latter disorder was ranked seventh in the leading causes of global years of life lost.[5] In contrast, excessive GWG[3] is associated with risks of gestational diabetes, caesarean delivery, giving birth to a large-for-gestational-age newborn, and postpartum weight retention.[6-8] Furthermore, excessive GWG has been linked to childhood obesity in the offspring,[9] which is associated with increased risk of chronic diseases in later life.[10]

Hence, it is important to identify modifiable factors associated with abnormal GWG. Such a modifiable factor could be dietary intake during pregnancy.[11] Several national and international dietary guidelines recommend higher energy intake but do not have specific recommendations for the macronutrient composition in pregnancy,[12] whereas other guidelines recommend slightly higher protein intake in addition to higher energy intake.[13] Nevertheless, the effects of different dietary components during pregnancy on GWG have not been fully elucidated. Several studies have reported that energy intake was associated with higher GWG in high-income countries,[14] but it is unclear whether weight gain during pregnancy is affected by specific sources of energy (e.g., energy from protein, carbohydrates, or fat). In addition, maternal nutrition might be differently associated with GWG in low- and middle-income countries than in high-income countries because maternal protein-energy malnutrition is prevalent in many low- and middle-income countries.[15,16]

In non-pregnant adult populations, weight gain is mainly determined by a positive energy balance, largely irrespective of the source of energy.[17] However, weight gain during pregnancy is a result of complex processes in both the mother and foetus and includes, for example, foetal weight gain, placental weight development, maternal fat accumulation, and changes in extracellular volume.[18] Therefore, the relation between macronutrient intake and weight gain may be more complex during pregnancy and may be different from that in non-pregnant subjects.

Previous systematic reviews on this topic have mainly focused on the effects of dietary factors in high-income countries[14] or were restricted to a specific type of study design (e.g., randomized controlled studies on dietary and lifestyle interventions).[19] Therefore, we aimed to conduct a systematic review that would provide a full overview of the effects of macronutrient intake on GWG worldwide. We systematically reviewed the association between energy and macronutrient intakes and weight gain during pregnancy in both observational and intervention studies in low-, middle-, and high-income countries.

Methods

A systematic review of the literature was performed to assess the association between energy and macronutrient intakes with weight gain during pregnancy. This review was performed according to a predefined protocol and reported in concordance with the PRISMA statement.[20]

Search strategy and selection criteria

We searched for articles without restrictions to the language and publication date in the following electronic databases: Embase (via embase.com), MEDLINE (via OvidSP), Cochrane central (via Wiley), Web-of-Science, CINAHL (via EBSCOhost), and PsycINFO (via OvidSP). Additional articles were retrieved from PubMed, where the subset "as supplied by publisher" contains the most-recent unindexed articles, and from Google Scholar. The search was last run on 12 August 2015 and was designed by an experienced medical information specialist. The search strategy combined controlled vocabulary terms (in MEDLINE, Embase, CINAHL, and PsycINFO) and free-text words in title or abstract related to the exposure (e.g., nutrition, diet, or food), outcome (e.g., weight gain, weight change, or change in BMI), and studied population (e.g., pregnancy, pregnant women, or maternal). The complete search strategy for all databases is provided in the **Supplementary Material 2.1.1.**

To minimize publication bias, we used the following 3 strategies: 1) we did not restrict to the language of publication; 2) we applied a broad search strategy that focused on all nutritional exposures in relation to pregnancy and weight gain; and 3) we searched through trial registries (clinicaltrials.gov and the International Clinical Trials Registry Platform) to identify unpublished relevant intervention trials.

Study selection

Randomized controlled trials (RCTs), intervention studies, cohort studies, and case-control studies were eligible for inclusion if the recruited participants were women with a singleton pregnancy either healthy or diseased. Studies were included if protein, fat, carbohydrate, or energy intake was measured or supplemented as the exposure or intervention and if the reported outcome was GWG (measured or self-reported) or the adequacy of GWG. In addition, we included studies that measured weight shortly after childbirth. The PICOS criteria are presented in **Table 2.1.1.**

We excluded studies if they included only mothers who had given birth to newborns with birth defects because birth defects might disproportionately contribute to GWG or to extremely preterm newborns (born <28 weeks of gestation) because of the short follow-up period. We excluded studies that were restricted to adolescents, and we excluded studies in which the mean age of the total population was <18 y because, during adolescence, additional energy is needed for the adolescents' own growth. Intervention studies in which the exclusive effects of macronutrients could not be determined were excluded (e.g., when the intervention was combined with micronutrients or physical activity). We also did not include studies on dietary counselling when actual dietary intake was not measured.

Two independent reviewers screened the titles and abstracts. The retrieved full-text articles were also evaluated by 2 independent reviewers and included if the selection criteria matched. To solve disagreements during the selection process, a third reviewer was contacted. In case of multiple articles including the same participants, we included the article that provided the most information or which was published most recently. Non-English articles were preferably evaluated by native speakers in the presence of one of the authors.

Reference lists of the 20% most recently published articles and of related systematic reviews were screened to identify relevant articles. Additional articles were identified through hand searches of related articles.

Data extraction

We extracted the data with the use of a predesigned template. The following information was extracted: 1) details of the study (e.g., study design, starting year of the study, country in which the study was performed, number of participants, inclusion and exclusion criteria, and source of funding); 2) data on study participants (e.g., age, ethnicity, and comorbidities); 3) pregnancy-related aspects (e.g., complications and parity); 4) type of dietary exposure (energy or macronutrient intake); 5) details of measurement methods of diet and GWG (e.g., method used, number of measurements, absolute GWG or adequacy of GWG, measured or self-reported, and pregnancy period covered); and 6) statistical analyses (e.g., statistical method, crude and adjusted effect estimates, 95%-confidence intervals, standard deviations, standard errors, P values, and covariates). The extracted data were checked by a second reviewer in a random sample of 20% of the studies included.

We stratified the studies by income level of the country in which each study was performed on the basis of the World Bank list of economies[21] because differences in maternal nutritional status might affect the association between maternal macronutrient intake and GWG.[15]

Table 2.1.1. PICOS criteria

	Selection criteria
Population	Women with singleton pregnancies
Intervention/Exposure	Macronutrient supplementation
	Energy intake
	Carbohydrate intake
	Protein intake
	Fat intake
Comparison	No supplementation
	Low-energy supplementation
	Not applicable for cohort and case-control studies
Outcome	Gestational weight gain
	Adequacy of gestational weight gain
Study design	Intervention studies
	Cohort studies
	Case-control studies

Assessment of study quality

The quality of each study was assessed with the use of a predefined scoring system **(Supplementary Material 2.1.2.)**. This quality score was developed on the basis of existing scoring frameworks.[22-24] We assigned a quality score to each article on the basis of 5 items,

namely the study design, population size, exposure measurement (or, in intervention studies, the adequacy of blinding), outcome measurement, and adjustment for confounders and energy adjustment (or, in intervention studies, the adequacy of random assignment). Each item received points from zero to 2, which led to a maximum of 10 points that represented the highest quality. Studies were considered of high quality when the quality score was ≥7. One reviewer ascribed a quality score to all studies, and a second reviewer checked the assigned quality scores in a random sample of the studies included. Quality scores were checked randomly for 17 studies (31%), and both reviewers agreed on 96% of the assigned scores on the individual items of the quality score.

Results

Study selection

For this systematic review, we identified 7623 distinctive references in the electronic databases. We excluded 7141 references on the basis of their titles and abstracts, and we retrieved full texts of the remaining 482 articles. After a critical evaluation, 433 references did not meet the selection criteria and were excluded. Seven full texts were identified by a reference check (*n*=4) or by a hand search (*n*=3). No additional studies were identified via trial registries. We included 56 studies in this systematic review of which 50 articles were written in English. Other languages of included studies were French,[25] German,[26] Italian,[27] Polish,[28] and Portuguese.[29,30] **Figure 2.1.1** shows the flow chart of the selection process.

Figure 2.1.1. Search strategy for the studies included in current systematic review (searched until 12 August 2015)

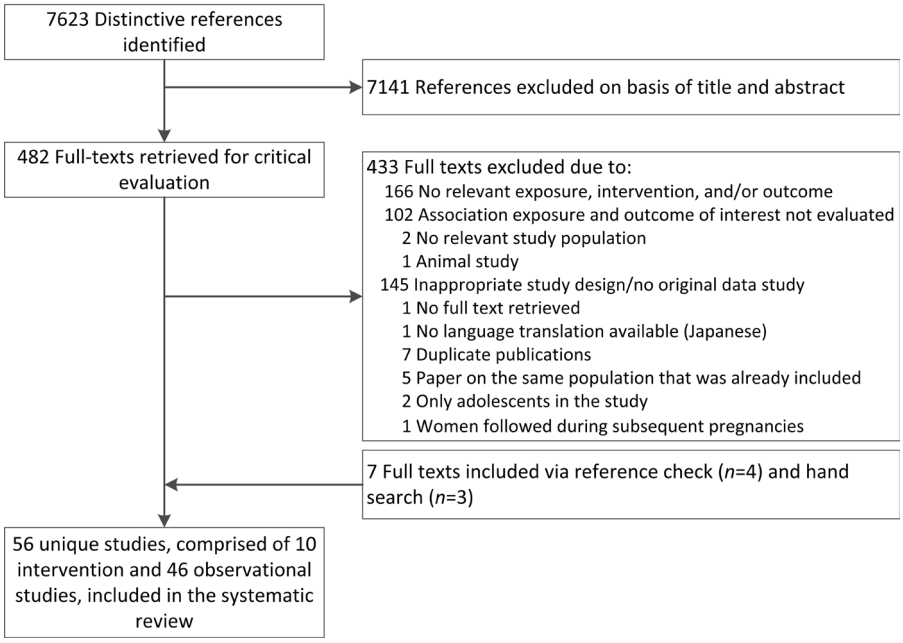


Table 2.1.2. Study characteristics, macronutrient composition, and GWG: a systematic review (*n*=56 studies)

Reference	Country	Study design	n	Mean age (y) (range)	Population characteristics	Dietary intake	Dietary assessment/ intervention (T)	GWG assessment (T)	QS
High-income countries									
Althuizen, 2009[34]	the Netherlands	Longitudinal	144	31 (NR)	Women with term child birth	Energy Total fat Saturated fat	FFQ (T3)	GWG and adequacy of GWG[a] (SR weight T3 minus SR pre-pregnancy weight)	4
Ancri, 1977[46]	USA	Longitudinal	98	NR (12-32)	Healthy women	Energy Protein	24hR (T3)	Measured and estimated[b] GWG (weight T3 minus weight T2)	4
Anderson, 1986[35]	UK	Longitudinal	49	28 (20-38)	Healthy women without obstetric complications	Energy Protein Total fat Carbohydrate	Weighed FR (T3)	Adequacy of GWG[c] (measured weight T3 minus SR pre-pregnancy weight)	4
Bellati, 1995[27]	Italy	Longitudinal	100	NR (NR)	Healthy women without pregnancy complications	Energy	24hR (T2 or T3)	GWG (assessment method and covered period NR)	3
Bergmann, 1997[36]	Germany	Longitudinal	156	NR (≤21-≥ 27)	Healthy women with child birth ≥35 weeks of gestation	Energy	Weighed FR (T1,T2 and T3)	Measured GWG (weight T3 minus weight T1 minus birth weight and placenta weight)	7
Blumfield, 2015[56]	Australia	Longitudinal	134	29 (18-41)	Pregnant women from general population	Energy Protein Total fat Saturated fat Monounsaturated fat Polyunsaturated fat Carbohydrate	FFQ (T1 or T2 and T2 or T3)	GWG & adequacy of GWG[d] (measured weight T3 minus SR pre-pregnancy weight)	4
Bompiani, 1986[33]	Italy	Intervention	20	NR (NR)	Women with type 2 diabetes mellitus	Energy	Low energy diet vs. control diet[e] (NR)	GWG (assessment method and covered period NR)	2
Chasan-Taber, 2008[47]	USA	Longitudinal	454	NR (16-39)	Healthy women with term child birth	Energy	FFQ (T2 or T3)	GWG (measured weight T3 minus SR pre-pregnancy weight)	3
Gaillard, 2013[37]	the Netherlands	Longitudinal	6959	30[f] (NR)	Pregnant women from general population	Energy Protein Total fat Carbohydrate	FFQ (T1 or T2)	Adequacy of GWG[d] (measured weight T3 minus SR pre-pregnancy weight)	7
Heery, 2015[38]	Ireland	Longitudinal	799	31 (18-44)	Pregnant women from general population	Energy	FFQ (T1 or T2)	GWG & adequacy of GWG[d] (last measured weight minus SR pre-pregnancy weight)	5

Table 2.1.2. (continued) Study characteristics, macronutrient composition, and GWG: a systematic review (*n*=56 studies)

Reference	Country	Study design	n	Mean age (y) (range)	Population characteristics	Dietary intake	Dietary assessment/ intervention (T)	Gestational weight gain assessment (T)	QS
Lagiou, 2004[48]	USA	Longitudinal	207	NR (18-39)	Healthy women with term child birth	Energy Protein Fat (source) Carbohydrate	FFQ (T2)	GWG (measured weight T2 minus SR pre-pregnancy weight)	6
Langhoff-Roos, 1987[39]	Sweden	Longitudinal	50	29 (21-40)	Healthy women with term child birth	Energy	FR (T2 and T3)	GWG (measured weight T3 minus SR pre-pregnancy weight)	4
Maple-Brown, 2013[g,49]	USA	Longitudinal	39	31 (NR)	Healthy women	Energy Protein Total fat Saturated fat Monounsaturated fat Polyunsaturated fat Carbohydrate	FR (T3)	GWG (assessment method and covered period NR)	4
Maslova, 2015[40]	Denmark	Longitudinal	46,262	30-31[f] (NR)	Pregnant women from general population	Protein (source)	FFQ (T2)	GWG (SR weight T3 minus SR weight T1)	8
Montpetit, 2012[50]	Canada	Longitudinal	59	32 (18-40)	Healthy women of high socio-economic position	Energy	24hR (NR)	GWG (measured weight T3 minus SR pre-pregnancy weight)	4
Olafsdottir, 2006[41]	Iceland	Longitudinal	194	28-29[f] (NR)	Women with term child birth	Energy Protein Total fat Carbohydrate	FFQ (T1 or T2, and T3)	Adequacy of GWG[i] (measured weight T3 minus measured weight T1 or T2)	6
Ostachowska-Gasior, 2003[28]	Poland	Longitudinal	27	NR (19-34)	NR	Energy Protein Total fat Carbohydrate	24hR (T1, T2 and T3)	Adequacy of GWG[a] (measured weight T3 minus SR pre-pregnancy weight)	4
Papoz, 1980[25]	France	Longitudinal	156	25 (16-42)	Women with preterm or term child birth	Energy Protein Total fat Carbohydrate	Dietary interview by dietician (T1, T2 and T3)	GWG (measured weight minus SR pre-pregnancy weight, unclear which period of GWG is covered)	4
Picone, 1982[51]	USA	Longitudinal	60	23 (NR)	Healthy women	Energy Protein Total fat Carbohydrate	24hR (NR)	Adequacy of GWG (measured weight T3 minus SR pre-pregnancy weight)	4
Rae, 2000[32]	Australia	Intervention	124	30-31[f] (NR)	Obese women with gestational diabetes mellitus	Energy	FR (NR)	GWG (weight T3 minus weight when intervention; was started assessment method NR)	6

Table 2.1.2. (continued) Study characteristics, macronutrient composition, and GWG: a systematic review (*n*=56 studies)

Reference	Country	Study design	n	Mean age (y) (range)	Population characteristics	Dietary intake	Dietary assessment/ intervention (T)	GWG assessment (T)	QS
Renault, 2015[42]	Denmark	Longitudinal embedded in intervention	366	31 (>18)	Obese women (BMI ≥30 kg/m^2)	Protein (source) Saturated fat Polyunsaturated fat	FFQ (T1 or T2)	GWG (measured weight T3 minus SR pre-pregnancy weight)	6
Scholl, 1993[52]	USA	Longitudinal	790	19 (12-29)	Healthy women with preterm or term child birth	Energy	24hR (T2 and T3)	Adequacy of GWG[a] (measured T3, first measurement NR)	8
Shin, 2014[53]	USA	Longitudinal	490	NR (16-43)	Pregnant women from general population	Energy Protein Total fat Saturated fat Carbohydrate	24hR (T1, T2 or T3)	Adequacy of GWG[d] (measured weight (T1,T2 or T3) minus SR pre-pregnancy weight)	5
Sloan, 2001[54]	USA	Longitudinal	2087	22 (NR)	Women with term childbirth	Protein	24hR (T1 or T2)	GWG (assessment method and period NR)	4
Stuebe, 2009[55]	USA	Longitudinal	1388	NR (≤24-≥35)	Women with child birth ≥ 34 weeks	Energy Protein Total fat Saturated fat Monounsaturated fat Polyunsaturated fat Trans-fat	FFQ (T1 or T2 and T2 or T3)	Adequacy of GWG[a] (last measured weight minus SR pre-pregnancy weight)	7
Uusitalo, 2009[43]	Finland	Longitudinal	3360	29 (≤24-≥35)	Women in families with newborn infants carrying increased human leucocyte antigen-conferred susceptibility to type 1 diabetes	Energy Protein Saturated fat	FFQ (pp)	GWG (measured weight T3 minus measured weight T1)	8
Viegas, 1982[44]	UK	Intervention	142	25 (NR)	Asian women	Energy suppl[k]	Consumed supplements (T2 and T3) recorded	Measured GWG (T3 minus T2)	6
Viegas, 1982[45]	UK	Intervention	128	23-25[f] (NR)	Asian women w	Energy suppl[k]	Consumed supplements (T3) recorded	Measured GWG (T3 minus T2)	6

Table 2.1.2. (continued) Study characteristics, macronutrient composition, and GWG: a systematic review (*n*=56 studies)

Reference	Country	Study design	n	Mean age (y) (range)	Population characteristics	Dietary intake	Dietary assessment/ intervention (T)	GWG assessment (T)	QS
Low- and middle-income countries									
Adair, 1984[62]	Taiwan	Intervention	125	26-27[f] (19-30)	Women with inadequate protein consumption and of low socio-economic position	Energy suppl[k]	Suppl vs. no suppl (T1, T2 and T3)	Measured GWG (weight T3 minus pre-pregnancy weight)	10
Ali, 2002[63]	India	Longitudinal	150	NR (NR)	Healthy women without pregnancy complications	Energy Protein	NR	GWG (assessment method and covered period NR)	3
Begum, 1991[64]	Pakistan	Intervention	30	25 (20-29)	Healthy women of very low socio-economic position	Energy suppl[f]	Suppl vs. no suppl (T3)	GWG (assessment method and covered period NR)	2
Castro, 2013[29]	Brazil	Longitudinal	224	NR (18-40)	Healthy women	Energy	FFQ (T1, T2 and T3)	Measured GWG (weight differences T1, T2 and T3)	7
Changamire, 2014[61]	Tanzania	Longitudinal embedded in intervention	6889	25 (≥18)	HIV-negative women	Energy Protein Total fat Carbohydrate	24hR (T3)	Measured GWG (difference in weight during T3)	7
Costa, 2011[30]	Brazil	Case-control	200	24-25[f] (22-28)	Healthy normal weight women with term child birth	Energy Protein Total fat Saturated fat Unsaturated fat Carbohydrate	FFQ (pp)	Adequacy of GWG[L] (measured weight T3 minus pre-pregnancy weight)	6
Das, 1976[65]	India	Longitudinal	60	23 (16-40)	Healthy (underprivileged) women	Energy Protein	24hR (T1, T2 and T3)	Measured GWG (weight T3 minus weight T1 or T2)	4
Drehmer, 2010[57]	Brazil	Longitudinal	570	25 (≥14)	Women with child birth ≥34 weeks	Energy	FFQ (T2 or T3)	Adequacy of GWG[d] (reported weight T3 minus SR pre-pregnancy weight)	4
Ebrahimi, 2015[66]	Iran	Longitudinal	308	27 (18-35)	Pregnant women from urban area	Energy Protein Total fat Carbohydrate	2-day 24hR (NR)	Adequacy of GWG[d] (measured and estimated weight T3 minus measured pre-pregnancy weight)	7

Table 2.1.2. (continued) Study characteristics, macronutrient composition, and GWG: a systematic review (n=56 studies)

Reference	Country	Study design	n	Mean age (y) (range)	Population characteristics	Dietary intake	Dietary assessment/ intervention (T)	GWG assessment (T)	QS
Ho, 2005[31]	China	Longitudinal	62	35 (NR)	Women with gestational diabetes mellitus	Energy	FR (NR)	GWG (measured weight T3 minus SR pre-pregnancy weight)	4
Hsu, 2013[67]	Taiwan	Longitudinal	451	29 (15-41)	Healthy women	Energy Protein Total fat	24hR (T1, T2 and T3)	Measured GWG (weight difference T1, T2 and T3)	4
Hussain, 1988[68]	Thailand	Intervention	29	22-24[f] (20-30)	Healthy women	Energy suppl[k]	Suppl vs. no suppl (T3)	Measured GWG (weight T3 minus weight T2)	4
Jaruratana-sirikul, 2009[69]	Thailand	Longitudinal	236	27 (17-42)	Women with child birth ≥ 34 weeks of gestation	Energy Protein Total fat Carbohydrate	24hR (NR) and FFQ (NR)	Adequacy of GWG[m] (measured weight T3 minus SR pre-pregnancy weight)	3
Kardjati, 1990[70]	Indonesia	Longitudinal nested in intervention	438	26 (NR)	Underprivileged women	Energy suppl[k] Energy[n] Protein[n] Total fat[n]	FR (T2 and T3)	Measured GWG[o] (specific period NR, weight measured with intervals ≥2 weeks)	5
Kaseb, 2002[71]	Iran	Intervention	53	26 (NR)	Healthy women with term child birth	Energy suppl[k]	Energy-protein suppl vs. no suppl (T2 and T3)	Measured GWG (weight T3 minus weight T2)	5
Lechtig, 1978[58]	Guatemala	Longitudinal nested in intervention	135	26[f] (14-46)	Underprivileged women	Energy (provided through suppl[p])	Daily measurement of individual suppl (NR)	Adequacy of GWG[q] (weight T3 minus T2)	5
Martins, 2011[59]	Brazil	Longitudinal	82	26 (>18)	Healthy women	Saturated fat	24hR (T1, T2 and T3)	Measured postpartum weight retention (weight ≤15 days postpartum minus weight T1 or T2)	6
Nahar, 2009[72]	Bangladesh	Longitudinal	1104	NR (NR)	Underprivileged women	Energy suppl[k]	Suppl vs. no suppl (T2 and T3)	Measured GWG (weight T3 minus weight T1 or T2)	4
Ortolano, 2003[73]	Bangladesh	Longitudinal	456	25-26[f] (NR)	NR	Energy suppl[k]	Suppl vs. no suppl (NR)	Measured GWG and adequacy of GWG[r] (covered period NR)	3
Popa, 2014[80]	Romania	Longitudinal	382	28 (NR)	Women without obstetrical complications	Energy Protein Total fat Carbohydrate	FFQ (pp)	Adequacy of GWG[d] (measured last weight minus measured first weight)	6

Table 2.1.2. (continued) Study characteristics, macronutrient composition, and GWG: a systematic review (*n*=56 studies)

Reference	Country	Study design	n	Mean age (y) (range)	Population characteristics	Dietary intake	Dietary assessment/ intervention (T)	GWG assessment (T)	QS
Qureshi, 1973 [74]	India	Intervention	76	NR (20-35)	Women with term child birth	Energy suppl[k]	NR	Measured GWG (T3 minus T2)	5
Rodrigues, 2008[60]	Brazil	Longitudinal	222	26 (18-41)	Healthy women	Energy Protein Total fat Carbohydrate	FFQ (T1)	Measured GWG (T3 minus T1)	5
Saowakontha, 1992[75]	Thailand	Longitudinal	Rural[s]: 130 Urban[s]: 52	Rural: 22[f] Urban: 28[f] (NR)	Women with preterm or term child birth	Energy Protein Total Fat Carbohydrate	24hR[t] (T1, T2 and T3)	Measured GWG (T3 minus T2)	5
Siega-Riz, 1993[76]	the Philippines	Longitudinal	715	24 (15-45)	Women from general population	Energy	24hR (NR)	Measured GWG (T3 minus pre-pregnancy weight)	7
Smith, 1997[77]	Nepal	Longitudinal	35	NR (18-43)	NR	Energy	24hR (T2 and T3)	Measured GWG (covered period NR)	4
Tontisirin, 1986[78]	Thailand	Intervention	43	22-25[f] (16-30)	Healthy women	Energy suppl[k]	FR (T2 or T3), 24hR (T3)	Measured GWG (T3)	6
Wagner, 1975[26]	Colombia	Longitudinal	145	NR	Women with at least 50% of their children being undernourished	Energy Protein	24hR (T1 or T2 and T3)	Measured GWG (T3 minus T1 or T2)	4
Zulfiqar, 2011[79]	Pakistan	Longitudinal	118	25 (NR)	Healthy women with term child birth	Energy Protein Total fat Carbohydrate	24hR (T2 and T3)	Measured GWG (T3 minus T2)	5

[a.] Adequacy of GWG according to Institute of Medicine recommendations 1990[102]; [b.] Estimation of weight gained between 20 weeks of pregnancy and enrolment and between the last measurement and delivery; [c.] Adequacy of GWG categorized as low GWG ≤8 kg and adequate GWG >8 kg; [d.] Adequacy of GWG according to Institute of Medicine recommendations 2009[3]; [e.] The low energy diet contained 1200 kcal/day and the control diet contained 30 kcal/kg of ideal pre-pregnancy weight; [f.] Median (instead of mean); [g.] Additional information received from the author; [f.] Mean age has been reported stratified by exposure (or by outcome in case-control studies); [i.] Adequacy of GWG according to Icelandic guidelines: optimal GWG for normal weight between 12.1 and 18.0 kg and for overweight between 7.1 and 12.0 kg; [j.] Adequacy of GWG: inadequate GWG ≤ 6.8 kg, adequate GWG > 6.8 kg; [k.] Details on supplementation are provided in supplementary table 2; [L.] Adequacy of GWG: recommended GWG 11.5 – 16 kg and excessive GWG > 16 kg; [m.] Adequacy of GWG: Low GWG < 6 kg, adequate GWG ≥6 kg; [n.] Macronutrient intake from home diet (not taking into account the supplementation); [o.] Authors assumed that the pregnancy duration was exactly 40 weeks for all participants; [p.] Content of supplementation: supplement 1 contains 59 kcal/180 mL (from carbohydrates), and supplement 2 contains 163 kcal/180 mL (from protein, fats and carbohydrates); [q.] Adequacy of GWG: Low GWG when GWG ≤0.5 kg/month; [r.] Adequacy of GWG: adequate GWG when GWG >1 kg/month; [s.] Living location of the women (rural vs. urban); [t.] Dietary intake was not measured for each individual but of a subgroup of women which was taken as representative for the whole population; Abbreviations: 24hR, 24-hour dietary recall; FFQ, food-frequency questionnaire; FR, food record; GWG, gestational weight gain; n, number of participants; NR, not reported; QS, quality score; suppl, supplementation; SR, self-reported; T, trimester of pregnancy; Vi, vitamin supplementation; pp, postpartum; y, year.

Characteristics of studies included

The characteristics of the included studies and population characteristics are presented in **Table 2.1.2**. The 56 articles included 78,362 participants (ranging from 20 to 46,262 participants/study) with an age range of 12-46 y. Most studies included healthy women, 2 studies were restricted to women with gestational diabetes,[31,32] and one study exclusively included women with type 2 diabetes.[33] The results of these studies have not been included in the figures. Twenty-nine studies did not report the health status of the participants. The absolute change of weight during pregnancy was reported in 42 studies, whereas 17 studies reported on the adequacy of GWG. Twenty-eight studies were performed in high-income countries,[21] namely countries in Europe,[25,27,28,33-45] North-America,[46-55] and Oceania.[32,56] The other 28 studies were conducted low- and middle-income countries in Central and South America,[26,29,30,57-60] Africa,[61] Asia,[31,62-79] and Europe.[80] Ten of 56 studies were intervention studies of which 2 studies were RCTs.[32,62] The remaining 46 studies were observational studies with a longitudinal design, except for one case-control study.[30]

The quality score of the studies ranged from 2 to 10 **(Table 2.1.2)**, and the median quality score was 5. Eleven of 56 studies received a quality score of ≥7 and, therefore, were considered as providing high-quality evidence. The majority of the studies did not receive any quality points for covariate adjustments because these studies did not adjust for relevant covariates, such as pre-pregnancy BMI, or energy intake.

High-income countries compared with low- and middle-income countries

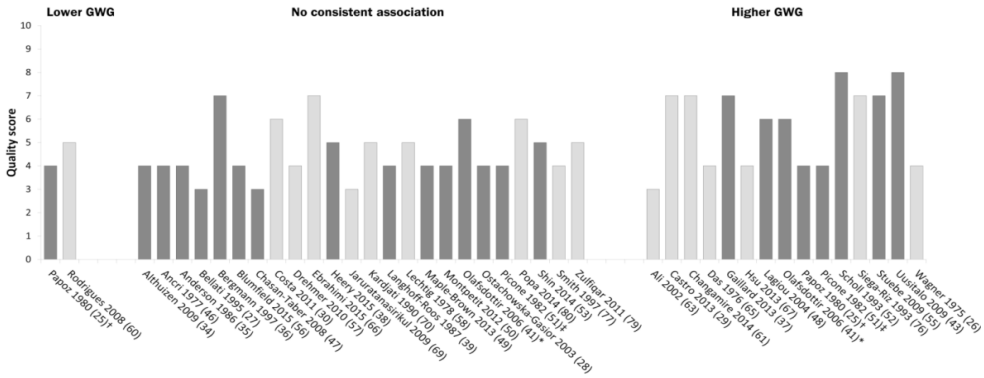
Fifty percent of the included studies (*n*=28) were performed in high-income countries. The majority (82%) of supplementation studies were located in low- and middle-income countries. The quality of the studies was comparable, with a median quality score of 4.5 for studies performed in high-income countries compared with a median score of 5 for studies conducted in low- and middle-income countries. We did not observe differences in reported associations between macronutrient intake and GWG in low- and middle-income countries or in high-income countries **(Supplementary Tables 2.1.1-2.1.8).**

Maternal energy intake and gestational weight gain

Forty-two studies reported on the association of energy intake with GWG **(Supplementary Table 2.1.1)**. Twenty-three studies (55%) were performed in high-income countries, and 9 studies (21%) were of high quality. **Figure 2.1.2** gives an overview of studies that reported on the association of energy intake with GWG. Four studies were not included in Figure 2.1.2 because the studies were restricted to diseased populations (*n*=3)[31-33] or did not report a directionality of the association (*n*=1).[75] Results from high-quality studies (*n*=9) suggested that higher energy intake was associated with higher GWG,[29,37,43,52,55,61,76] except in 2 studies that reported no consistent association.[36,66] Of the 33 low-quality studies, statistically significantly higher GWG was reported in 5 studies[26,48,63,65,67] and 3 studies reported this association in subgroups only[25,41,51] [e.g., in overweight women but not in normal-weight women[41] and in non-smoking women, whereas no association was shown in the total population[51]]. No consistent association was shown in 20 low-quality studies.[27,28,30,34,35,38,39,46,47,49,50,53,56-58,69,70,77,79,80] One study showed that energy intake during

the first trimester was positively correlated with GWG, whereas a change in energy intake between 3 and 8 months of pregnancy was negatively correlated with GWG.[25] Another study showed that energy intake

Figure 2.1.2. Harvest plot of the evidence of an association between energy intake and GWG (*n*=38 observational studies)



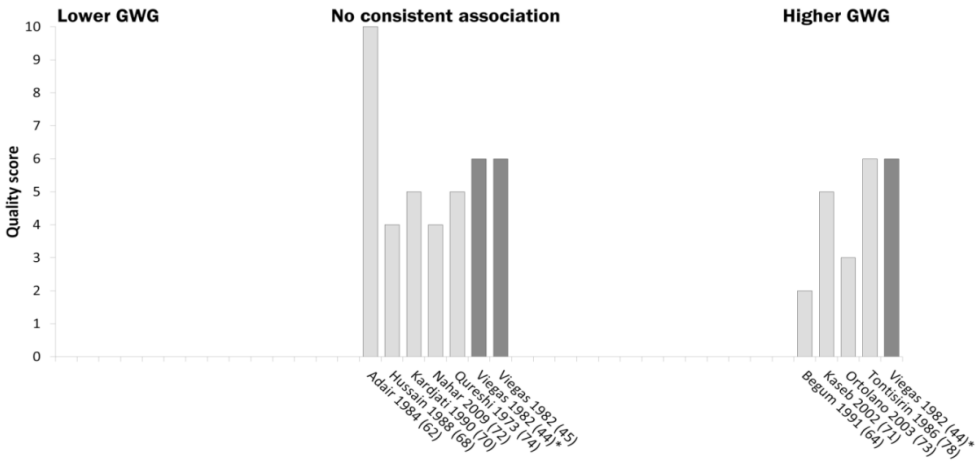
Each study that was not restricted to diseased women and reported a directionality of the association between energy intake and GWG was added to the x axis [consequently, the studies by Bompiani and Botta,[33] Ho et at.,[31] Rae et al.,[32] and Saowakontha et al.[75] are not displayed]. The height of each bar represents the quality score, and the colour shows whether studies were performed in high-income countries (dark grey) or in low- and middle-income countries (light grey). Magnitudes of the associations are quantified in Supplementary Table 2.1.1. * Higher GWG was shown in women with BMI (in kg/m^2) ≥25, but no association was shown in women with BMI <25. † A positive correlation was shown between energy intake during first trimester and the change of intake during first trimester with GWG, and a negative correlation was shown between the change in energy intake later in pregnancy with GWG. ‡ No association was reported in the total population, and a higher GWG in non-smokers. Abbreviation: GWG, gestational weight gain in women who were living in urban areas, but not in rural areas, contributed statistically significantly to the explained variance of GWG although no directionality of this association was reported.[75]

Three studies included only diseased women, namely women with diabetes[33] or gestational diabetes.[31,32] In the RCT by Rae et al.,[32] obese women with gestational diabetes had comparable GWG regardless of their diet (a moderately energy-restricted diet compared with a control diet that was not energy restricted). Nonetheless, the actual energy intake between these groups was comparable. A second intervention study was performed in women with type 2 diabetes and showed that those who received a low-energy diet (1200 kcal/d) gained less weight during pregnancy than did those who received a control diet (30 kcal/d per kg ideal pre-pregnancy weight).[33] The third study that had an observational design was in women with gestational diabetes and showed no association between energy intake and GWG.[31] Higher energy intake may be associated with excessive GWG (*n*=9),[30,37,41,51,52,55,58,66,80] although at some occasions only in subgroups. However, 8 studies did not find associations of energy intake with GWG adequacy.[28,34,38,53,56-58,69]

Maternal macronutrient supplementation and gestational weight gain

Eleven studies reported on GWG differences between participants with macronutrient supplementation and those who received either no supplementation or a macronutrient supplement that was low in energy **(Supplementary Table 2.1.2, Figure 2.1.3)**. The macronutrient supplementation in the included studies consisted of different macronutrients compositions.

Figure 2.1.3. Harvest plot of the evidence of an association between macronutrient supplementation and GWG (*n*=9 intervention studies and *n*=2 longitudinal studies)



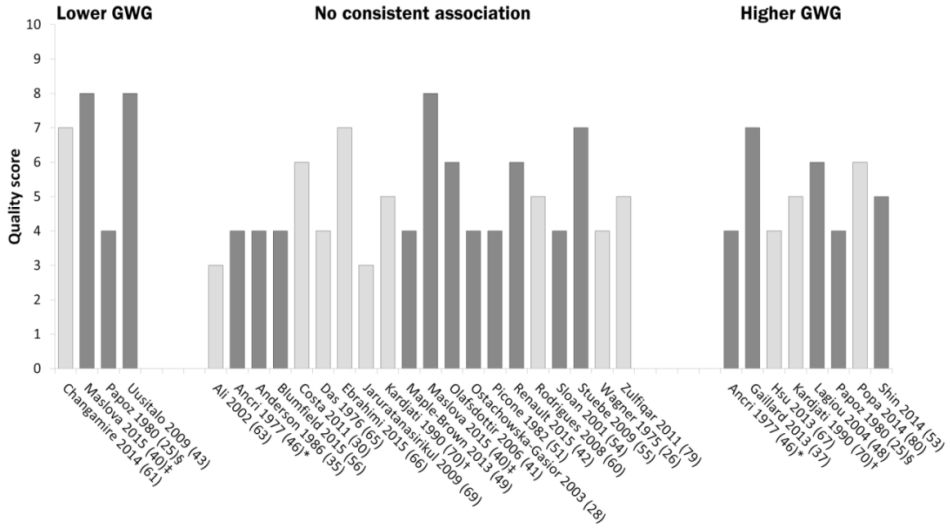
Each study that reported a directionality of the association between macronutrient supplementation and GWG was added to the x axis. The height of each bar represents the quality score, and the colour shows whether studies have been performed in high-income countries (dark grey) or in low- and middle-income countries (light grey). Magnitudes of the associations are quantified in Supplementary Table 2.1.2. * Supplementation during the second trimester of pregnancy was associated with higher GWG, but supplementation during the third trimester was not. Abbreviation: GWG, gestational weight gain.

Nine studies (82%) were performed in low- and middle-income countries,[62,64,68,70-74,78] and only one study[62] was of high quality (9%). In this high-quality trial by Adair et al.,[62] no difference in GWG was shown between women who received a macronutrient supplement that contained 400 kcal/d throughout pregnancy and women who received a supplement that contained <50 kcal/d with similar food intake besides the supplementation. The 10 studies of low quality reported either no consistent difference in GWG (*n*=5),[45,68,70,72,74] higher GWG (*n*=4) in the intervention group than in the control group,[64,71,73,78] or significant associations in subanalyses only (*n*=1).[44] In this latter study by Viegas et al.,[44] statistically significantly higher GWG was reported during second-trimester supplementation but not during third-trimester supplementation.

Maternal protein intake and gestational weight gain

Twenty-nine studies reported on the association between maternal protein intake and GWG **(Supplementary Table 2.1.3)**, all of which had a longitudinal design, and 23 studies (79%) were of low quality. **Figure 2.1.4** displays the 28 studies that reported a direction of the association. The 6 studies of high quality reported conflicting results.[37,40,43,55,61,66] Although higher protein intake was associated with lower GWG in 2 studies,[43,61] one study showed that protein intake was associated with lower GWG in normal-weight and overweight women only.[40]

Figure 2.1.4. Harvest plot of the evidence of an association between protein intake and GWG (*n*=28 observational studies)



Each study that reported a directionality of the association between protein intake and GWG was added to the x axis [consequently, the study by Saowakontha et al.[75] is not displayed]. The height of each bar represents the quality score, and the colour shows whether studies have been performed in high-income countries (dark grey) or in low- and middle-income countries (light grey). Magnitudes of the associations are quantified in Supplementary Table 2.1.3. * A positive correlation was shown in women aged 25-32 y, but no correlation was shown in women ages 18-24 y. † Higher GWG was shown in the second trimester of pregnancy, but no significant association was shown in the third trimester. ‡ There was lower GWG in normal and overweight women, but no association in obese women. § A positive correlation was shown during the first trimester of pregnancy, but a negative correlation was shown with the change of protein intake later in pregnancy. Abbreviation: GWG, gestational weight gain.

In addition, one study reported that higher protein intake was associated with higher GWG,[37] and one study showed no association between protein intake and GWG.[55] In 15 low-quality studies, protein intake was not associated with GWG,[26,28,30,35,41,42,49,51,54,56,60,63,65,69,79] whereas higher protein intake was associated with higher GWG in 4 studies.[48,53,67,80] In 4 low-quality studies, one of which is not included in Figure 2.1.4,[75] authors showed associations in subgroups only [e.g., older ages,[46] urban-living women,[75] or different associations during a specific trimester of pregnancy (*n*=2).[25,70] The effect of substituting fat or carbohydrate

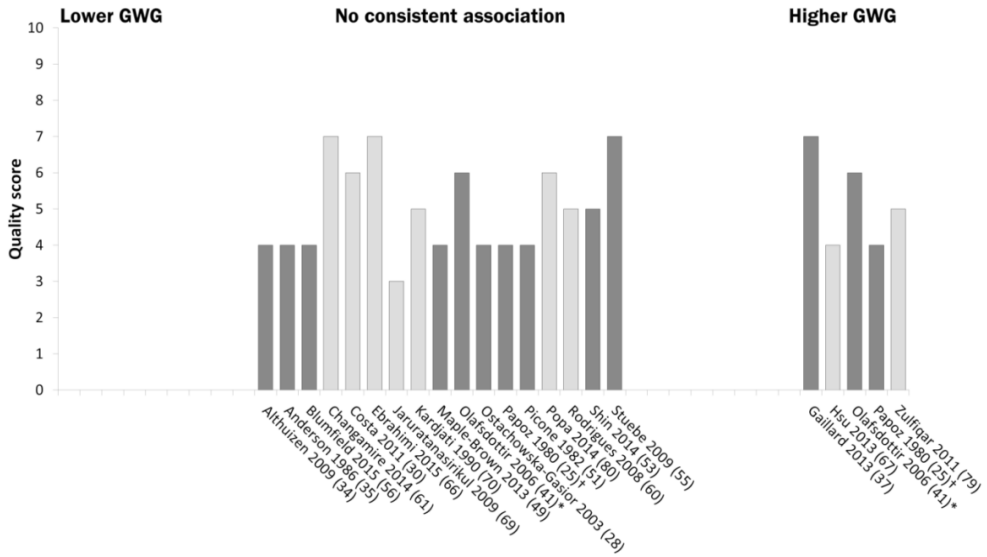
for protein was examined in 2 studies,[40,61] and both studies reported that the substitution of both fat and carbohydrate for protein was associated with lower GWG. The source of protein (e.g., animal compared with vegetable protein) was also not consistently associated with GWG (*n*=2)[40,42] (**Supplemental Table 2.1.4**).

Of the 11 studies that evaluated higher protein intake and the adequacy of GWG, 8 studies showed no association.[28,30,41,51,55,56,66,69] Although Gaillard et al.[37] and Popa et al.[80] showed a higher prevalence of excessive GWG, Shin et al.[53] reported a lower prevalence of inadequate GWG.

Maternal fat intake and gestational weight gain

The association of total fat intake with GWG was assessed in 21 observational studies **(Supplementary Table 2.1.5)** and is summarized in **Figure 2.1.5** when the directionally of the association was reported (*n*=20).

Figure 2.1.5. Harvest plot of the evidence of an association between total fat intake and GWG (*n*=20 observational studies)



Each study that reported a directionality of the association between total fat intake and GWG was added to the x axis [consequently, the study by Saowakontha[75] is not displayed]. The height of each bar represents the quality score, and the colour shows whether studies were performed in high-income countries (dark grey) or in low- and middle-income countries (light grey). Magnitudes of the associations are quantified in Supplementary Table 2.1.5. * Higher GWG was shown in women with a BMI (in kg/m^2) ≥25, but no association was shown in women with a BMI <25. †A positive correlation was shown during the first trimester of pregnancy, but no correlation was shown later in pregnancy. Abbreviation: GWG, gestational weight gain.

The 4 studies of high quality (19%) reported either no association (*n*=3)[55,61,66] or that higher fat intake was associated with higher GWG (*n*=1).[37] Of the low-quality studies (*n*=17), no association was shown in 12 studies,[28,30,34,35,49,51,53,56,60,69,70,80] and in one study[75] the direction

of the association was not reported. In addition, 2 low-quality studies[67,79] reported that higher fat intake was associated with higher GWG, one study[41] showed an association in overweight women but not in normal-weight women, and, in one study,[25] fat intake during the first trimester, but not in other periods, was associated with GWG.

The majority of studies (*n*=9) did not show an association between total fat intake and the prevalence of inadequate or excessive GWG.[28,34,51,53,55,56,66,69,80] A higher prevalence of excessive GWG was shown in the total population in one study[37] and, in a second study, in overweight women only.[41]

In nine studies,[30,34,42,43,49,53,55,56,59] an association was reported of specific fatty acids (e.g., saturated fatty acids, unsaturated fatty acids and trans fatty acids) with GWG **(Supplementary Table 2.1.6).** Of the 2 high-quality studies,[43,55] one study reported an association of higher saturated fatty acid intake with marginally higher GWG,[43] whereas the second study showed no association.[55] In the 7 low-quality studies, no association was shown in 5 studies,[30,34,42,49,56] and in one study,[59] higher saturated fat intake was associated with higher GWG. No association was reported of trans-fat intake with GWG[55] or for unsaturated fat.[30] One study[55] reported that higher monounsaturated fat intake was associated with lower GWG, whereas 3 studies showed no association.[42,49,56] There was no evidence that polyunsaturated fat intake was associated with GWG (*n*=3).[49,55,56] One study [48] stratified fat intake on the basis of its source and showed that higher fat intake from animal sources was associated with higher GWG, whereas higher vegetable-based fat intake was not associated with higher GWG **(Supplementary Table 2.1.7)**.

Maternal carbohydrate intake and gestational weight gain

The association of carbohydrate intake with GWG was described in 18 observational studies **(Supplementary Table 2.1.8)**, which reported inconsistent associations (**Figure 2.1.6** shows the 17 studies that reported directionality of the association). The 3 studies of high quality (17%) reported either that higher carbohydrate intake was associated with higher GWG[37] or reported no association.[61,66] In the 15 low-quality studies, higher carbohydrate intake was either associated with higher GWG (*n*=1),[79] with lower GWG (*n*=1),[48] or was not associated with GWG (*n*=10).[28,30,35,49,51,53,56,60,69,80] Three low-quality studies reported associations in subgroups only [e.g., overweight women,[41] urban-living women,[75] or different correlations in specific trimesters of pregnancy[25]].

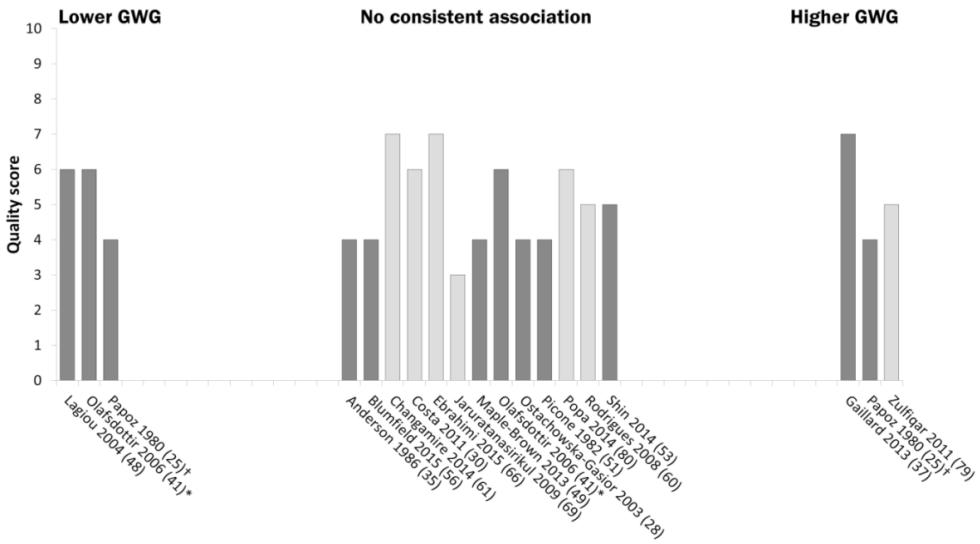
Of the 9 studies on GWG adequacy, carbohydrate intake was not associated with the prevalence of inadequate or excessive GWG in 7 studies.[28,51,53,56,66,69,80] In addition, Gaillard et al.[37] reported a higher prevalence of excessive GWG, and Olafsdottir et al.[41] showed a lower prevalence of excessive GWG with higher intake of carbohydrate in overweight women.

Discussion

This systematic review suggests that higher total energy intake during pregnancy is associated with higher GWG. More specifically, results on the effect of maternal fat intake were inconsistent, and the effect might be restricted to subgroups of women (e.g., overweight) or to specific types of fat (e.g., saturated fat). The effects of maternal protein and carbohydrate

intakes on GWG remain unclear. No differences were shown between studies performed in high-income countries and studies performed in low- and middle-income countries.

Figure 2.1.6. Harvest plot of the evidence of an association between carbohydrate intake and GWG (*n*=17 observational studies)



Each study that reported a directionality of the association between carbohydrate intake and GWG was added to the x axis [consequently, the study by Saowakontha [75] is not displayed]. The height of each bar represents the quality score, and the colour shows whether studies have been performed in high-income countries (dark grey) or in low- and middle-income countries (light grey). Magnitudes of the associations are quantified in Supplementary Table 2.1.8. * Higher GWG was shown in women with a BMI (in kg/m^2) ≥25, but no association was shown in women with BMI <25. † A positive correlation was shown between carbohydrate intake during the first trimester and the change of intake during first trimester with GWG, and a negative correlation was shown between the change in carbohydrate intake later in pregnancy with GWG. Abbreviation: GWG, gestational weight gain.

Our results for energy intake are in line a previous systematic review that was restricted to high-income countries.[14] Although most observational studies showed that energy intake was associated with higher GWG, trials with macronutrient supplementation did not always report differences in GWG. The discrepancy between results from trials may be explained by differences in the energy content of supplements, the duration of the intervention, or the variation in macronutrient composition.

Fats, carbohydrates, and proteins are the 3 primary energy-providing nutrients. However, it is unclear whether different macronutrients have different effects on GWG and its specific components independent of their energy contents. Although we showed no association of protein intake with GWG, higher protein intake might decrease GWG as a result of higher energy expenditure because the thermogenesis of protein is higher than that of carbohydrate or fat.[81,82] Also, higher protein intake might increase satiety because it has been shown that protein, and specifically animal-based protein, provides a higher level

of satiety than does carbohydrate or fat, which may affect overall energy intake.[83,84]

In our systematic review, we showed no consistent associations between total fat intake and GWG. A recent meta-analysis in non-pregnant adults reported weight reduction in participants who consumed a diet that was low in fat compared with diets with normal fat contents.[85] Fat is the macronutrient that is most efficiently stored in the body, although evidence has suggested differences in fat storage for different types of fats.[86] For example, saturated fatty acids might be more likely to be stored in adipose tissue than are unsaturated fats.[87] The results of 2 studies included in this systematic review[43,59] implied that saturated fat intake may increase GWG, which is in line with non-pregnant populations.[88,89]

We showed no consistent association of carbohydrate intake with GWG. This finding may be explained by differences in the carbohydrate quality [e.g., differences in the glycaemic index (GI)]. Consumption of high-GI foods could lead to decreased fat utilization and provide a lower satiety than does intake of low-GI foods.[90,91] Some studies described that higher GI-diets were associated with higher GWG,[92,93] but additional studies are needed to confirm this association.

The effect of the macronutrient content and composition of the diet could differ between malnourished populations and persons with an adequate nutrient status. For example, maternal protein-energy malnutrition is prevalent in many low- and middle-income countries.[16] We observed similar associations between macronutrient intake and GWG in studies in low-, middle-income countries and those in high-income countries. However, we were unable to take into account the nutritional status at baseline, because this information was often not reported. In addition, dietary behaviour is shaped by regional, cultural, and economic influences,[94] which could not be captured completely by our distinction between high-income countries and low- and middle-income countries.

Strengths and limitations

A strength of this review was the inclusion of studies performed in low-, middle- and high-income countries, which enhanced the external validity of the review. Another strength of this systematic review was the comprehensive systematic search strategy in 8 different databases as well as the reviewing process by 2 independent collaborators and the use of a comprehensive quality-scoring system.

A limitation of this review was the overall low quality of the studies and, in particular, the insufficient adjustment for confounding factors in many of the included studies. Many studies did not adjust for potential confounding factors such as pre-pregnancy BMI and physical activity levels. Therefore, residual confounding might still remain. Another limitation was the measurement of energy intake with the use of self-reporting methods such as food-frequency questionnaires.[95] Energy intake can be either overestimated or underestimated,[95] but the relation between macronutrient composition and GWG may also depend on total energy intake. To reduce the magnitude of this measurement error related to energy intake or to assess whether the relation between macronutrient intake and GWG is independent of energy intake, energy adjustment can be applied.[96] However, only 13 of 31 observational studies that assessed the association of a macronutrient with GWG used a form of energy adjustment. In addition, changes in energy intake during pregnancy were

not evaluated in relation to increased energy requirements or altered physical activity levels in pregnancy.[12,97]

Also, we were not able to study the associations of macronutrient intake with different components of GWG such as the effect on maternal fat accretion, which is an important determinant of postpartum weight retention.[98] In addition, many studies did not take into account pregnancy complications. Pre-eclampsia, e.g., often coexists with excess amounts of extracellular fluid (oedema), which leads to high GWG. Also, we were not able to present results by maternal weight status because most studies did not report analyses stratified by BMI.

We were not able to perform a meta-analysis of the findings because of large heterogeneity in the studies (e.g., different assessment methods). Also, dietary assessments were conducted during different periods in pregnancy. However, other studies have shown that macronutrient intake (relative to total energy intake) remains relatively stable during pregnancy.[99,100] Hence, we believe that the different measurement periods during pregnancy may not have influenced the results to a great extent. The studies also applied different definitions for GWG, used various cut-offs for the adequacy of GWG, and used diverse measurement strategies for GWG.

Finally, only a few studies reported the association of macronutrients on GWG adjusted for each other or for other foods or nutrients. Because individuals consume combined macronutrients in food products and not individual macronutrients, the effects of the macronutrients may depend on the source and interaction with other foods or nutrients.[101]

Conclusions

In conclusion, increased energy intake is associated with higher GWG, whereas the effect is not conclusive for specific macronutrients. Higher intake of fat, mainly saturated fat, might be associated with higher GWG, whereas the effects of protein intake and carbohydrate intake remain unclear. We did not observe different associations between macronutrient intake and GWG in low-, middle-, and high-income countries. However, the included studies had, on average, a low quality. Results from this systematic review implicate that higher-quality research is needed as is the studying of the effect of maternal diet as a whole including the macronutrient composition.

Supplementary Material can be found online: http://hdl.handle.net/1765/80025

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Chapter 2.2

A Priori and *a posteriori* dietary patterns during pregnancy and gestational weight gain: The Generation R Study

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Abstract

Abnormal gestational weight gain (GWG) is associated with adverse pregnancy outcomes. We examined whether dietary patterns are associated with GWG. Participants included 3374 pregnant women from a population-based cohort in the Netherlands. Dietary intake during pregnancy was assessed with food-frequency questionnaires. Three *a posteriori*-derived dietary patterns were identified using principal component analysis: a 'Vegetable, oil and fish', a 'Nuts, high-fibre cereals and soy', and a 'Margarine, sugar and snacks' pattern. The *a priori*-defined dietary pattern was based on national dietary recommendations. Weight was repeatedly measured around 13, 20 and 30 weeks of pregnancy; pre-pregnancy and maximum weight were self-reported. Normal weight women with high adherence to the 'Vegetable, oil and fish' pattern had higher early-pregnancy GWG than those with low adherence (43 g/week [95%CI 16;69] for highest *vs.* lowest quartile (Q)). Adherence to the 'Margarine, sugar and snacks' pattern was associated with a higher prevalence of excessive GWG (OR 1.45 [95%CI 1.06;1.99] Q4 *vs.* Q1). Normal weight women with higher scores on the 'Nuts, high-fibre cereals and soy' pattern had more moderate GWG than women with lower scores (-0.01 [95%CI -0.02;-0.00] per SD). The *a priori*-defined pattern was not associated with GWG. To conclude, specific dietary patterns may play a role in early pregnancy but are not consistently associated with GWG.

Introduction

Abnormal maternal weight gain during pregnancy (i.e., too little or too much) has been associated with unfavourable pregnancy outcomes in both mother and child. Insufficient gestational weight gain (GWG) is associated with both preterm birth and low birthweight,[1] and excessive GWG increases the risk of giving birth to large-for-gestational-age infants.[2] Excessive GWG is also associated with maternal pregnancy complications, including hypertensive disorders[3,4] and gestational diabetes,[5] which can increase the risk of the mother developing cardiometabolic diseases after pregnancy.[6,7]

Energy intake during pregnancy is associated with GWG,[4,8] but literature is scarce on whether GWG could be influenced by dietary composition. Some studies have examined the influence of food groups on GWG.[9-11] These studies found no association of fruit or vegetable intake with GWG[9,11] but unhealthier foods (e.g.,, sweets and processed foods) were associated with higher prevalence of excessive GWG.[9-11] Weight gain during pregnancy involves both maternal components (e.g., blood volume increase, fat accretion) and foetal components (e.g., weight of the foetus, amniotic fluid).[12] Therefore, the effect of diet on weight gain may differ between pregnant and non-pregnant women.

Assessing overall diet in relation to GWG has several advantages over studying individual foods or nutrients. First, the intakes of different nutrients are often highly correlated, which complicates the assessment of individual nutrients.[13] Second, possible associations between nutrient intake and GWG might be affected by biological interactions between nutrients.[13] For these reasons, evaluating diet using a dietary pattern approach may improve our understanding of which dietary pattern is most beneficial during pregnancy. Also, this approach can facilitate future food-based dietary guidelines.[14]

Only a few studies have focused on the relationship between dietary patterns and GWG.[15-18] However, no study evaluated dietary patterns and longitudinal development of weight during pregnancy. We hypothesized that specific dietary patterns may influence the development of maternal weight during pregnancy. In addition, dietary patterns are likely to differ between countries and populations,[13] so it is important to identify country-specific dietary patterns that may be associated with GWG.

Hence, the purpose of our study was to determine whether *a posteriori*-derived and *a priori*-defined dietary patterns are associated with GWG during different phases in pregnancy, adequacy of GWG and weight development during pregnancy in Dutch women participating in a population-based cohort.

Methods

Study design

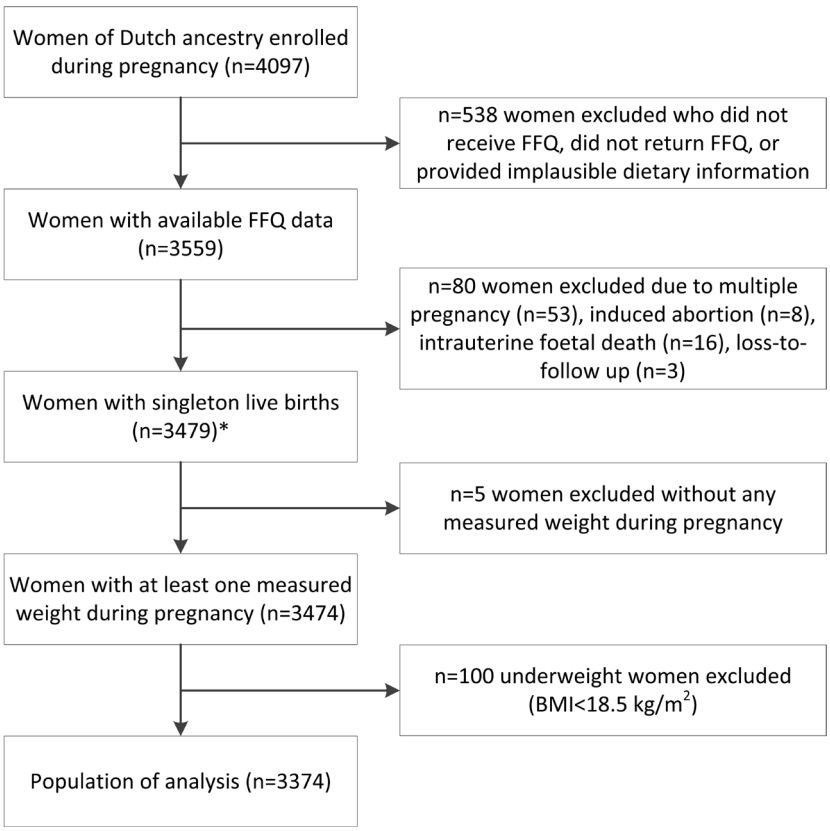
This study was embedded in the Generation R Study, a population-based prospective cohort from foetal life onwards in Rotterdam (the Netherlands). Details of this study have been described previously.[19] Briefly, pregnant women with an expected delivery date between April 2002 and January 2006, living in the urban area around Rotterdam were approached to participate. All participants provided written informed consent. The study was conducted

according to the World Medical Association Declaration of Helsinki and was approved by the Medical Ethics Committee, Erasmus Medical Center Rotterdam (the Netherlands, MEC 198.782.2001.31).

Population of analysis

For the current analysis, we included women of Dutch ancestry who entered the Generation R Study during pregnancy (n=4097). We did not include women of non-Dutch ancestry because the dietary assessment method that we used was designed to evaluate a Dutch diet. We excluded women with missing dietary information (n=538) and restricted our analysis to women with singleton live births (n=3479). We excluded 5 women whose weight was not measured during pregnancy. Finally, we excluded women who were underweight before pregnancy (body mass index (BMI) <18.5 kg/m^2; n=100), leaving 3374 women for the current analysis (**Figure 2.2.1**).

Figure 2.2.1. Flow chart of the study population: the Generation R Study (2002–2006)



* Population in which the *a posteriori*-derived dietary patterns were determined. Abbreviations: BMI, body mass index; GWG, gestational weight gain.

Dietary assessment

Dietary intake in early pregnancy was assessed at enrolment (median 13.4 weeks of gestation [Inter quartile range (IQR) 12.2-15.5]) using a 293-item semi-quantitative food-frequency questionnaire (FFQ) that covered dietary intake over the previous three months. The FFQ contained questions regarding foods that are frequently consumed in a traditionally Dutch diet, their consumption frequency, portion size,[20] preparation methods, and additions to foods. The average daily intake of energy and nutrients was calculated using the Dutch food-composition table (2006).[21] The FFQ was designed for and validated in an elderly population,[22] and has additionally been validated against three 24-hour dietary recalls in 71 Dutch pregnant women who visited a midwifery in Rotterdam. The intra-class correlation coefficients for energy-adjusted macronutrients ranged between 0.48 and 0.68.

A posteriori-derived dietary patterns

We used principal component analysis (PCA) with Varimax rotation to identify *a posteriori*-derived dietary patterns.[13,23] Our dietary patterns have been described in detail previously.[24] Briefly, the 293 individual food items from the FFQ were aggregated into 23 food groups (**Supplementary Table 2.2.1**). Subsequently, we extracted those factors (i.e., dietary patterns) of the PCA that had an Eigenvalue of ≥ 1.5.[25] The factor loadings, which described how strong the association between the food groups and each of the extracted patterns is, are presented in **Table 2.2.1**. Finally, we determined factor scores (i.e., adherence scores) for each participant and each pattern, by calculating the individual sum of the intake of the food groups, weighted with their factor loadings and standardizing those weighted sums to have mean zero and standard deviation one (standard deviation score). A higher factor score indicated that a woman's diet was closer to that dietary pattern.

Three *a posteriori*-derived dietary patterns were identified, namely a 'Vegetable, oil and fish' pattern, a 'Nuts, high-fibre cereals and soy' pattern and a 'Margarine, sugar and snacks' pattern, together explaining 25.8% of the variance in maternal dietary intake (**Table 2.2.1**).

A priori-defined dietary pattern

The *a priori*-defined dietary pattern was based on the Dutch Healthy Diet-index.[26] This index was developed to measure adherence to the Dutch guidelines for a healthy diet[27] and consisted of ten components: physical activity, vegetable, fruit, dietary fibre, fish, saturated fatty acids, *trans*-fatty acids, consumption of acidic drinks and foods, sodium, and alcohol. We omitted the components physical activity, *trans*-fatty acids, and the consumption of acidic drinks and foods because this information had not been collected. Furthermore, we did not include the alcohol component because alcohol abstinence is recommended during pregnancy. The score of each component ranged between 0 and 10 points, resulting in a total score ranging from 0 to 60 points (**Supplementary Table 2.2.2**). A higher score on the Dutch Healthy Diet-index corresponds with a higher adherence to the 2006 Dutch healthy diet guidelines and thus reflects a healthier diet. Finally, to facilitate comparison between all dietary patterns, we standardized the 'Dutch Healthy Diet-index' pattern to a standard deviation score.

Maternal weight gain

Information on pre-pregnancy weight was collected at enrolment using a questionnaire and was used to calculate a pre-pregnancy BMI (kg/m^2). Women visited our research centre three times at median (IQR) gestational ages of 12.9 (12.1-14.4) weeks (*first visit*), 20.4 (19.9-21.1) weeks (*second visit*), and 30.2 (29.9-30.8) weeks (*third visit*). During each visit, maternal height and weight were measured without shoes and heavy clothing. Six weeks after childbirth, women were asked to report their highest weight during pregnancy using a questionnaire, which we used as maximum weight in pregnancy.

Pre-pregnancy weight was highly correlated with weight measured during *the first visit* (R=0.96, p-value <0.001, n=2425), and there was no indication for systematic measurement error (**Supplementary Figure 2.2.1**). Also, a high correlation was found between weight during *the third visit* and maximum weight in pregnancy (R=0.89, p-value <0.001, n=2177) without an indication for systematic measurement error (**Supplementary Figure 2.2.1**). To evaluate long-term maternal weight gain, we measured maternal weight at our research centre six years after childbirth.

Table 2.2.1. Factor loadings food groups in *a posteriori*-derived dietary patterns[a]

Food group	'Vegetable, oil and fish' dietary pattern	'Nuts, high-fibre cereals and soy' dietary pattern	'Margarine, sugar and snacks' dietary pattern
Potatoes and other tubers	0.05	**-0.53**	**0.21**
Vegetables	**0.78***	0.17	-0.03
Fruits	0.13	**0.37**	0.02
Dairy products – high fat	**0.26**	**-0.26**	**0.29**
Dairy products – low fat	-0.15	**0.29**	0.16
Cereals – high fibre	0.24	**0.43***	**0.36**
Cereals – low fibre	**0.23**	-0.16	**0.25**
Meat and meat products	0.08	**-0.54**	**0.33**
Fish and shellfish	**0.45***	**0.24**	-0.11
Eggs and egg products	**0.27**	0.05	0.19
Vegetable oils	0.74*	0.08	-0.12
Margarine and butter	-0.06	-0.03	**0.61***
Sugar and confectionary and cakes	-0.11	0.13	**0.56***
Snacks	0.05	0.08	**0.40***
Coffee and tea	**0.28**	**0.34**	0.10
Sugar-containing beverages	-0.14	**-0.28**	**0.29**
Light soft drinks	0.13	**0.28**	0.02
Alcoholic beverages	**0.35**	-0.00	-0.04
Condiments and sauces	0.05	-0.09	**0.39**
Soups and bouillon	0.19	-0.02	0.15
Nuts, seeds and olives	0.03	**0.64***	**0.30**
Soy products	0.01	**0.39***	-0.10
Legumes	**0.44**	-0.02	0.07

[a.] Reprinted with permission from Van den Broek et al.[24] The food groups that are considered to have a strong association with a dietary pattern (factor loading ≥0.2 or ≤-0.2) are shown in bold. The three factor loadings with

the highest positive factor loading are used to name the dietary pattern and are presented with an asterisk (*). The three dietary patterns together explained 25.8% of the total variance in maternal dietary intake.

Gestational weight gain during different phases in pregnancy

GWG in different phases of pregnancy was calculated for three consecutive periods, namely early-pregnancy GWG (calculated as weight at *the first visit* minus pre-pregnancy weight, divided by follow-up duration (g/week), n=2425), mid-pregnancy GWG (calculated as weight at *the second visit* minus weight *the first visit*, divided by follow-up duration (g/week), n=2748), and late-pregnancy GWG (calculated as weight at *the third visit* minus weight at *the second visit*, divided by follow-up duration (g/week), n=3158). GWG until early-third trimester was calculated as weight at *the third visit* minus pre-pregnancy weight, divided by follow up duration (g/week, n=2815).

Adequacy of gestational weight gain

Women's total GWG (calculated as maximum weight in pregnancy minus pre-pregnancy weight, n=1917) was used to classify their GWG into inadequate, adequate, or excessive GWG. Cut-off values of GWG adequacy were based on recommendations published by the US Institute of Medicine (2009) and were BMI-specific.[28] Normal weight women (BMI 18.5-24.9 kg/m^2) were categorized as having an adequate GWG with a GWG between 11.5 and 16 kg, overweight women (BMI 25-29.9 kg/m^2) were classified as adequate GWG with GWG between 7 and 11.5 kg, and adequate GWG for obese women (BMI ≥30 kg/m^2) was between 5 and 9 kg.

Covariates

Several maternal sociodemographic and lifestyle characteristics were considered as potential confounders. We obtained information from prenatal questionnaires that were sent in different trimesters regarding maternal age, educational level,[29] household income (≤2200 *vs.* >2200 Euros/month), parity (no child *vs*. ≥1 child), pre-pregnancy weight, pre-existing comorbidities, vomiting, smoking or alcohol consumption (both categorized as never during pregnancy, stopped when pregnancy was known, or continued throughout pregnancy), folic acid supplementation (started periconceptionally, started first 10 weeks, or no supplementation), energy intake, and stress during pregnancy (using the Global Severity Index[30]). To calculate pre-pregnancy BMI, height was measured at enrolment. Gestational age was determined based on ultrasound examination, and during *the third visit* an ultrasound was performed to estimate foetal weight. Information on foetal sex was obtained from delivery reports.

Statistical analyses

We considered two sets of possible confounders in the analysis. *Model 1* was adjusted for median gestational age at follow-up and pre-pregnancy BMI. *Model 2* was further adjusted for age, educational level, household income, parity, smoking during pregnancy, alcohol consumption during pregnancy, stress during pregnancy, and foetal sex. The selection of potential confounders was based on factors found in the literature and on a change of at least 10% in effect estimate in a preliminary analysis assessing the association of dietary patterns

with GWG until early-third trimester. As GWG is related to BMI[28] and the preliminary analysis showed significant interaction terms for the 'Vegetable, oil and fish' pattern (p-value <0.01) and the 'Nuts, high-fibre cereals and soy' pattern (p-value = 0.01) with pre-pregnancy BMI, we stratified all analyses on the basis of weight status (normal weight (BMI <25 kg/m^2) and overweight (BMI ≥25 kg/m^2)).

In order to adequately estimate the relationship between diet and trajectories of gestational weight in the presence of incomplete covariates, we performed a longitudinal analysis using linear mixed modelling in the Bayesian framework. This method has been described in detail previously.[31] Briefly, by modelling the joint distribution of exposure, outcome and covariates, all available information is used to impute the missing values and estimate the parameters of interest simultaneously.

In the Bayesian linear mixed model, all main effects from *Model 2*, interaction terms between the dietary pattern variables (as derived by PCA) and a linear and quadratic effect for gestational age were included in the fixed effects structure. The correlation between the weight measurements within an individual was modelled by including random effects for the intercept and slope (for gestational age) into the model. No additional correlation structure was assumed for the error terms. For this analysis, the reported parameter estimates and 95% credible intervals were obtained by taking the mean and 2.5% and 97.5% quantiles of the posterior sample of the respective parameters.

To analyse the association of the *a priori*-defined and *a posteriori*-derived dietary patterns with GWG during different phases in pregnancy, GWG until early third trimester and maximal GWG, we performed multivariable linear regression analysis. Missing covariate values were multiply imputed by randomly drawing ten values from the posterior samples of each incomplete covariate derived in the Bayesian analysis. Missing observations of gestational weight were not imputed. The reported results from the cross-sectional models were pooled over all ten completed datasets. Separate models were fitted with the dietary patterns discretized in quartiles, with the lowest quartile (quartile 1) as a reference category, as well as continuously per SD score. Quartiles were constructed separately for normal weight and overweight women; each of these analyses was done for *Model 1* and *Model 2*. To identify cases that have an influence on the regression models we calculated Cook's distance.[32]

Because GWG is a physiological process in pregnancy and resulting in weight gain in almost all women during pregnancy,[28] we also evaluated the associations between dietary patterns and GWG adequacy (inadequate, adequate *vs.* excessive GWG) using multinomial regression models. We included all covariates from *Model 2* and used 'adequate GWG' as a reference category.

Sensitivity analyses

To test the stability of our results, we performed four sensitivity analyses in *Model 2* for the association between dietary patterns and GWG until early-third trimester. First, because energy intake may be an intermediate factor in the association of maternal diet with GWG, we further adjusted for energy intake (kcal/day). Second, we further adjusted for estimated foetal weight in the early-third trimester to evaluate whether higher GWG could be explained

by greater foetal growth because we previously found that specific dietary patterns may be associated with foetal weight.[33] Third, we excluded women with pre-existing comorbidities (n=182) and women with hypertensive complications in pregnancy[34] or gestational diabetes (n=272) since these conditions may influence both dietary intake and GWG. Fourth, we excluded women who reported vomiting more than once per week during the three months prior to enrolment (n=421), since this might alter dietary intake and GWG. Also, we explored effect modification of the association between dietary patterns and GWG with educational level and household income.

Additionally, we evaluated whether the associations of dietary patterns with GWG would markedly change when using self-reported maximum weight during pregnancy instead of measured weight at *the third visit* (n=1917). Furthermore, we evaluated whether the associations found between the dietary patterns and adequacy of weekly GWG (between *the first* and *the third visit*) were similar to those with adequacy of total GWG (n=2745), because some measurement error was found in the self-reported weights (**Supplementary Figure 2.2.1**). The cut-off values of adequate weekly GWG were 0.35-0.50 kg/week for normal weight women, 0.23-0.33 kg/week of overweight women, and 0.17-0.27 kg/week for obese women.[28] In addition, we explored long-term maternal weight gain and evaluated whether this long-term weight gain differed in women with inadequate, adequate or excessive GWG using Analysis of Variance (ANOVA). Finally, we calculated the correlation between weight at *the third visit* and weight 6 years after childbirth.

All statistical analyses were performed in SPSS version 21.0 (IBM Corp., Armonk, NY, USA), R version 3.2.1 (R Foundation for Statistical Computing, Vienna, Austria) and JAGS version 3.4.0.[35]

Results

Study population

Baseline characteristics for normal weight women (n=2544; 75%) and overweight women (n=830; 25%) are presented in **Table 2.2.2**. The mean score ± SD on the Dutch Healthy Diet-index was 32 ± 8 and ranged from 8 to 59. Overall, 43% of women had excessive GWG (n=826); excessive GWG was found in 37% of the normal weight women (n=557) and in 63% of the overweight women (n=269).

We did not identify women with large influence on the effect estimates of the association between dietary patterns and GWG (all Cook's distances were <1).

Table 2.2.2. Subject characteristics (n=3374), the Generation R Study (2002-2006)[a]

Subject characteristics		Normal weight women (n = 2544)	Overweight women (n = 830)
Age (years)		31.6 ± 4.3	31.0 ± 4.4
Educational level, n (%)	Low and midlow	307 (12.1)	201 (24.2)
	Midhigh	1283 (50.4)	436 (52.5)
	High	954 (37.5)	193 (23.3)
Household income, n (%)	< 2200 Euros/month	620 (24.4)	266 (32.1)
	≥ 2200 Euros/month	1924 (75.6)	564 (67.9)
Parity, n (%)	0	1554 (61.1)	465 (56.0)
	≥1	990 (38.9)	365 (44.0)
Pre-pregnancy BMI (kg/m^2)		21.6 (20.3-23.0)	27.7 (26.0-30.5)
Smoking, n (%)	Never during pregnancy	1911 (75.1)	612 (73.7)
	Until pregnancy was known	233 (9.2)	61 (7.3)
	Continued throughout pregnancy	400 (15.7)	157 (19.0)
Alcohol consumption, n (%)	Never during pregnancy	764 (30.0)	359 (43.2)
	Until pregnancy was known	416 (16.4)	138 (16.6)
	Continued throughout pregnancy	1364 (53.6)	334 (40.2)
Stress during pregnancy (score 0-4)		0.11 (0.06-0.24)	0.13 (0.06-0.25)
Energy intake (kcal/day)		2162 ± 507	2090 ± 514
Dutch Healthy Diet-index (score 0-60)		32 ± 8	30 ± 8
Foetal sex, n (%)	Male	1287 (50.6)	415 (50.0)
	Female	1257 (49.4)	415 (50.0)
Gestational weight gain (kg)		14.7 ± 7.3	12.9 ± 7.7
Adequacy of GWG, n (%)	Inadequate	370 (24.8)	89 (20.9)
	Adequate	565 (37.9)	67 (15.8)
	Excessive	557 (37.3)	269 (63.3)

[a.] Values represent n (%) for categorical variables, and for continuous variables they represent mean ± SD or median (interquartile range). Missing data: educational level (1.3%), household income (10.3%), parity (0.2%), pre-pregnancy BMI (14.2%), smoking during pregnancy (7.4%), alcohol consumption during pregnancy (8.1%), stress during pregnancy (12.0%), gestational weight gain (43.2%), adequacy of gestational weight gain (43.2%). No missing data for maternal age, energy intake, Dutch Healthy Diet-index or foetal sex. Numbers may not add up to total due to rounding after imputation. Abbreviations: BMI, body mass index; GWG, gestational weight gain.

Dietary patterns and gestational weight gain in different phases in pregnancy

Normal weight women in the highest quartile of the 'Vegetable, oil and fish' pattern had a 43 g/week (95%CI 16;69) greater early-pregnancy GWG than women in the lowest quartile, independent of lifestyle and sociodemographic variables. We observed no such association in overweight women (**Table 2.2.3**). The 'Nuts, high-fibre cereals and soy' pattern was associated with a lower early-pregnancy GWG in *Model 1* in both normal weight and overweight women. However, after additional adjustment (*Model 2*) this pattern was no longer significantly associated with early-pregnancy GWG. Neither the 'Margarine, sugar and snacks' pattern nor the 'Dutch Healthy Diet-index' pattern was associated with early-pregnancy GWG.

Table 2.2.3. Association of dietary patterns with gestational weight gain in early pregnancy (n=2425)[a]

	Early-pregnancy weight gain (g/week)			
	Normal weight women (n=1849)		**Overweight women** (n=576)	
	Model 1	*Model 2*	*Model 1*	*Model 2*
		'Vegetable, oil and fish' pattern		
Q1 (low)	Reference	Reference	Reference	Reference
Q2	-8 (-34; 17)	-3 (-28; 23)	18 (-44; 80)	29 (-34; 91)
Q3	-14 (-40; 11)	-4 (-30; 22)	59 (-3; 121)	**77 (14; 141)**
Q4 (high)	**38 (12; 63)***	**43 (16; 69)***	4 (-58; 66)	31 (-37; 99)
Per SD	***p < 0.01****	***p < 0.01****	*p = 0.63*	*p = 0.24*
		'Nuts, high-fibre cereals and soy' pattern		
Q1 (low)	Reference	Reference	Reference	Reference
Q2	-10 (-36; 15)	5 (-21; 30)	-19 (-81; 43)	-17 (-79; 45)
Q3	**-26 (-52; -1)**	-4 (-31; 23)	-54 (-117; 10)	-44 (-109; 21)
Q4 (high)	**-31 (-57; -6)**	-10 (-37; 18)	-64 (-128; 1)	-52 (-120; 15)
Per SD	***p < 0.01****	*p = 0.22*	***p = 0.02***	*p = 0.06*
		'Margarine, sugar and snacks' pattern		
Q1 (low)	Reference	Reference	Reference	Reference
Q2	3 (-22; 29)	2 (-23; 27)	33 (-28; 94)	28 (-32; 88)
Q3	5 (-21; 30)	-1 (-26; 24)	35 (-29; 98)	41 (-22; 103)
Q4 (high)	20 (-6; 46)	13 (-12;39)	52 (-9; 114)	45 (-17; 106)
Per SD	*p = 0.11*	*p = 0.36*	*p = 0.20*	*p = 0.24*
		'Dutch Healthy Diet-index' pattern		
Q1 (low)	Reference	Reference	Reference	Reference
Q2	0 (-25; 26)	-2 (-27; 23)	4 (-58; 65)	3 (-58; 64)
Q3	16 (-10; 42)	7 (-19; 32)	48 (-14; 110)	38 (-23; 100)
Q4 (high)	3 (-22; 29)	-14 (-40; 12)	34 (-28; 96)	11 (-54; 75)
Per SD	*p = 0.86*	*p = 0.17*	*p = 0.32*	*p = 0.86*

[a.] Results from multivariable linear regression analyses, based on imputed data. Values (regression coefficients with 95%-confidence interval) reflect the difference in early-pregnancy weight gain (g/week) for quartile 2 until 4 relative to quartile 1. p-Values correspond to the effect of 1SD increase in dietary pattern score. *Model 1*: adjusted for pre-pregnancy BMI and median gestational age at follow-up. *Model 2*: *Model 1* further adjusted for age, educational level, household income, parity, smoking during pregnancy, alcohol consumption during pregnancy, stress during pregnancy, and foetal sex. P for interaction between dietary patterns and pre-pregnancy BMI was <0.10 for the

'Vegetable, oil and fish' pattern and for the other patterns >0.10. Significant results are presented in bold (p-value <0.05) and results with a p-value <0.0125 with an asterisk (*). Abbreviations: BMI, body mass index; Q, quartile; SD, standard deviation.

Table 2.2.4. Association of dietary patterns with gestational weight gain in mid-pregnancy (n=2748)[a]

	Mid-pregnancy weight gain (g/week)			
	Normal weight women (n=2079)		**Overweight women** (n=669)	
	Model 1	*Model 2*	*Model 1*	*Model 2*
	'Vegetable, oil and fish' pattern			
Q1 (low)	Reference	Reference	Reference	Reference
Q2	-0 (-39; 38)	6 (-33; 45)	37 (-42; 115)	17 (-64; 97)
Q3	2 (-36; 39)	12 (-27; 51)	21 (-59; 101)	7 (-76; 90)
Q4 (high)	-13 (-52; 25)	-4 (-44; 36)	23 (-56; 103)	-19 (-105; 68)
Per SD	*p = 0.48*	*p = 0.72*	*p = 0.36*	*p = 0.92*
	'Nuts, high-fibre cereals and soy' pattern			
Q1 (low)	Reference	Reference	Reference	Reference
Q2	22 (-16; 60)	25 (-14; 64)	28 (-52; 107)	8 (-73; 89)
Q3	-7 (-46; 31)	-2 (-42; 39)	47 (-33; 128)	19 (-65; 102)
Q4 (high)	25 (-13; 64)	30 (-11; 70)	62 (-19; 142)	17 (-68; 103)
Per SD	*p = 0.38*	*p = 0.32*	*p = 0.14*	*p = 0.72*
	'Margarine, sugar and snacks' pattern			
Q1 (low)	Reference	Reference	Reference	Reference
Q2	31 (-7; 68)	31 (-6; 69)	25 (-53; 102)	30 (-47; 107)
Q3	15 (-23; 53)	18 (-21; 56)	8 (-71; 87)	17 (-62; 96)
Q4 (high)	16 (-22; 54)	18 (-20; 57)	14 (-64; 92)	24 (-54; 103)
Per SD	*p = 0.44*	*p = 0.40*	*p = 0.65*	*p = 0.48*
	'Dutch Healthy Diet-index' pattern			
Q1 (low)	Reference	Reference	Reference	Reference
Q2	-15 (-53; 23)	-14 (-52; 24)	-36 (-113; 41)	-31 (-109; 47)
Q3	-0 (-38; 37)	-1 (-39; 36)	-23 (-101; 54)	-4 (-81; 74)
Q4 (high)	-7 (-46; 31)	-10 (-49; 30)	-9 (-89; 70)	27 (-56; 109)
Per SD	*p = 0.66*	*p = 0.76*	*p = 0.43*	*p = 0.88*

[a.] Results from multivariable linear regression analyses, based on imputed data. Values (regression coefficients with 95%-confidence interval) reflect the difference in mid-pregnancy weight gain (g/week) for quartile 2 until 4 relative to quartile 1. p-Values correspond to the effect of 1SD increase in dietary pattern score. *Model 1*: adjusted for pre-pregnancy BMI and median gestational age at follow-up. *Model 2*: *Model 1* further adjusted for age, educational level, household income, parity, smoking during pregnancy, alcohol consumption during pregnancy, stress during pregnancy, and foetal sex. P for interaction between dietary patterns and pre-pregnancy BMI was <0.10 for the 'Dutch Healthy Diet-index' pattern and for the other patterns >0.10. Significant results are presented in bold (p-value <0.05) and results with a p-value <0.0125 with an asterisk (*). Abbreviations: BMI, body mass index; Q, quartile; SD, standard deviation.

Table 2.2.5. Association of dietary patterns with gestational weight gain in late pregnancy (n=3158)[a]

	Late-pregnancy weight gain (g/week)			
	Normal weight women (n=2384)		**Overweight women** (n=774)	
	Model 1	*Model 2*	*Model 1*	*Model 2*
		'Vegetable, oil and fish' pattern		
Q1 (low)	Reference	Reference	Reference	Reference
Q2	-3 (-32; 26)	10 (-20; 39)	21 (-35; 78)	36 (-21; 93)
Q3	-18 (-47; 10)	-4 (-33; 26)	-3 (-60; 55)	21 (-38; 80)
Q4 (high)	-19 (-47; 10)	-0 (-31; 30)	-8 (-64; 49)	24 (-38; 86)
Per SD	*p = 0.09*	*p = 0.54*	*p = 0.42*	*p = 0.82*
		'Nuts, high-fibre cereals and soy' pattern		
Q1 (low)	Reference	Reference	Reference	Reference
Q2	-2 (-30; 27)	14 (-15; 44)	4 (-54; 62)	18 (-41; 77)
Q3	-16 (-45; 12)	8 (-22; 38)	-3 (-60; 55)	15 (-45; 74)
Q4 (high)	**-37 (-65; -8)**	-13 (-43; 18)	3 (-55; 61)	21 (-41; 83)
Per SD	*p < 0.01**	*p = 0.48*	*p = 0.91*	*p = 0.66*
		'Margarine, sugar and snacks' pattern		
Q1 (low)	Reference	Reference	Reference	Reference
Q2	-21 (-49; 8)	-20 (-48; 8)	-8 (-65; 49)	-7 (-63; 50)
Q3	-12 (-41; 16)	-12 (-40; 17)	7 (-49; 64)	17 (-40; 74)
Q4 (high)	-5 (-34; 24)	-6 (-35; 23)	8 (-49; 65)	10 (-48; 68)
Per SD	*p = 0.86*	*p = 0.76*	*p = 0.64*	*p = 0.66*
		'Dutch Healthy Diet-index' pattern		
Q1 (low)	Reference	Reference	Reference	Reference
Q2	-14 (-43; 14)	-13 (-41;15)	46 (-10; 102)	51 (-5; 108)
Q3	-2 (-31; 27)	-10 (-39; 18)	23 (-34; 81)	25 (-33; 82)
Q4 (high)	-3 (-31; 26)	-14 (-43; 15)	33 (-24; 90)	28 (-31; 88)
Per SD	*p = 0.61*	*p = 0.57*	*p = 0.46*	*p = 0.58*

[a.] Results from multivariable linear regression analyses, based on imputed data. Values (regression coefficients with 95%-confidence interval) reflect the difference in late-pregnancy weight gain (g/week) for quartile 2 until 4 relative to quartile 1. p-Values correspond to the effect of 1SD increase in dietary pattern score. *Model 1*: adjusted for pre-pregnancy BMI and median gestational age at follow-up. *Model 2*: *Model 1* further adjusted for age, educational level, household income, parity, smoking during pregnancy, alcohol consumption during pregnancy, stress during pregnancy, and foetal sex. P for interaction between dietary patterns and pre-pregnancy BMI was >0.10 for the 'Nuts, high-fibre cereals and soy' and the 'Dutch Healthy Diet-index' pattern, but <0.10 for the 'Vegetable, oil and fish' and the 'Margarine, sugar and snacks' pattern. Significant results are presented in bold (p-value <0.05) and results with a p-value <0.0125 with an asterisk (*). Abbreviations: BMI, body mass index; Q, quartile; SD, standard deviation.

No significant associations were found for any of the dietary patterns with mid-pregnancy GWG in normal weight or overweight women (**Table 2.2.4**). **Table 2.2.5** shows that in normal weight women, only the 'Nuts, high-fibre cereals and soy' pattern was inversely associated with late-pregnancy GWG in *Model 1* (p-value for 1 SD increase <0.01), but these results largely attenuated after adjustment for sociodemographic and lifestyle factors (p-value = 0.48). In overweight women, none of the dietary patterns were significantly associated with late-pregnancy GWG. In line with the results from early-pregnancy GWG, normal weight

women in the highest quartile of the 'Vegetable, oil and fish' pattern had higher GWG by 25 g/week (95%CI 9;42) until the early-third trimester than women in the lowest quartile (**Supplementary Table 2.2.3**), whereas no association was found in overweight women (**Supplementary Table 2.2.4**). The other dietary patterns were not associated with GWG until the early-third trimester.

Dietary patterns and gestational weight gain adequacy

Higher adherence to the dietary patterns was not associated with the prevalence of inadequate GWG (**Table 2.2.6**). The 'Vegetable, oil and fish', the 'Nuts, high-fibre cereals and soy' and the 'Dutch Healthy Diet-index' pattern were also not associated with prevalence of excessive GWG. Yet, women with higher scores on the 'Margarine, sugar and snacks' pattern had a higher prevalence of excessive GWG than women in the lowest quartile (ORs Q2: 1.40 [95%CI 1.04;1.90], Q3: 1.37 [95%CI 1.00;1.87], and Q4: 1.45 [95%CI 1.06;1.99]).

Dietary patterns and trajectories of gestational weight

Figure 2.2.2 shows the longitudinal relationship between the *a posteriori*-derived dietary patterns with trajectories of maternal weight during pregnancy, as the difference in weight (kg) between the 12.5% quantile ('quartile 1') and the 37.5%, 62.5% and 87.5% quantiles ('quartiles' 2, 3 and 4, respectively) of adherence to the dietary pattern in normal weight women. Corresponding results for overweight women are displayed in **Figure 2.2.3**. In both normal weight and overweight women, most of the main effects of diet as well as the interaction terms with gestational age were not significant (**Supplementary Table 2.2.5**). Only the 'Margarine, sugar and snacks' pattern was significantly associated with higher weight in normal weight women (0.30 [95%CI 0.07;0.52]) throughout pregnancy and the 'Nuts, high-fibre cereals and soy' pattern was associated with slightly slower weight gain in normal weight women (-0.01 [95%CI-0.02;-0.00]).

Sensitivity analyses

The results of the sensitivity analyses are presented in **Supplementary Table 2.2.3** for normal weight women and in **Supplementary Table 2.2.4** for overweight women. Additional adjustment for energy intake resulted in little attenuation of the effect estimate of the 'Vegetable, oil and fish' pattern with GWG until early-third trimester, however the association remained statistically significant. Further adjustment of estimated foetal weight did not change the effect estimates of any dietary pattern with GWG in normal weight or overweight women. The results did not alter greatly after exclusion of women who vomited more than once per week, or exclusion of women with pre-existing comorbidities or pregnancy complications. The evaluation of maximum GWG showed that normal weight women with high adherence to the 'Vegetable, oil and fish' pattern had 29 g/week (95%CI 2;57) higher maximal GWG than women with low adherence. In addition, normal weight women in the highest quartile of the 'Dutch Healthy Diet-index' had a 28 g/week (95%CI -55;-1) lower maximal GWG than women in quartile 1. The association between dietary patterns and GWG was not modified by educational level. For household income, women with higher household income (≥2200 Euro/month) and higher scores on the 'Dutch Healthy

Diet-index' pattern had lower GWG (p-value = 0.01 per 1 SD score) than women with higher income and lower scores on this dietary pattern, whereas no association was found in women with lower household income (p-value = 0.11).

Table 2.2.6. Association of dietary patterns with gestational weight gain adequacy (n=1917)[a]

	Inadequate GWG (n=459)	**Adequate GWG** (n=632)	**Excessive GWG** (n=826)
	OR (95%CI)		OR (95%CI)
		'Vegetable, oil and fish' pattern	
Q1 (low)	Reference	Reference	Reference
Q2	0.85 (0.60;1.22)	Reference	1.08 (0.79;1.48)
Q3	0.86 (0.60;1.23)	Reference	1.05 (0.76;1.46)
Q4 (high)	0.84 (0.58;1.22)	Reference	1.06 (0.76;1.48)
Per SD	*p = 0.21*		*p = 0.91*
		'Nuts, high-fibre cereals and soy' pattern	
Q1 (low)	Reference	Reference	Reference
Q2	0.77 (0.53;1.13)	Reference	**1.16 (0.82;1.62)**
Q3	0.86 (0.59;1.25)	Reference	**1.26 (0.89;1.77)**
Q4 (high)	0.85 (0.58;1.24)	Reference	**1.09 (0.77;1.53)**
Per SD	*p = 0.76*		*p = 0.46*
		'Margarine, sugar and snacks' pattern	
Q1 (low)	Reference	Reference	Reference
Q2	0.97 (0.69;1.36)	Reference	1.40 (1.04;1.90)
Q3	0.93 (0.66;1.32)	Reference	1.37 (1.00;1.87)
Q4 (high)	0.98 (0.69;1.40)	Reference	1.45 (1.06;1.99)
Per SD	*p = 0.73*		*p = 0.09*
		'Dutch Healthy Diet-index' pattern	
Q1 (low)	Reference	Reference	Reference
Q2	1.04 (0.74;1.45)	Reference	0.92 (0.69;1.24)
Q3	0.84 (0.59;1.20)	Reference	0.95 (0.70;1.27)
Q4 (high)	1.32 (0.92;1.90)	Reference	1.11 (0.80;1.53)
Per SD	*p = 0.07*		*p = 0.66*

[a.] Results (OR with 95%-confidence interval) from multivariable multinomial logistic regression analyses, based on imputed data. Low dietary pattern adherence (Q1) is the reference category for diet and adequate GWG is the reference category for adequacy of GWG in the multinomial regression model. P-values correspond to the effect of 1SD increase in dietary pattern score. Adjusted for pre-pregnancy BMI, age, educational level, household income, parity, smoking during pregnancy, alcohol consumption during pregnancy, stress during pregnancy, and foetal sex. Significant results are presented in bold (p<0.05) and results with a p-value <0.0125 with an asterisk (*). Abbreviations: GWG, gestational weight gain; OR, odds ratio; Q, quartile; SD, standard deviation.

Evaluating adequacy of GWG using weekly GWG instead of total GWG resulted in a higher percentage women being classified as having 'excessive GWG' (57% *vs.* 43%). However, also with this different definition a high adherence to the 'Margarine, sugar and snacks' pattern was associated with a higher prevalence excessive weekly GWG. Nonetheless, high adherence to this pattern was also associated with higher prevalence of inadequate weekly GWG, although without dose-response association (**Supplementary Table 2.2.5**).

Six years after childbirth, women had gained on average 3.4 kg (IQR: 0.4;7.0) compared to their pre-pregnancy weight (*n*=2247). The median (IQR) long-term weight gain was significantly different between the categories of GWG adequacy: women with inadequate GWG gained 2.2 kg (-0.6;5.2), those with adequate GWG gained on average 2.6 kg (0.2;5.2), and women with excessive GWG were 4.6 kg (1.4;8.8) heavier (F-test 27.5, p-value <0.001). The weight 6 years after childbirth was highly correlated with the weight at *the third visit* in pregnancy (R=0.85; p-value <0.001).

Discussion

Summary of main findings

Our results from a population-based Dutch cohort suggest that specific *a posteriori*-derived dietary patterns have a limited influence in early-pregnancy GWG, the prevalence of excessive GWG, and weight development in pregnancy. We found neither consistent associations of any dietary pattern with the prevalence of inadequate GWG, nor was the association of the *a priori*-defined dietary pattern with GWG.

Interpretation and comparison with other studies

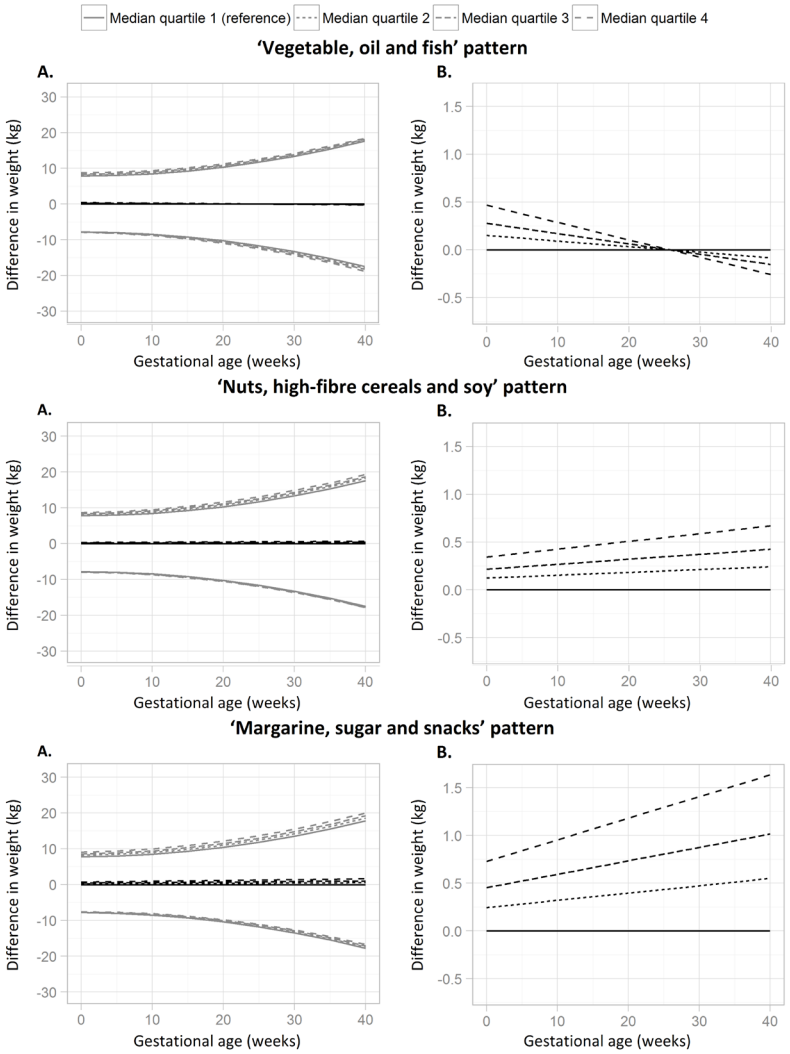
The association of dietary patterns during pregnancy with GWG has been evaluated previously in a few studies,[15-18] but these studies did not evaluate longitudinal development of gestational weight and were conducted in different populations. Uusitalo et al., found that higher adherence to an *a posteriori*-derived dietary pattern characterized by high intake of sweets, fast food and snacks was associated with higher weekly GWG.[15] In line with these results,[15] we found that higher adherence to the unhealthy 'Margarine, sugar and snacks' pattern was associated with higher prevalence of excessive GWG. Additionally, Uusitalo et al. reported that a pattern that was high in vegetables, fish and fruits was not associated with GWG.[15] In contrast, we found that the 'Vegetable, oil and fish' pattern, a relatively healthy pattern, was associated with higher GWG, particularly in early pregnancy.

In our study, the *a priori*-defined 'Dutch Healthy Diet-index' pattern was not consistently associated with any measure of GWG. This result was in accordance with two studies showing no relationship between the *a priori*-defined 'US healthy eating index of 2005' (HEI-2005) and the 'Alternate Healthy Eating Index, slightly modified for pregnancy' (AHEI-P) with the prevalence of inadequate or excessive GWG.[16,17] Nevertheless, a large population-based cohort study of over 66,000 participants found that high adherence to the *a priori*-defined 'New Nordic Diet score' was associated with a 7% lower prevalence of excessive GWG in normal weight women, compared with low adherence.[18] The inconsistent significant associations between the *a priori*-defined dietary patterns may be due to different items that were included in the diet scores, whereas the 'New Nordic Diet score' contained items on meal patterns and the type of beverages consumed, among others;[18] these items were not evaluated in our Dutch Healthy Diet-index, nor in other *a priori*-defined patterns.[16,17]

The association of *a posteriori*-derived dietary patterns with weight trajectories over pregnancy has not been evaluated previously, to our knowledge. Studying this association

longitudinally has the advantage that all available weight measurements can be used, and takes into account the correlation between these measurements. In addition, weekly GWG is not constant over pregnancy and differs considerably by individual,[28,36] which complicates cross-sectional comparisons of GWG. Our longitudinal analysis showed that women with higher adherence to the 'Nuts, high-fibre and soy' pattern had a more moderate increase in weight during pregnancy than did women with low adherence to this dietary pattern, although absolute differences were small.

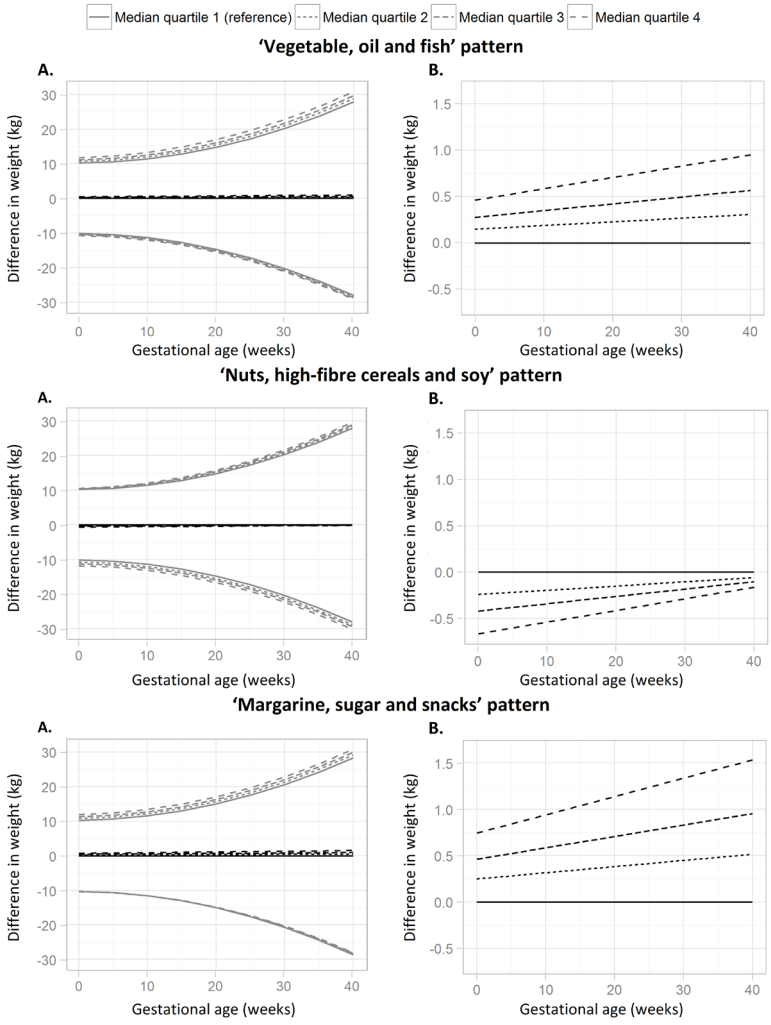
Figure 2.2.2. Trajectories of gestational weight in normal weight women (n=2564)



The figure shows the development of weight during pregnancy in normal weight women as estimated by the linear mixed model. Trends are plotted for 37.5%, 62.5%, and 87.5% quantiles (denoted as quartiles 2 until 4)

as compared with the 12.5% quantile (denoted quartile 1). Adjusted for gestational age at measurements, age, educational level, household income, parity, smoking during pregnancy, alcohol consumption during pregnancy, stress during pregnancy, and foetal sex. Panel A displays effect estimates with 95% CI. Panel B zooms in on the effect estimates.

Figure 2.2.3. Trajectories of gestational weight in overweight women (n=810)



The figure shows the development of weight during pregnancy in overweight women as estimated by the linear mixed model. Trends are plotted for 37.5%, 62.5%, and 87.5% quantiles (denoted as quartiles 2 until 4) as compared with the 12.5% quantile (denoted quartile 1). Adjusted for gestational age at measurements, age, educational level, household income, parity, smoking during pregnancy, alcohol consumption during pregnancy, stress during pregnancy, and foetal sex. Panel A displays effect estimates with 95% CI. Panel B zooms in on the effect estimates.

Results from both observational and interventional studies indicated that women with higher energy intake had higher GWG compared with women who have lower energy intake,[8] results that were also found in our cohort.[4] In our analyses, the association of the 'Vegetable, oil and fish' pattern remained significantly associated with GWG after additional adjustment for energy intake. This may indicate that dietary patterns are associated with GWG beyond energy intake.

Evaluating weight gain in pregnancy is important because GWG has been associated with many adverse pregnancy and birth outcomes. Gaining excessive weight during pregnancy can have short-term consequences such as delivery complications, and giving birth to a child that is large for its gestational age.[3,4,28] Additionally, it has been associated with long-term health consequences including post-partum obesity of the mother[37] due to retaining their excess fat mass, and childhood obesity.[4] Indeed, in our population, six years after childbirth women had gained on average 3.4 kg from their pre-pregnancy weight.

Weight gain during pregnancy consists of several maternal and foetal components that contribute differently to GWG over time.[12] For example, during the first half of pregnancy, maternal fat gain is a major contributor of GWG,[38,39] and most of the fat gain that takes place during pregnancy is in that period.[40] In our study, the higher GWG in women with high adherence to the 'Vegetable, oil and fish' pattern could not be explained by foetal growth and was mainly found in early pregnancy, meaning this higher GWG is likely due to maternal components, e.g., fat mass.

Our results for normal weight women differed from those for overweight women particularly for the 'Vegetable, oil and fish' pattern and for the 'Nuts, high-fibre cereals and soy' pattern. Similarly, Hillesund et al. reported differential associations for women below and above a BMI of 25 kg/m^2.[18] These differential findings may be explained by different reporting of dietary intake[41] or by differing contribution of the individual components of GWG for normal weight and overweight women.[42] In addition, our longitudinal analyses showed that over the whole course of pregnancy, normal weight women with higher adherence to the 'Margarine, sugar and snacks' pattern tend to be heavier than women with lower adherence.

Strengths and limitations

A strength of our study is that we used a comprehensive approach to analyse the relation between diet and GWG by evaluating the associations of dietary patterns with 1) GWG during different phases in pregnancy, 2) adequacy of GWG, and 3) trajectories of gestational weight. Another strength is the use of two distinct methods to define dietary patterns, which enabled us to evaluate the effects of dietary patterns derived by a data-driven and by a hypothesis-driven approach. Dietary patterns represent the combined effects of all foods consumed,[13] which may lead to a more powerful effect than the effects of the individual components, although it may also have led to a dilution of the effects of individual components that are associated with GWG.[43] For example, the food groups of vegetables and high-fat dairy products were strongly associated with the 'Vegetable, oil and fish' dietary pattern. Yet, higher intake of fruits and vegetables has been associated with lower GWG,[44] whereas dairy products were associated with higher GWG.[9,10] Consequently, this may result in an

overall null effect of the dietary pattern. Furthermore, imputing the missing covariate values in the Bayesian framework allowed us to use all available information in the imputation. Especially in settings with a longitudinal outcome, imputation methods that are available in standard software and, hence, are more commonly used, often fail to appropriately include the outcome into the imputation procedure which may lead to severely biased results.[31] Other strengths of our study are its population-based design, the collection of numerous covariates, and that the population was restricted to women of Dutch ancestry. We excluded women with other ethnicities to minimize measurement error, since the FFQ was designed to evaluate a Dutch diet. However, this restriction may have reduced the generalizability of our results to other ethnicities.

Our study also has some limitations. First, maternal weight before pregnancy as well as maximum weight were obtained using questionnaires, which may have resulted in a larger measurement error. Although we found no indication of systematic measurement error, random error may have resulted in loss of precision in GWG assessment. Furthermore, we were not able to calculate GWG per trimester because we did not have weight measurements at the required time points and the available data was insufficient for imputing those values. Another limitation is the lack of information on the separate components of GWG, in particular maternal fat mass, and the lack of information on postpartum maternal weight. Future studies should collect detailed information on maternal body composition during pregnancy or measure the participants' weight a few weeks postpartum to evaluate associations with the different components of GWG. Also, we could not use information on absolute dietary intake because dietary information collected using an FFQ does not provide this information. However, FFQs have been shown to be accurate in ranking participants according to their dietary intake.[45] Furthermore, we assessed maternal diet only once during pregnancy and were therefore not able to account for changes in dietary intake. Nevertheless, dietary patterns and macronutrient composition may not change largely during pregnancy despite an increased energy intake.[46,47] Additionally, we found that our results did not change after excluding women who may have altered their dietary intake due to illness or vomiting. Finally, the numerous statistical analyses performed may have resulted in chance findings (*type I error*). However, our results for weight trajectories and early-pregnancy GWG remained statistically significant when a more stringent alpha-level was used (alpha-level 0.05/4 = 0.0125).

Conclusions and implications

In conclusion, our results suggest that dietary composition during pregnancy may play a role in GWG in early pregnancy but has limited influence on total GWG in a population of Dutch women. The strength of the associations between dietary patterns and GWG differs for different definitions of dietary patterns and GWG. This suggests that the relationship between dietary patterns and GWG may be complex and may need further elucidation in order to facilitate the development of dietary guidelines during pregnancy and to adequately advise pregnant women on their diet.

Supplementary Material can be found online: http://hdl.handle.net/1765/80025

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Chapter 2.3

Dietary acid load and blood pressure development in pregnancy: The Generation R Study

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Abstract

Background: Dietary intake could induce a mild maternal metabolic acidosis that might lead to a higher level of blood pressure.

Objective: To evaluate the association between maternal dietary acid load and changes in blood pressure during pregnancy, pregnancy-induced hypertension and pre-eclampsia.

Methods: We included 3411 pregnant women of Dutch ancestry from a prospective population-based cohort in Rotterdam (the Netherlands). Dietary data was self-reported in early pregnancy via a food-frequency questionnaire. Four dietary acid load measurements were calculated: dietary potential renal acid load (dPRAL), net endogenous acid production (NEAP), animal protein/potassium ratio, and vegetable protein/potassium ratio. Diastolic blood pressure (DBP) and systolic blood pressure (SBP) were measured 3 times during pregnancy. Information on pregnancy-induced hypertension and pre-eclampsia was obtained from medical records. Linear mixed models and logistic regression were used and adjusted for sociodemographic and lifestyle factors.

Results: dPRAL, NEAP and animal protein/potassium ratio were not associated with DBP or SBP in pregnancy. One standard deviation higher vegetable protein/potassium ratio was associated with lower DBP (-0.30 mmHg [95%CI -0.54; -0.06]) but not with SBP (-0.29 mmHg [95% CI -0.60; 0.01]). This association did not greatly alter when the model was additionally adjusted for diet quality. Dietary acid load measurement was neither associated with the prevalence of pregnancy-induced hypertension nor with pre-eclampsia.

Conclusions: Dietary acid load was not associated with changes in DBP or SBP during pregnancy, although women with a higher vegetable protein/potassium ratio had a slightly lower DBP. Dietary acid load was not associated with pregnancy-induced hypertension or pre-eclampsia.

Introduction

Hypertensive disorders during pregnancy occur in 2-8% of all pregnancies and have been associated with adverse perinatal outcomes, such as preterm birth and foetal growth restriction, and with the development of cardiovascular disease of the mother later in life.[1]

Studies have suggested that the development of hypertension may be influenced by the acid-base balance in the body.[2] This acid-base homeostasis needs to remain within a small range, and is therefore controlled by the lungs on the short term by altering the rate of CO2 excretion.[3] On a longer term, the kidney's contribute to a stable acid-base homeostasis by eliminating abundant acids.[3] Additionally, dietary intake can influence this acid-base homeostasis.[3] Whereas a higher intake of sulphur-containing amino acids and phosphorus will increase a diet-dependent acid load, a higher intake of potassium, magnesium and calcium will increase a diet-dependent base load.[2] Foods with a high diet-dependent acid load such as meat, fish, cheese, grain products and rice will increase dietary acid load, and foods with a high diet-dependent base load such as fruits, vegetables and potatoes will reduce the dietary acid load.[2] A typical Western diet contains predominantly foods with high diet-dependent acid loads, which has been suggested to induce a mild metabolic acidosis.[4] Observational studies in non-pregnant populations have suggested that a higher dietary acid load (e.g., a high diet-dependent acid load) may be associated with hypertension,[5,6] although another study found no association.[7] Studies have also indicated that the severity of metabolic acidosis may increase independent of diet, due to a decline in kidney function.[8] Currently, it is unknown whether dietary acid load may be associated with blood pressure increase in pregnancy. During pregnancy, physiological changes occur in the acid-base homeostasis due to changes in the respiratory system resulting in lower arterial CO^2 tension and higher oxygen tension and to changes in the renal system, resulting in a reduction in plasma bicarbonate amongst others.

To our knowledge, there are no studies that have evaluated the influence of dietary acid load on blood pressure or hypertensive complications in pregnancy. Yet, individual food groups or food components that contribute to dietary acid load have been studied in relation to pre-eclampsia.[10-13] For example, calcium supplementation, which is a contributor of diet-dependent base load, has been shown to reduce the risk to develop pre-eclampsia.[10] Considering this, we evaluated whether maternal dietary acid load during the first trimester of pregnancy is associated with higher systolic and diastolic blood pressures during pregnancy. We also evaluated whether maternal dietary acid load is associated with pregnancy-induced hypertension and pre-eclampsia.

Methods

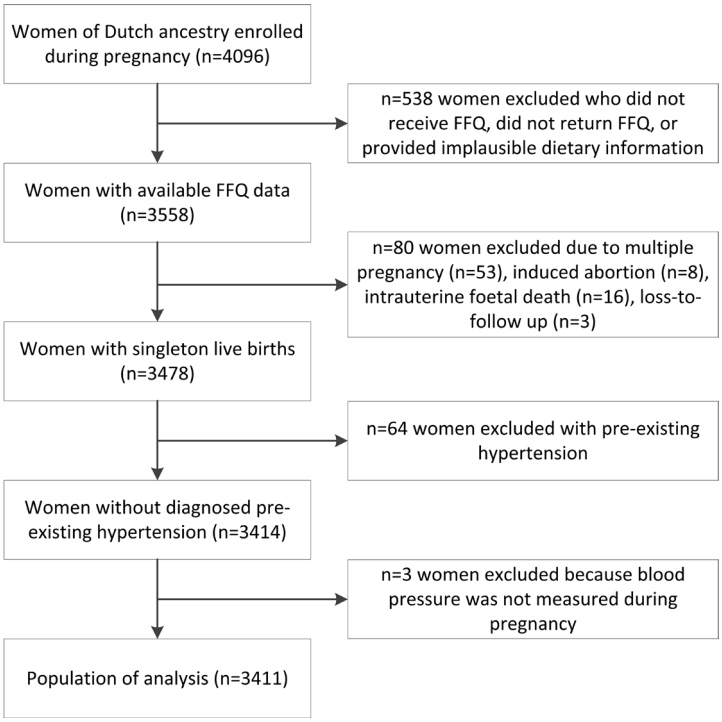
Study design

The project is embedded in the Generation R Study, an ongoing multi-ethnic prospective birth cohort. Pregnant women who lived in the area of the city Rotterdam (the Netherlands) and who had an expected delivery date between April 2002 and January 2006 were approached to participate. Details of the Generation R Study have been described in detail previously.[14,15] Written informed consent was obtained from all women. The study was approved by the Medical Ethics Committee of the Erasmus Medical Center Rotterdam (the Netherlands) and was in accordance with the World Medical Association Declaration of Helsinki.

Population of analysis

During pregnancy, 4096 women of Dutch ancestry enrolled into the Generation R Study and were therefore eligible for this analysis. We restricted our population of analysis to women with valid dietary information and who gave birth to a live singleton new born (n=3478). Women with pre-existing hypertension (n=64) were excluded as well as women (n=3) who did not have their blood pressure measured, leaving 3411 women for the study at hand **(Figure 2.3.1).**

Figure 2.3.1. Selection process study population: the Generation R Study (2002 – 2006)



Abbreviation: FFQ, food-frequency questionnaire.

Dietary acid load

Dietary intake was assessed using a 290-item semi-quantitative food-frequency questionnaire (FFQ) which covered the average dietary intake over the previous three months. Women received the FFQ at enrolment at a median gestational age of 13.4 (interquartile range (IQR): 12.2-15.5) weeks. The FFQ included questions on foods that are frequently consumed in a Dutch diet, their portion size and consumption frequency as well as the methods of preparation, and additions to the foods. To calculate individual macronutrient and micronutrient intake from the FFQ, we used the Dutch food composition database from 2006.[16] The FFQ was originally developed to assess the dietary intake in an elderly Dutch population.[17] A validation study was carried out in 80 elderly comparing the FFQ with fifteen 24h food records collected over a 1-y period and 24h urinary urea secretion collected over 4 non-consecutive days.[17] This validation study showed Pearson correlation coefficients for the within-person variation of 0.66 for total protein, 0.59 for vegetable protein, 0.52 for potassium, 0.72 for calcium, 0.74 for phosphorus, and 0.71 for magnesium. The Spearman correlation coefficient between protein intake estimated from the FFQ and from urinary urea was 0.67.[17]

Dietary acid load was calculated using four different formulas. First, we estimated dietary acid load using the following formula: dPRAL (mEq/d) = 0.4888*protein(g/d) + 0.0366* – phosphorus(mg/d) – 0.0205*potassium(mg/d) – 0.0263*magnesium(mg/d) - 0.0125*calcium (mg/d).[7,18] The second formula was NEAP (mEq/d) = 54.5*protein(g/d) /potassium(mEq/d) – 10.2.[19] Third, we calculated the animal protein/potassium ratio as follows: animal protein/ potassium ratio = animal protein (g/d)/potassium (g/d).[20] Finally, we calculated the vegetable protein/potassium ratio by using the following formula: vegetable protein/potassium ratio = vegetable protein (g/d)/potassium (g/d).[20]

The dPRAL formula proposed by Remer[18] takes into account average intestinal absorption rates of acid precursors (protein and phosphorus) and of base precursors (potassium, magnesium and calcium). This formula has been previously validated against urine pH in healthy adults.[21] The NEAP and the animal protein/potassium ratio take into account only protein (as acid precursor) and potassium (as base precursor) for their estimation of dietary acid load. The NEAP formula has been validated previously in healthy adults using renal net acid excretion.[19] The animal protein/potassium ratio has been found to be a predictor for resorption of bone, an important reservoir of ions that can reduce excess acid loads.[20]

Blood pressure

Maternal blood pressure was measured using the validated Omron 907® automated digital oscillometric sphygmanometer (OMRON Healthcare Europe B.V. Hoofddorp, the Netherlands)[22] at the research centre that women visited at median gestational ages (IQR) of 12.9 (12.1-14.4), 20.4 (19.9-21.1), and 30.2 (29.9-30.8) weeks of gestation. During each visit, women were seated 5-10 minutes before the systolic blood pressure (SBP, mmHg) and diastolic blood pressure (DBP, mmHg) were measured twice over a 60 seconds interval.[23] The mean value was documented and used in the analysis. We collected 2831 (83%) blood pressure measurements during the first visit, 3299 (97%) measurements during the second

visit, and 3321 (97%) measurements during the third visit. The blood pressure was measured during all three visits in 2706 women (79.3%), 628 women (18.4%) had two measurements, and 77 (2.3%) women had their blood pressure measured once.

Pregnancy-induced hypertension and pre-eclampsia

Information of hypertensive complications during pregnancy was obtained from medical records. Medical records of women who were suspected to have any kind of hypertensive complication or foetal growth retardation were reviewed in detail to confirm the presence of pregnancy-induced hypertension or pre-eclampsia.[24] Pregnancy-induced hypertension was defined as the development of a SBP ≥140 mmHg and/or a DBP ≥90 mmHg without proteinuria after 20 weeks of gestation in previous normotensive women.[25] Pre-eclampsia was defined as the development of a SBP ≥140 mmHg and/or a DBP ≥90 mmHg and proteinuria (defined as ≥2 dipstick readings of ≥2+, one catheter sample reading ≥1+, or a 24-hour urine collection containing ≥300 mg of protein) after 20 weeks of gestation in previous normotensive women.[25] In addition, women diagnosed with haemolysis, elevated liver enzyme and low platelet (HELLP) syndrome[24] were included in the group with pre-eclampsia.

Covariates

At enrolment, information was collected on maternal age, educational level,[26] household income, pre-pregnancy weight, parity, and folic acid supplementation[27] using questionnaires. Information on energy intake, dietary intake of food groups, and the Dutch Healthy Diet index (e.g., adherence to the Dutch healthy dietary guidelines,[28] adapted for pregnancy[29]) was derived from the FFQ described previously.[17] During each trimester, women reported their smoking behaviour and alcohol consumption by postal questionnaires. Pre-existing maternal comorbidities (including diabetes, hypercholesterolemia, heart disease, thyroid disease and systemic lupus erythematosus) were self-reported in the questionnaires that were filled in at enrolment. At enrolment, maternal height was measured at the research centre and used to calculate a body mass index (BMI, kg/m2). In addition, a foetal ultrasound was performed to determine gestational age.[30] Gestational weight gain was calculated using maternal weight measured during the third visit (median 30.2 (IQR: 29.9-30.8) weeks of gestation) minus self-reported pre-pregnancy weight.

Statistical methods

First, we compared women that were included in the analysis (n=3411) with women not included in the analysis (n=685), using independent student t-tests or Mann Whitney U tests for continuous variables and Chi-square statistics for the categorical variables.

The dietary acid load formulas (e.g., dPRAL, NEAP, the animal protein/potassium ratio and the vegetable protein/potassium ratio) were adjusted for total energy using the nutrient residual method.[31] We used energy-adjusted dietary acid load to reduce systematic measurement error because measurement error of dietary components is strongly correlated with errors in the measurement of total energy intake.[31] After energy adjustment, we created standard deviation (SD) scores of the four dietary acid load measures (with a

mean of zero and a SD of one) to facilitate comparison between the measures.

Pearson correlation coefficients were calculated to evaluate the correlation between the four dietary acid load formulas. In addition, we performed variable selection using lasso regression[32] to identify which food groups were the main contributors to the dietary acid load measures. By posing an additional constraint on the estimation of a regression model, lasso tends to shrink some coefficients to exactly zero. We report the standardized regression coefficients of the food groups that remained in the models and the explained variance (R^2) of the final models.

To reduce bias due to missing covariates (0-18%), we imputed missing covariates using multiple imputation **(Supplementary Table 2.3.1)**.[33] Briefly, we created ten different datasets that differed only in the imputed values and performed the analysis in each of the sets. The derived effect estimates were pooled using Rubin's rules[34] to obtain the final results that we report here.

Our main analyses were performed both with crude models (i.e., no covariates in the model), as well as with multivariable models. The decision which covariates to include in the multivariable models was based on a ≥10% change of the fixed effects estimate in preliminary analysis of the dietary acid load measurements with change in SBP and DBP in linear mixed models. This resulted in including the following covariates for the multivariable model: age, educational level, household income, pre-pregnancy BMI, parity, alcohol consumption and smoking behaviour.

For the main analysis, we analysed the association between exposure (i.e., dietary acid load measures) and the trajectories of SBP and DBP during pregnancy using linear mixed models. Details on the modelling choices have been added to the **Supplementary Material 2.3.** The final model for DBP included a random intercept and natural cubic splines for gestational age with 2 *df*, the final model for SBP included a random intercept and random slope, and natural cubic splines for gestational age with 4 *df*. Both models contain linear effects of the dietary acid load measures, and no interaction terms. Additionally, we evaluated the association of the dietary acid load measures with pregnancy-induced hypertension and pre-eclampsia using logistic regression analysis. We tested for non-linearity of the association between the dietary acid load measures and pregnancy-induced hypertension and pre-eclampsia using natural cubic splines.[35]

As additional analyses, first, we further adjusted our multivariable model with the Dutch Health Diet-index, to evaluate the effect of the associations after taking into account overall quality of dietary intake. Second, we restricted our analysis to women without pre-existing comorbidities, because they may have altered their dietary intake following diagnosis (n=3290).

Results were considered statistically significant when the p-value was less than 0.05. The analyses were performed in R version 3.2.1 (2015-06-18, R Foundation for Statistical Computing, Vienna, Austria) using the R packages lme4[36] and splines for the repeated measurement analysis, and in SPSS version 21.0 (IBM Corp., Armonk, NY, USA).

Results

Participant characteristics

The characteristics of the 3411 participants are described in **Table 2.3.1.** The mean blood pressure at enrolment was 117/68 mmHg. In total, 232 women were diagnosed with a hypertensive complication during pregnancy, e.g., 173 women (5.3%) with pregnancy-induced hypertension and 59 women with pre-eclampsia (1.9%). A graphical display of the DBP and SBP development in our participants can be found in **Supplementary Figures 2.3.1 and 2.3.2.** The dietary acid load formulas dPRAL, NEAP and the animal protein/potassium ratio were highly correlated with Pearson correlation coefficients between 0.72 and 0.93 (p-value <0.001). The vegetable protein/potassium ratio was less correlated with the other formulas (Pearson correlation coefficients were 0.48 for dPRAL, 0.42 for NEAP and -0.16 for the animal protein/potassium ratio). Comparison of included women with women that were not included showed that women included in the analysis were higher educated, continued smoking less frequently, and had a slightly lower SBP and DPB than women not included in the analysis **(Supplementary Table 2.3.2)**.

Contributors to dietary acid load

The food groups that contributed to the dietary acid load algorithms have been listed in **Table 2.3.2.** The main contributors to higher values of dPRAL, NEAP and the animal protein potassium ratio were cheese, meat and meat products, and fish and shellfish whereas the main contributors to a higher vegetable protein/potassium ratio were nuts and seeds, and bread. Whereas higher intake of meat and meat products was associated with a lower vegetable protein/potassium ratio, meat intake increased dietary acid load using the other measurements.

Table 2.3.1. Participants characteristics (n=3411)[a]

		Original data	Imputed data[b]
Age (years)		31.4 ± 4.4	No missing values
Educational level, % (n)	Low and midlow	15.0 (505)	15.2 (519)
	Midhigh	50.8 (1712)	50.8 (1734)
	High	34.2 (1150)	34.0 (1158)
Household income, % (n)	< 2,200 Euros/month	25.2 (769)	27.2 (928)
	≥ 2,200 Euros/month	74.8 (2281)	72.8 (2483)
Parity, % (n)	0	59.9 (2038)	59.9 (2042)
	≥1	40.1 (1365)	40.1 (1369)
Pre-pregnancy BMI (kg/m^2)		22.2 (20.6 – 24.6)	22.4 (20.8 – 24.6)
Gestational weight gain (kg)		10.8 ± 4.4	11.0 ± 5.9
Smoking, % (n)	Never during pregnancy	74.2 (2346)	74.4 (2539)
	Until pregnancy was known	8.9 (280)	8.9 (302)
	Continued throughout pregnancy	17.0 (536)	16.7 (570)
Alcohol consumption, % (n)	Never during pregnancy	33.8 (1059)	33.6 (1145)

Table 2.3.1. (continued) Participants characteristics (n=3411)[a]

		Original data	Imputed data[b]
	Until pregnancy was known	16.2 (509)	16.2 (552)
	Continued throughout pregnancy	50.0 (1569)	50.2 (1714)
Folic acid suppl., % (n)	Started periconceptional	56.1 (1569)	55.2 (1882)
	Started first 10 weeks	33.0 (923)	33.5 (1143)
	No supplementation	10.9 (304)	11.3 (386)
Gestational age at enrolment (weeks)		13.5 (12.4 – 16.1)	No missing values
Diastolic blood pressure at enrolment (mmHg)		68 ± 9	Not imputed
Systolic blood pressure at enrolment (mmHg)		117 ± 12	Not imputed
Maternal diet	Energy intake (kcal/day)	2147 ± 511	No missing values
	Animal protein (E%)	9.2 ±2.4	No missing values
	Vegetable protein (E%)	5.8 ± 1.1	No missing values
	Dietary potential acid load (mEq/d)	0.8 ± 11.2	No missing values
	Net endogenous acid production	39.2 ± 7.7	No missing values
	Animal protein/ potassium ratio	14.3 ± 3.3	No missing values
	Vegetable protein / potassium ratio	9.1 ± 2.1	No missing values
	Dutch Healthy diet-index (max score: 60)	31.6 ± 7.8	No missing values
Hypertensive complications, % (n)	Pre-eclampsia	59 (1.9)	Not imputed
	Pregnancy-induced hypertension	173 (5.3)	Not imputed

[a.] Values represent n (%), mean ± SD, median (interquartile range). [b.] Number of participants may not add up to total number of subjects due to rounding of imputation. Percentage of missing variables: educational level (1.3%), household income (10.6%), parity (0.2%), pre-pregnancy body mass index (13.7%), gestational weight gain (16.0%), smoking (7.3%), alcohol consumption (8.0%), folic acid supplementation (18.0%), systolic blood pressure at enrolment (0.8%), diastolic blood pressure at enrolment (0.8%), pre-eclampsia (7.4%), and pregnancy-induced hypertension (4.1%). Abbreviations: suppl., supplementation

Table 2.3.2. Dietary components in relation to the four dietary acid load measurements

	Dietary acid load			
Food group	**dPRAL**	**NEAP**	**AP/P ratio**	**VP/P ratio**
Potatoes and other tubers	-0.0044	-0.0058	-0.0051	-0.0019
Vegetables	-0.0034	-0.0033	-0.0031	-0.0014
Legumes			-0.0027	0.0033
Fruits	-0.0025	-0.0025	-0.0019	-0.0013
Nuts and seeds			-0.0096	0.0194
Pasta, rice and other grains	0.0008	0.0003		0.0025
Bread	0.0037	0.0016	-0.0026	0.0077
Breakfast cereals			-0.0028	0.0049
Meat and meat products	0.0063	0.0092	0.0139	-0.0046
Fish and shellfish	0.0048	0.0060	0.0111	-0.0039
Dairy other than cheese	-0.0002	-0.0001	0.0009	-0.0021
Cheese	0.0163	0.0191	0.0214	-0.0007

Table 2.3.2.(continued) Dietary components in relation to the four dietary acid load measurements

	Dietary acid load			
Food group	**dPRAL**	**NEAP**	**AP/P ratio**	**VP/P ratio**
Eggs and egg products			0.0042	-0.0021
Vegetable oils				-0.0054
Butter and margarine				
Soy products			-0.0018	0.0069
Explained variance (%)	**54**	**58**	**71**	**78**

The values are standardized coefficients of the lasso regression models. A standardized value that deviates more from the zero indicates that a food group contributes more to that dietary acid load measurement. Abbreviations: dPRAL, dietary potential renal acid load; NEAP, net endogenous acid production; AP/P ratio, animal protein/potassium ratio; VP/P ratio, vegetable protein/potassium ratio.

Table 2.3.3. Pooled results of dietary acid load with systolic blood pressure and with diastolic blood pressure (n=3411)[a]

	Diastolic blood pressure	Systolic blood pressure
Dietary acid load	Difference in mmHg (95% CI)	Difference in mmHg (95% CI)
dPRAL		
Crude (per SD)	-0.08 (-0.34; 0.18)	0.00 (-0.32; 0.33)
Adjusted (per SD)	-0.11 (-0.35; 0.13)	0.06 (-0.24; 0.36)
NEAP		
Crude (per SD)	-0.12 (-0.38; 0.14)	0.02 (-0.31; 0.34)
Adjusted (per SD)	-0.17 (-0.41; 0.07)	0.02 (-0.28; 0.32)
Animal protein/potassium ratio		
Crude (per SD)	0.19 (-0.07; 0.45)	**0.45 (0.13; 0.78)***
Adjusted (per SD)	-0.01 (-0.24; 0.23)	0.20 (-0.10; 0.50)
Vegetable protein/potassium ratio		
Crude (per SD)	**-0.50 (-0.76; -0.24)***	**-0.71 (-1.03; -0.38)***
Adjusted (per SD)	**-0.30 (-0.54; -0.06)***	-0.29 (-0.60; 0.01)

[a.] Results (coefficients with 95% confidence interval (CI)) from linear mixed model analysis. The coefficient reflects the difference in systolic blood pressure (mmHg) or diastolic blood pressure (mmHg) per one standard deviation (SD) increase in dietary acid load. The crude models include the natural cubic splines for gestational age (2 *df* for DBP and 4 *df* for SBP), a random intercept for DBP and a random intercept and slope for SBP. For the adjusted model we further adjusted for maternal age, pre-pregnancy BMI, parity, educational level, household income, alcohol consumption during pregnancy and smoking behaviour during pregnancy. Significant associations (p-value <0.05) are presented with an asterisk (*). Abbreviations: DBP, diastolic blood pressure; dPRAL, dietary potential renal acid load; NEAP, net endogenous acid production; SBP, systolic blood pressure.

Dietary acid load and blood pressure

Dietary acid load in early pregnancy, measured using dPRAL, was not associated with SBP and DPB during pregnancy, neither in crude analysis, nor after adjustment for sociodemographic

and lifestyle factors **(Table 2.3.3)**. Similarly, NEAP and the animal protein/potassium ratio were also not associated with increases of SBP and DBP during pregnancy. In contrast, a one SD higher vegetable protein/potassium ratio was associated with a 0.50 mmHg lower DBP (95% CI -0.76; -0.24) and with a 0.71 mmHg lower SBP (95% CI -1.03; -0.38) in the crude analysis. After adjustment for sociodemographic and lifestyle factors, the vegetable protein/potassium ratio remained only associated with lower DBP (-0.30 mmHg (95% CI -0.54; -0.06 per SD) but not with SBP (-0.29 mmHg (95% CI -0.60; 0.01) per SD) (**Table 2.3.3**).

Dietary acid load and hypertensive complications in pregnancy

Dietary acid load measurements in early pregnancy were not associated with the development of pregnancy-inducted hypertension or with pre-eclampsia (**Table 2.3.4**). Yet, a higher animal protein/potassium ratio resulted in higher odds to develop pregnancy-induced hypertension (OR 1.12 (95%CI 0.96; 1.30) per SD) and pre-eclampsia (OR 1.18 (95%CI 0.92; 1.53)) in multivariable models. Higher vegetable protein/potassium ratio was not associated with pre-eclampsia (OR 0.86 (95%CI 0.66; 1.13)).

Additional analysis

Further adjustment of the multivariable model for diet quality (the Dutch Healthy Diet-index), did not greatly alter the associations between dietary acid load and DBP or SBP **(Supplementary Table 2.3.3)**. Excluding women with pre-existing comorbidities did also not change the results **(Supplementary Table 2.3.4)**.

Table 2.3.4. The association between dietary acid load and hypertensive complications in pregnancy[a]

		Pregnancy-induced hypertension (173 cases)	**Pre-eclampsia** (59 cases)
Dietary acid load		OR (95% CI)	OR (95% CI)
dPRAL	Crude (per SD)	1.07 (0.92; 1.24)	1.00 (0.78; 1.30)
	Adjusted (per SD)	1.08 (0.93; 1.27)	1.06 (0.81; 1.37)
NEAP	Crude (per SD)	1.05 (0.90; 1.22)	1.03 (0.80; 1.33)
	Adjusted (per SD)	1.06 (0.91; 1.23)	1.08 (0.84; 1.39)
Animal protein/ potassium ratio	Crude (per SD)	1.14 (0.98; 1.33)	1.15 (0.89; 1.48)
	Adjusted (per SD)	1.12 (0.96; 1.30)	1.18 (0.92; 1.53)
Vegetable protein/ potassium ratio	Crude (per SD)	0.87 (0.74; 1.02)	0.83 (0.63; 1.08)
	Adjusted (per SD)	0.92 (0.78; 1.08)	0.86 (0.66; 1.13)

[a.] Results (odds ratio (OR) with 95% confidence interval (CI)) from multivariable logistic regression. The odds ratio reflects the odds of pregnancy-induced hypertension or pre-eclampsia per 1 standard deviation (SD) increase in dietary acid load. For the adjusted model we added to our model: maternal age, pre-pregnancy BMI, parity, educational level, household income, alcohol consumption during pregnancy and smoking behaviour during pregnancy. Significant associations (p-value <0.05) are presented with an asterisk (*). Abbreviations: dPRAL, dietary potential renal acid load; NEAP, net endogenous acid production.

Discussion

The results of this observational study do not suggest that higher dietary acid load is neither associated with higher blood pressure during pregnancy, nor with pregnancy-induced hypertension or with pre-eclampsia. Higher vegetable protein/potassium ratio was associated with lower DBP during pregnancy in our study, but not with SBP, and this association was not fully explained by sociodemographic or lifestyle factors or by overall dietary quality.

This is one of the first studies to evaluate the association between dietary acid load and blood pressure development during pregnancy. In non-pregnant populations results have been inconsistent. Whereas some studies in middle-aged and elderly have suggested that a higher dietary acid load increases the risk to develop hypertension,[5,6] another study did not confirm these results.[7] Additionally, a cohort in children reported that higher dPRAL was associated with higher SBP, but was not associated with DBP.[37]

In our study, higher vegetable protein/potassium ratio was consistently associated with a lower DBP, but not lower SBP, during pregnancy in contrast to our null findings for dPRAL, NEAP and the animal protein/potassium ratio. Moreover, the effect estimates of the animal protein/potassium ratio and the vegetable protein/potassium ratio were in opposite directions. Both formulas have the same denominator, namely potassium. Potassium supplementation has been found to lower blood pressure.[38] Increased serum potassium levels lead to vasodilation and endothelium-dependent relaxation, which may result in a lower blood pressure.[39] With the same denominator, the difference between the two dietary acid load formulas is the numerator; animal protein vs. vegetable protein. Protein intake contributes to a higher dietary acid load because sulfuric acid is produced during protein metabolism.[2] However, not all proteins contain the same amount of sulphur, more specifically the sulphur content is more variable in vegetable protein than in animal protein.[40] In addition, protein can also have a base potential, for example glutamine regulates renal ammonia metabolism which corrects acid-base balance in case of increased acid load.[41] Consequently, the amino acid composition influences to which extent protein influences the dietary acid load. The different findings between the animal protein/potassium ratio and vegetable protein/potassium ratio may also be explained by a healthier diet and lifestyle in women with higher scores on the vegetable protein/potassium ratio, because a healthy diet contains relatively more vegetable protein than animal protein. However, after taking into account sociodemographic, lifestyle and dietary quality, the vegetable protein/potassium ratio remained beneficial for lower DPB, although the association did not remain for SBP.

Several mechanisms have been suggested in which metabolic acidosis may increase blood pressure. For example, acid-base status is an important regulator of urinary citrate excretion. Metabolic acidosis reduces the pH in proximal tubule cells of the kidneys. Accordingly, the reabsorption of citrate in the kidney tubules increases and thus less citrate will be excreted.[2] The reabsorption may influence blood pressure because citrate levels have been shown to alter salt sensitivity and may thus influence blood pressure in salt-sensitive subjects.[42] Another mechanism in which metabolic acidosis may influence blood pressure is via an elevated serum anion gap. Observational studies have reported that participants with

a higher anion gap have a higher blood pressure, nevertheless the exact underlying pathway has not been unraveled.[43]

Although the association between dietary acid load and hypertensive complications has not been evaluated previously, individual components of the dietary acid load have been studied in relation to hypertensive complications in pregnancy as well as food groups that contribute to lowering of the dietary acid load. For example, calcium supplementation has been found to reduce the risk of pre-eclampsia with 64% in women with a low calcium intake,[10] whereas no reduction of pre-eclampsia has been found with magnesium supplementation.[11] In addition, there is no evidence that a balanced protein/energy supplementation with protein is related to the risk of pre-eclampsia.[12] Yet, results from a case-control study indicated that high intake of fruits and vegetables, and of fibre was associated with a lower risk to develop pre-eclampsia.[13] Considering that associations were found with individual components that lower dietary acid load, it is puzzling why we did not find an association between dietary acid load and hypertensive complications in pregnancy in our cohort. This could have been the case because of several reasons, namely: first, due to increased glomerular filtration rate and changes in renal reabsorption during pregnancy, dietary acid load may not be an optimal reflection of the dietary acid-base balance during pregnancy. Second, maybe only some components of dietary acid load, in particular of dPRAL and NEAP, are associated with hypertensive complications, but due to the combined score these effects have been diluted. Third, lack of precision of dietary acid load assessment might have resulted in non-differential misclassification and consequently dilution of the effect estimates. Lastly, it might be that we did not have enough power to identify an association between dietary acid load and hypertensive complications, because only 5.3% of the women in our population were diagnosed with pregnancy-induced hypertension, and 1.9% with pre-eclampsia.

Strengths of this study include the availability of repeated measurements of blood pressure and the use of linear mixed models to model them, since mixed models not only allow for different numbers of measurements, taken at different time points for different individuals, but also take into account correlations between the repeated blood pressure measurements within each individual. Other strengths are its prospective population-based study design, the large sample size, and the collection of a broad range of potential confounders. We restricted this analysis to women of Dutch ancestry, because the FFQ was designed to evaluate a Dutch diet. This may have reduced the generalizability of our findings; nevertheless it may have increased the validity and precision of the exposure measurements.

Our study has also some limitations that we should address. First, we estimated the dietary acid load measurements using information derived from an FFQ, which is known to be a rather imprecise measurement tool.[44] However, dietary intake estimated from an FFQ has been shown to be accurate in ranking participants according to their dietary intake.[44] In addition, we used energy-adjusted values of dietary acid load, a method that has been shown to reduce the magnitude of measurement error.[31] Another limitation may be that the dietary acid load measurements were designed in non-pregnant populations and have not yet been validated in pregnant women. Another limitation is that we did not collect information on

kidney function during pregnancy. This information may be important because suboptimal functioning of the kidneys may decrease the ability to excrete excess acids via the kidneys, resulting in a less stable acid-base balance. Consequently, the association between dietary acid load and blood pressure may be stronger in women with suboptimal kidney function.

In conclusion, we found that dietary acid load in early pregnancy was not consistently associated with neither blood pressure, nor with pregnancy-induced hypertension or pre-eclampsia in a population of Dutch pregnant women. Our results do not suggest any specific recommendation for nutritional guidelines in pregnancy. To what extent diet-dependent acid load may have a function in acid-base balance in pregnancy requires further study.

Supplementary Material can be found online: http://hdl.handle.net/1765/80025

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Chapter 3

Maternal biomarkers & Pregnancy and birth outcomes

Chapter 3.1

Maternal fish consumption, fatty acid levels and angiogenic factors: The Generation R Study

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Abstract

Introduction: Angiogenic factors, such as placental growth factor (PlGF) and soluble Flt-1 (sFlt-1), are key regulators of placental vascular development. Evidence from in vitro studies indicates that fatty acids can affect angiogenesis. We investigated the associations of maternal fish consumption and fatty acids levels with angiogenic factors during pregnancy and in cord blood in a large population-based prospective cohort.

Methods: First trimester fish consumption was assessed among 3134 pregnant women using a food-frequency questionnaire. Plasma fatty acid levels were measured in second trimester. Plasma PlGF and sFlt-1 were measured in first and second trimester and in cord blood. Associations of fish consumption or fatty acid levels with angiogenic factors were assessed by multivariable linear regression analyses.

Results: There were no consistent associations of total fish or lean fish consumption with levels of PlGF, sFlt-1, or sFlt-1/PlGF ratio. Neither fatty fish nor shellfish were associated with angiogenic factors. Plasma n-3 polyunsaturated fatty acids, which are the main type of fatty acids in fish, were inconsistently associated with angiogenic factors in second trimester and cord blood. Yet, higher levels of arachidonic acid, an n-6 polyunsaturated fatty acid, were associated with lower levels of PlGF and sFlt-1.

Discussion: We found no consistent associations of fish consumption or fatty acids levels with angiogenic factors in a population with low fish consumption. Studies including populations with higher fish consumption are required to fully grasp the potential effects of maternal fish consumption on placental angiogenesis.

Introduction

Important regulators of placental vascular development are angiogenic factors.[1] Well-known proangiogenic proteins are Vascular Endothelial Growth Factor (VEGF) and Placental Growth Factor (PlGF). These proangiogenic factors might promote placental development by binding to its transmembrane receptor Flt-1, thereby inducing gene expression to promote proliferation and migration of trophoblasts.[2,3] A soluble variant of Flt-1, sFlt-1, binds to circulating proangiogenic factors with high affinity, thus diminishing PlGF and VEGF availability for the transmembrane Flt-1 receptor and inhibiting its proangiogenic signal.[4,5] Hence, sFlt-1 has an antiangiogenic effect. An imbalance between the levels of proangiogenic and antiangiogenic factors has been associated with pregnancy complications such as preeclampsia and intra-uterine growth restriction.[5-7] Additionally, plasma levels of PlGF and sFlt-1 have also been associated with offspring growth in our cohort.[8] As a measure of anti-angiogenesis the sFlt-1/PlGF ratio could be used.[9]

Multiple environmental factors are known to affect placental angiogenesis, including smoking, diabetes and maternal diet.[10-12] Maternal diet is also known to influence blood pressure in pregnancy and foetal outcomes as has been suggested in cohort and case-control studies.[13-15] Among other nutritional exposures, higher fish consumption before and during pregnancy has been associated with higher birth weight and lower risk of foetal growth retardation in two European observational studies,[16,17] although this association was not found in our cohort.[18] Potential effects of fish may be mediated through polyunsaturated fatty acids (PUFAs), in particular omega-3 (n-3) PUFAs. n-3 PUFA supplementation during pregnancy has been shown to increase pregnancy duration, head circumference and birth weight.[19,20] Evidence from in vitro studies indicates that some PUFAs stimulated angiogenesis in a cell line of first trimester human trophoblasts potentially by increasing the expression of angiogenic factors.[21,22]

So far, no studies have evaluated the association between maternal dietary intake of foods high in PUFAs, such as fish, and human placental angiogenesis. Therefore, we investigated the association of maternal fish consumption with the angiogenic factors PlGF and sFlt-1 measured in the first and second trimester, and in cord blood. In addition, we evaluated the association of maternal plasma fatty acids levels in second trimester with PlGF and sFlt-1 levels in the second trimester and in cord blood in a large population-based birth cohort.

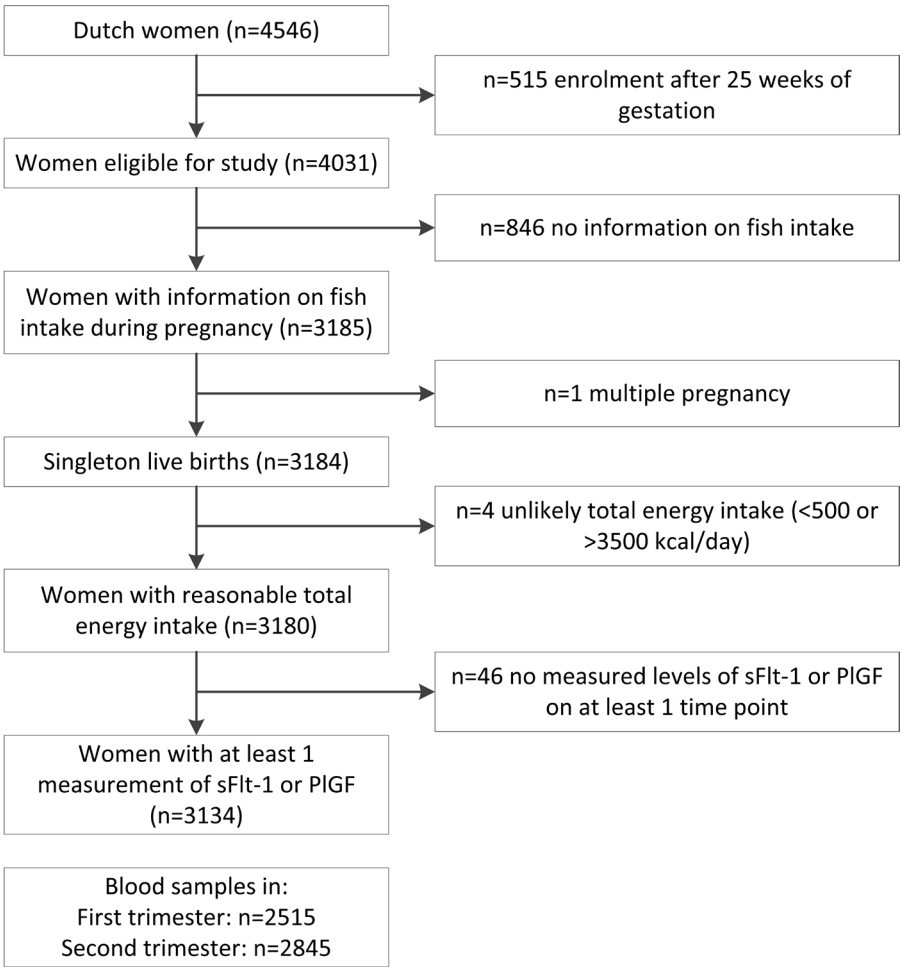
Methods

Study design and population

The present study was embedded in the Generation R Study, a population-based prospective cohort study from foetal life onwards in the city of Rotterdam, the Netherlands. Details of the study have been described before.[23] All participants provided written informed consent. The study was carried out in accordance with the World Medical Association Declaration of Helsinki and approved by the medical ethics committee of the Erasmus University Medical Center, Rotterdam, the Netherlands.

For this particular study, we included only Dutch women, because the food-frequency questionnaire (FFQ) was designed to evaluate a Dutch diet. Of the total of 4546 participating Dutch women, we excluded those enrolled after the 25th week of gestation (n=515), with missing information on fish consumption (n=846), multiple pregnancies (n=1), unlikely total daily energy intake (n=4) and those in whom no data on any of the angiogenic factors at any time point were available (n=46), leaving 3134 women in our analyses (**Figure 3.1.1**).

Figure 3.1.1. Flowchart of participants included for analyses



Fish intake

Maternal dietary intake, including fish consumption, was evaluated at enrolment (median 13.2 (95% range 9.9–21.6) weeks of gestation) using a self-administered semi-quantitative FFQ adapted from the validated FFQ of Klipstein-Grobusch et al.[24] This FFQ measured food

intake over the prior three months, and covered dietary intake during the first trimester of pregnancy. The FFQ considered consumption frequency, portion sizes,[25] preparation method and additions. To calculate average daily nutritional values, the 2006 version of the Dutch food composition table was used.[26]

Fish consumption (in grams per week) was assessed for total fish intake and for specific types of fish.[18] Fish consumption was grouped based on the nutrient content into: lean fish (codfish, plaice, catfish, sole fish, tuna, whiting and haddock), fatty fish (salmon, herring, mackerel, eel, sardines, halibut, bloater, trout, anchovy and gurnard) and shellfish (crab, lobster, shrimps and mussels). Processed fish, roe and fish derived from liver were not used in this analysis. Total fish includes all the types of fish consumed.

Blood sampling

Peripheral venous blood samples were drawn during the visits to the research centre in the first (median 12.9 weeks (95% range 9.8–17.3)) and second trimester (median 20.4 weeks (95% range 18.6–22.9)). Umbilical venous cord blood samples were obtained by midwives and obstetricians immediately after delivery (median gestational age at delivery 40.3 weeks (95% range 36.0–42.3)). All blood samples were transported to the regional laboratory, centrifuged and the plasma distributed into 250µL aliquots and stored at –80°C.[27]

Fatty acid levels

The analysis of plasma glycerophospholipid fatty acid (FA) composition was performed on plasma samples derived during second trimester of pregnancy in the Division of Metabolic Diseases and Nutritional Medicine, Dr. von Hauner Children's Hospital, Ludwig-Maximilians-University of Munich following the high-throughput method described by Glaser et al.[28] The average coefficient of variation was 15.7%. Plasma FA concentration reflects only current nutritional status.

Plasma PUFA levels are a result of both dietary intake and metabolism. Plasma n-3 and n-6 PUFAs may compete in these metabolic processes because the same rate-limiting enzymes are needed.[29] Therefore, we did not only take into account n-3 PUFAs but also evaluated the associations with n-6 PUFAs which are derived from other dietary sources than fish.

For the analyses, we used relative concentrations of the individual FAs as weight percentage of the total sum of FAs (%) instead of absolute concentrations (mg/L), because fatty acid levels are not only a reflection of dietary intake but also of metabolism, the complex interplay between individual fatty acids, and multiple additional factors.[30] The analysed n-3 PUFAs were docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and alpha-linolenic acid (ALA). The analysed n-6 PUFAs were linoleic acid (LA) and arachidonic acid (AA). Also n-3/n-6, EPA/AA and DHA/AA ratios were evaluated. The n-3/n-6 ratio was calculated by the sum of five n-3 PUFAs (ALA, eicosatreionic acid, EPA, docasapentaenoic acid and DHA) divided by the sum of the n-6 PUFAs matching the carbon chain length of the n-3 PUFAs (LA, dihomo-gamma-linolenic acid, AA, adrenic acid and osbond acid). The EPA/AA and DHA/AA ratios have been used as cardiovascular risk biomarkers.[31]

Angiogenic factors

Levels of sFlt-1 and PlGF were measured in first and second trimester plasma and cord blood plasma at the Department of Clinical Chemistry of the Erasmus University Medical Center using a two-step chemi-luminescent microparticle immunoassay (CMIA) on the Architect System (Abbot Diagnostics B.V., Hoofddorp, the Netherlands). Cord blood plasma, originating from the foetal circulation, was evaluated in addition to maternal plasma because studies have suggested a discordant release of sFlt-1 into both circulations.[32] Plasma levels of sFlt-1 and PlGF were evaluated individually and additionally combined within the sFlt-1/PlGF ratio, which can be used as a measure of antiangiogenesis.[9] The between-run coefficients of variation for plasma sFlt-1 were 2.8% at 5.5 ng/mL and 2.3% at 34.0 ng/mL; and 4.7% at 24 pg/mL, and 3.8% at 113 pg/mL for plasma PlGF. The highest detected level was 150 ng/mL for sFlt-1 and 1500 pg/mL for PlGF.7

Covariates

Information on maternal age, folic acid supplementation, parity and educational level was obtained from questionnaires completed by the mothers at enrolment. Information on smoking and alcohol consumption was obtained from questionnaires in each trimester. As season of conception might influence maternal fish consumption we back-calculated the month of conception from the gestational age and then categorized it into seasons: winter (December to February), spring (March to May), summer (June to August) and fall (September to November).

Maternal height and weight were measured at enrolment to calculate body mass index (BMI; kg/m^2) (correlation coefficient with pre-pregnancy BMI 0.96). Gestational age at delivery, birth weight, foetal sex and pregnancy complications (pregnancy-induced hypertension and preeclampsia) were obtained from medical records. The occurrence of pregnancy complications was cross-validated using medical record review.[7]

Statistical analysis

We adjusted fish consumption for total energy intake to reduce measurement error, to control for confounding, and to evaluate the effect of fish consumption independent of energy intake using the nutrient residual method.[33] Energy-adjusted fish consumption was derived by first regressing fish consumption on total energy intake, saving the residuals from this regression and then adding the median fish consumption to these residuals. This gave us an energy-adjusted fish intake for all women.[33]

Subsequently, to reduce the influence of outlying data points, fish consumption was divided in categories. The energy-adjusted total fish consumption was grouped into five categories (0; 1–69; 70–139; 140–209; and >210 g per week), lean fish and fatty fish consumption into four categories each (0; 1–34; 35–69; and >70 g per week), and shellfish consumption into three categories (0; 1–13; >14 g per week) as previously described.[18] The category of “no fish consumption” was considered the reference category. The plasma levels of sFlt-1 and PlGF as well as the sFlt-1/PlGF ratio were natural logarithm (ln)-transformed to obtain a normal distribution.

We studied the association of maternal fish consumption with levels of angiogenic

factors and the association of plasma FA levels with angiogenic factors using multivariable linear regression models.

Each of the following variables was evaluated as potential confounders in univariable models: age, parity, BMI, smoking, educational level, foetal sex, season of conception and energy intake. All these variables yielded a change of at least 5% in the effect estimate and were therefore included in the adjusted models. Tests for trend were done by including the fish consumption categories as a continuous variable in the linear regression models. A sensitivity analysis was performed in women without known diabetes mellitus or gestational diabetes (n=2771) because these women may have received dietary advice that altered their dietary intake and additionally levels of angiogenic factors may differ.[34]

Missing values for the covariates included in the regression models (0.4% for BMI, 0.2% for parity, 0.6% for educational level and 7% for smoking) were imputed using multiple imputation.[35] Five datasets were created and analysed independently and then the results were pooled. All analyses were performed using both the original data set and the imputed data sets. As the effect estimates were very similar, we present only the pooled effect estimates with their 95% confidence intervals.

All statistical analyses were performed using SPSS Statistics version 20 for Windows (PASW Inc., Chicago, IL, USA). P-values of < 0.05 were considered statistically significant.

Results

Maternal and child characteristics are presented in **Table 3.1.1** (Characteristics after imputations are presented in S**upplementary Table 3.1.1**). Median total fish intake was 75 gram/week (95% range 0–318), mainly of fatty fish and lean fish consumption.

Fish intake and angiogenic factors

Associations of energy-adjusted total fish intake and angiogenic factors are presented in **Table 3.1.2.** We found no association of maternal energy-adjusted total fish intake with PlGF levels at any of the three time points. The association of total fish intake with sFlt-1 levels was inconsistent. In the first trimester, women with a total fish intake of 1-69 gram per week had 0.11 (95%CI 0.03; 0.20) ng/mL higher Ln-sFlt-1 levels than women who did not consume fish. However, the other categories of total fish intake were not associated with sFlt-1. In the second trimester, total fish intake was associated with higher sFlt-1 levels in all categories, although not statistically significant in the highest category, which may be due to the lower number of women in this category (n=196) than in the other categories (between 470 and 1063 women). We found no dose-response relation and no association was found in cord blood.

The associations of total fish intake with the sFlt-1/PlGF ratio were also inconsistent. In the first trimester, an energy-adjusted intake of 1-69 g per week of total fish intake, but none of the other categories, was associated with an increased ratio. In the second trimester total fish intake was associated with increased ratios in all categories except for 70-139 g per week, but there was no dose-response relation.

The association of lean fish consumption with levels of PlGF, sFlt-1 and the sFlt-1/

PlGF ratio is presented in **Supplementary Table 3.1.2**. There were some scattered significant associations for single categories of intake of lean fish, but no clear pattern could be distinguished. Neither fatty fish nor shellfish intake was associated with angiogenic factors (**Supplementary Tables 3.1.3** and **3.1.4**). Restricting the analyses to women without diabetes resulted in a minor attenuation of the effect estimates *(data not shown)*.

Table 3.1.1. Characteristics of participants (n=3134)[a]

Maternal characteristics			**Mean ± SD or Median (95% range)**
Age (years)			31.4 ± 4.3[b]
Gestational age at enrolment (weeks)			13.2 (9.9-21.6)[b]
BMI at enrolment (kg/m2)			24.1 ± 3.9
Parity, % (n)		Nullipara	60.1 (1883)
		Multipara	39.9 (1251)
Highest education finished, % (n)		Primary	3.2 (100)
		Secondary	36.4 (1140)
		Higher	60.5 (1894)
Smoking during pregnancy, % (n)		Yes	28.5 (894)
		No	71.5 (2240)
Alcohol use during pregnancy, % (n)		Yes	67.2 (2105)
		No	32.8 (1029)
Folic acid supplementation use, % (n)		Yes	89.0 (2789)
		No	11.0 (345)
Pregnancy-induced hypertension, % (n)		Yes	5.3 (164)
		No	94.7 (2914)
Preeclampsia, % (n)		Yes	1.9 (56)
		No	98.1 (2914)
Season of conception, % (n)		Winter	28.3 (888)
		Spring	23.6 (738)
		Summer	21.8 (684)
		Fall	26.3 (823)
Maternal diet	Total daily energy intake (kJ/day)[b]		8972 ± 2127[b,c]
	Total fish intake (gram/week)[d]		75 (0-318)[b]
	Lean fish intake (gram/week)[d]		24 (0-149)[b]
	Fatty fish intake (gram/week)[d]		30 (0-150)[b]
	Shellfish intake (gram/week)[d]		0 (0-34.5)[b]
Plasma fatty acids	Docosahexaenoic acid (mg/L)		80.1 ± 20.0
	Eicosapentaenoic acid (mg/L)		13.0 ± 4.2
	Alpha-Linolenic acid (mg/L)		5.5 ± 1.8
	Linoleic acid (mg/L)		352.3 ± 59.5
	Arachidonic acid (mg/L)		154.5 ± 31.2
	n-3/n-6 ratio		0.2 ± 0.05
	EPA/AA ratio		0.06 ± 0.04
	DHA/AA ratio		0.5 ± 0.14

Table 3.1.1. (continued) Characteristics of participants (n=3134)[a]

Maternal characteristics		**Mean ± SD or Median (95% range)**
Angiogenic factors levels		
First trimester	PlGF (pg/ mL)	37.8 (14.4-154.7)
	sFlt-1 (ng/mL)	4.9 (1.9-12.8)
	sFlt-1/PlGF ratio	0.13 (0.02-0.43)
Second trimester	PlGF (pg/mL)	185.9 (73.2-569.3)
	sFlt-1 (ng/mL)	4.7 (1.5-16.0)
	sFlt-1/PlGF ratio	0.02 (0.01-0.11)
Cord blood	PlGF (pg/mL)	8.6 (0.0-20.7)
	sFlt-1 (ng/mL)	0.45 (0.08-4.01)
	sFlt-1/PlGF ratio	0.05 (0.01-0.42)
Child characteristics		
Sex, % (n)	Boy	50.8 (1592)
	Girl	49.2 (1542)
Gestational age at birth (weeks)		40.3 (36.0-42.4)
Birth weight (grams)		3497 ± 550

[a.] Values are mean ± SD when normally distributed or median (95% range) when not normally distributed for continuous variables and percentages (n) for categorical outcomes. The number of missing values were 0.4% (n= 4) for BMI at enrolment, 0.2% (n=7) for parity, 0.6% (n=19) for maternal education, 7.0% (n=219) for smoking, 7.6% (n=238) for alcohol use, 17.6% (n = 552) for folic acid supplementation use, 1.8% (n=56) for pregnancy-induced hypertension, 5.2% (n=164) for preeclampsia, 0.03% (n=1) for season of conception, 13.9% (n=435) for plasma fatty acids, 0.03% (n=1) for child sex, 0.03% (n=1) gestational age at birth and 0.2 % (n=5) for birth weight. Abbreviations: AA, arachidonic acid; DHA, docosahexanoic acid; EPA, eicosapentanoic acid; PlGF, Placental growth factor; s-Flt1, soluble Flt-1. [b.] No missing values. [c.] Values correspond to a daily energy intake of 2143 ± 508 kcal/day. [d.] Absolute values of fish consumption.

Plasma fatty acids and angiogenic factors

Of the n-3 PUFAs, higher levels of EPA were associated with low PlGF levels in the second trimester and higher levels of ALA were associated with increased sFlt-1 levels in cord blood (**Table 3.1.3**). Higher levels of the n-6 PUFA arachidonic acid were associated with significantly lower PlGF and sFlt-1 levels in the second trimester and with lower sFlt-1 levels in cord blood. We found no associations of any of the fatty acid ratios with PlGF or sFlt-1.

Discussion

We found no consistent associations of first trimester maternal fish consumption with levels the proangiogenic factor PlGF or the anti-angiogenic factor sFlt-1. Higher total fish intake increased sFlt-1 levels in some categories of intake, but there was not a dose-response effect. The association of fish consumption with the sFlt-1/PlGF ratio showed a similar pattern to that for sFlt-1. When we evaluated the effect of the plasma FA levels on angiogenesis, higher EPA was associated with lower PlGF levels in second trimester and ALA was associated with higher sFlt-1 levels in cord blood. Higher arachidonic acid was associated with lower sFlt-1 levels in both measurements and in second trimester PlGF levels.

Maternal overall diet has been found to influence foetal growth,[14] also consumption of food items such as fish has been associated with foetal growth in some studies.[16,17,36] Similarly, results from a systematic review have indicated that supplementation of n-3 PUFAs resulted in lower prevalence of preterm birth and higher birthweight,[20] although these results were not confirmed in another systematic review.[19] Results from several in vitro and animal studies, mostly in the field of cancer research, have suggested that food or food-derived compounds, such as components of cinnamon or grape seed, may influence angiogenesis.[37,38] However, whether maternal fish intake, or the intake of fish-derived compounds, is associated with markers of human placental angiogenesis has not yet been studied, to the best or our knowledge.

Our results suggest a potential anti-angiogenic effect of total fish intake in the second trimester reflected in the increased sFlt-1 levels and sFlt-1/PlGF ratio. In this association, the absence of a significant p-value for trend may not preclude the possibility of a threshold effect rather than a dose-response association.[39] Arachidonic acid (AA), an n-6 PUFA, was significantly associated with lower levels of both PlGF and sFlt-1, however fish is not a source of AA. Two n-3 PUFAs EPA and ALA were associated with anti-angiogenesis, EPA through lower PlGF levels in second trimester and ALA through higher sFlt-1 levels in cord blood. These findings are in agreement with the effect of PUFAs on angiogenesis in cancer cell lines where n-3 PUFAs, such as EPA, exert anti-inflammatory and antineoplastic effects, whereas n-6 PUFAs, such as AA, promote inflammation and carcinogenesis.[40,41] In addition, n-3 PUFAs were found to down-regulate the production of VEGF and thus inhibit angiogenesis in human tumours implanted in mice and rats. However, EPA, DHA and AA increased angiogenesis in first trimester trophoblast cells[22] and Basak et al.[21] reported that these PUFAs also increased the expression of COX-2 gene, which may be associated with induction of angiogenesis.

Although the mechanisms underlying the different effects of PUFAs in cancer cells and in trophoblasts remain to be established, distinct fatty acids metabolism could be expected in different cell lines, because differences in the handling of fatty acids have been shown between first trimester trophoblasts and third trimester trophoblasts.[21] In our study, the increase in sFlt-1 levels, secreted by trophoblasts,[42] may be explained by increased trophoblastic volume rather than anti-angiogenesis, which would be beneficial.

Table 3.1.2. Associations between weekly maternal total fish consumption and levels of angiogenic factors[a]

Total fish consumption (energy-adjusted g/week)	**First Trimester** n	β (95% CI)	**Second Trimester** n	β (95% CI)	**Cord Blood** n	β (95% CI)
	Ln-PlGF pg/mL (n = 2515)		**Ln-PlGF pg/mL** (n = 2845)		**Ln-PlGF pg/mL** (n = 1552)	
0	155	Reference	181	Reference	108	Reference
1-69	935	-0.028 (-0.105; 0.049)	1063	-0.017 (-0.094; 0.060)	560	-0.090 (-0.464; 0.284)
70-139	849	-0.008 (-0.086; 0.069)	934	-0.002 (-0.080; 0.076)	508	0.006 (-0.195; 0.206)
140-209	416	-0.007 (-0.090; 0.077)	470	-0.007 (-0.091; 0.077)	255	0.069 (-0.349; 0.487)
>210	160	-0.015 (-0.113; 0.084)	197	-0.044 (-0.141; 0.053)	121	-0.156 (-0.402; 0.090)
P for trend		*0.61*		*0.73*		*0.79*
	Ln-sFlt-1 ng/mL (n = 2513)		**Ln-sFlt-1 ng/mL** (n = 2844)		**Ln-sFlt-1 ng/mL** (n = 1695)	
0	155	Reference	181	Reference	111	Reference
1-69	936	**0.114 (0.025; 0.203)†**	1063	**0.157 (0.060; 0.255)***	613	0.129 (-0.095; 0.353)
70-139	846	0.064 (-0.025; 0.152)	934	**0.106 (0.007; 0.204)†**	552	0.098 (-0.128; 0.325)
140-209	415	0.092 (-0.004; 0.189)	470	**0.141 (0.035; 0.247)***	294	0.124 (-0.118; 0.365)
>210	161	0.059 (-0.055; 0.173)	196	0.108 (-0.015; 0.232)	125	0.279 (-0.002; 0.559)
P for trend		*0.74*		*0.78*		*0.18*
	Ln-sFlt-1/PlGF ratio (n = 2505)		**Ln-sFlt-1/PlGF ratio** (n = 2844)		**Ln-sFlt-1/PlGF ratio** (n = 1469)	
0	155	Reference	181	Reference	102	Reference
1-69	932	**0.142 (0.034; 0.250)†**	1063	**0.175 (0.059; 0.290)***	522	0.159 (-0.055; 0.372)
70-139	845	0.070 (-0.039; 0.178)	934	0.108 (-0.009; 0.225)	487	0.152 (-0.063; 0.366)
140-209	414	0.099 (-0.018; 0.216)	470	**0.148 (0.023; 0.273)†**	245	0.192 (-0.039; 0.423)
>210	159	0.063 (-0.075; 0.201)	196	**0.151 (0.006; 0.297)†**	113	**0.266 (0.001; 0.532)†**
P for trend		*0.44*		*0.65*		*0.10*

[a.] Results from multivariable linear regression model, based on imputed data. *P* for trend was conducted by including fish consumption categories in the multivariable linear regression model. Adjusted for total energy intake, maternal age, gestational age at measurement, BMI at enrolment, foetal sex, parity, maternal education, smoking, and season of conception. † P-value < 0.05, * P-value < 0.01. Abbreviations: CI, confidence interval; n, number of participants; PlGF, placental growth factor; sFlt-1, soluble Flt-1.

Table 3.1.3. Associations between maternal fatty acid plasma levels and levels of angiogenic factors[a]

Fatty acids (weighed percentage)	Second Trimester	Cord Blood
	β (95% CI)	β (95% CI)
	Ln-PlGF pg/mL (n=2699)	**Ln-PlGF pg/mL** (n=1311)
DHA (22:6n-3)	-0.006 (-0.024; 0.011)	0.012 (-0.089; 0.113)
EPA (20:5n-3)	-0.062 (-0.116; -0.007)†	-0.077 (-0.397; 0.243)
ALA (18:3n-3)	0.158 (-0.026; 0.342)	0.853 (-0.268; 1.973)
LA (18:2n-6)	0.007 (-0.001; 0.014)	-0.015 (-0.055; 0.025)
AA (20:4n-6)	-0.022 (-0.035; -0.009)*	-0.029 (-0.093; 0.035)
n-3/n-6 ratio	-0.157 (-0.542; 0.227)	1.003 (-1.266; 3.271)
EPA/AA ratio	-0.285 (-0.754; 0.183)	-0.146 (-2.893; 2.601)
DHA/AA ratio	0.087 (-0.049; 0.223)	0.341 (-0.429; 1.112)
	Ln-sFlt-1 ng/mL (n=2699)	**Ln-sFlt-1 ng/mL** (n=1444)
DHA (22:6n-3)	-0.011 (-0.032; 0.011)	-0.051 (-0.106; 0.005)
EPA (20:5n-3)	-0.044 (-0.112; 0.025)	0.055 (-0.127; 0.236)
ALA (18:3n-3)	0.066 (-0.166; 0.298)	0.676 (0.068; 1.284)†
LA (18:2n-6)	-0.001 (-0.010; 0.008)	0.005 (-0.017; 0.027)
AA (20:4n-6)	-0.022 (-0.038; -0.007)*	-0.044 (-0.084; -0.005)†
n-3/n-6 ratio	-0.021 (-0.505; 0.463)	-0.211 (-1.466; 1.044)
EPA/AA ratio	-0.129 (-0.718; 0.461)	1.001 (-0.548; 2.550)
DHA/AA ratio	0.074 (-0.095; 0.243)	0.029 (-0.396; 0.455)
	Ln-sFlt-1/PlGF ratio (n=2699)	**Ln-sFlt-1/PlGF ratio** (n= 238)
DHA (22:6n-3)	-0.005 (-0.031; 0.022)	-0.023 (-0.077; 0.032)
EPA (20:5n-3)	0.018 (-0.065; 0.101)	0.086 (-0.088; 0.261)
ALA (18:3n-3)	-0.092 (-0.372; 0.187)	0.564 (-0.031; 1.159)
LA (18:2n-6)	-0.008 (-0.019; 0.003)	-0.008 (-0.029; 0.014)
AA (20:4n-6)	0.000 (-0.020; 0.019)	-0.032 (-0.071; 0.007)
n-3/n-6 ratio	0.136 (-0.447; 0.719)	0.324 (-0.901; 1.549)
EPA/AA ratio	0.157 (-0.553; 0.867)	1.043 (-0.432; 2.518)
DHA/AA ratio	-0.013 (-0.219; 0.192)	0.071 (-0.342; 0.484)

[a.] Results from multivariable linear regression model, based on imputed data. Adjusted for total energy intake, maternal age, gestational age at measurement, BMI at enrolment, foetal sex, parity, maternal education and smoking. † P-value < 0.05, * P-value < 0.01. Abbreviations: AA, arachidonic acid; ALA, alpha-linolenic acid; CI, confidence interval; DHA, docosahexanoic acid; EPA, eicosapentanoic acid; LA, linoneic acid; n, number of participants; n-3/n-6, omega-3/omega-6 ratio; PlGF, placental growth factor; sFlt-1, soluble Flt-1.

Unfortunately, we were not able to evaluate the role of trophoblast or trophoblastic volume in the association of fish consumption with angiogenic factors, because this information was not collected in our participants. Our finding that AA was associated with both proangiogenic and antiangiogenic factors during pregnancy needs further study whether AA in pregnancy may indeed be involved in both processes.

Besides the intake of fatty acids, an important aspect of fish consumption is the bioaccumulation of polychlorinated biphenyls (PCBs) and dioxins. Foetal exposure to PCBs and dioxins increases the risk for intra-uterine growth retardation, leads to changes in thyroid hormone metabolism, immunosuppression and neurological deficits.[17,36,43] Also,

plasma PCB levels have been associated with lower syncytiotrophoblast volume and increased PlGF levels in pregnant women.[44] It is possible that potential beneficial effects of PUFAs on placental angiogenesis might be counterbalanced by the effects of PCBs or other substances contained in fish. Also, interactions of the components of fish with other dietary and metabolic factors, as well as the effects of the preparation method of the fish are yet to be explored.

Strengths and limitations

Our study has several strengths, including its large sample size, the population-based design, the in-depth information about fish intake and the extensive information on a large number of potential confounders. However, the study also has limitations. Our study population had a low fish consumption. The median total fish intake was 75 g (half a serving) per week and only 7% of pregnant women consumed more than 210 g of fish per week which was lower than the mean total fish intake in the general Dutch population of 88-104g per week.[45] Also, total fish intake in the Netherlands was the lowest in a study across 10 European countries.[45] Therefore, we were likely limited in our ability to study the full effects of fish intake, mainly due to a low variation in fish consumption between the subjects, especially in subgroups of specific types of fish. Also, the exact amount of fish intake measured by an FFQ might not be very precise. However, it does permit adequate ranking of participants by intake, as participants with a high intake will also report a higher intake of fish than those with a low intake.[46] Also, as mentioned above, there may be further aspects to fish intake beyond fatty acids that also influence levels of angiogenic factors and clinical outcomes that we were not able to take into account. Lastly, we got some scattered significant results. As we performed a large number of statistical tests, some significant results might arise by chance (multiple testing). However, our significant results were clustered within the second trimester and in two specific (and related) outcomes, so we feel that multiple testing is unlikely to explain all our findings.

Conclusions

In a population with low fish consumption, we found no consistent associations of total fish or specific groups of fish consumption with PlGF and sFlt-1 levels or the sFlt-1/PlGF ratio. These associations need further evaluation in studies including populations with a higher fish intake, and taking into account other components of fish intake, method of preparation and further dietary components to fully grasp the potential effects of maternal fish intake on placental angiogenesis.

Supplementary Material can be found online: http://hdl.handle.net/1765/80025

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Chapter 3.2

Circulating fatty acid patterns, gestational weight gain and hypertensive complications in pregnancy: The Generation R Study

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Abstract

Background: Dietary fat composition has been associated with gestational weight gain (GWG) and pregnancy outcomes. Dietary fat intake influences circulating fatty acids, however the composition circulating fatty acids result from a complex interplay of diet and metabolism, resulting in specific fatty acid patterns that may influence pregnancy outcomes.

Objective: To evaluate whether circulating fatty acid patterns are associated with GWG, adequacy of GWG, and pregnancy outcomes (pregnancy-induced hypertension, preeclampsia).

Methods: We included 6567 pregnant women who participated in the Generation R Study, a population-based birth cohort (Rotterdam, the Netherlands). Three circulating fatty acid patterns were identified using principal component analysis from 22 individual plasma fatty acids: a '*High n-6 poly-unsaturated fatty acid (PUFA)*', a '*Mono-unsaturated fatty acids (MUFA) and saturated fatty acids (SFA),* and a *'High n-3 PUFA*' pattern. Weekly GWG was measured between 20 and 30 weeks of pregnancy and used to categorize women as having an inadequate, adequate or excessive GWG. Information on pregnancy outcomes were collected from medical records. Analyses were adjusted for sociodemographic and lifestyle factors.

Results: A higher score on the '*High n-6 PUFA*' pattern (per standard deviation) was associated with a 42 g/week higher GWG (95%CI 36;49), higher prevalence of excessive GWG (OR 1.11 [95%CI 1.04; 1.19]) and lower prevalence of inadequate GWG (0.78 [95%CI 0.72; 0.85]). The '*MUFA and SFA*' pattern was not associated with GWG. A U-shaped association between the '*High n-3 PUFA*' pattern and GWG was found ($p<0.001$), indicating that both women with low and high scores on the '*High n-3 PUFA*' pattern had higher GWG, but no association was found for GWG adequacy. Circulating fatty acid patterns were not independently associated with pregnancy-induced hypertension or preeclampsia.

Conclusions: These results suggest that specific circulating fatty acid patterns are associated with gestational weight gain, but not with pregnancy-induced hypertension or preeclampsia.

Introduction

Excessive weight gain during pregnancy has been associated with pregnancy complications such as pregnancy-induced hypertension[1] and preeclampsia.[2,3] In observational studies, dietary fat composition has been described as one of the factors to be associated with gestational weight gain (GWG).[4-6] In these studies, dietary fat intake was assessed using self-reporting methods such as food-frequency questionnaires (FFQ).[7] Yet, these methods are prone to measurement error due to misreporting of dietary intake.[7] Alternatively, plasma fatty acids could be evaluated, diminishing measurement error and permitting a more objective assessment that also takes metabolic processes into account.[8,9] Plasma fatty acids could be examined using fatty acid patterns, which enables us to account for correlations between individual fatty acids and that it reflects metabolism as well as dietary fat intake.[10,11] Additionally, studying fatty acid patterns may provide new hypotheses regarding the associations of fatty acids with GWG.[10]

Adipose tissue fatty acid patterns have been previously examined in association with weight gain in one study in non-pregnant individuals. This analysis showed that women with a high score on a fatty acid pattern characterized by shorter-chain fatty acids and n-6 poly-unsaturated fatty acids (n-6 PUFAs) had higher weight gain than those with low scores.[12] However, the association of plasma fatty acid patterns with weight gain in pregnant women has not been studied previously. Fatty acid patterns may differ between pregnant and non-pregnant women due to the specific physiological and metabolic characteristics and changes occurring during gestation.[13] In addition, circulating fatty acid concentrations change during pregnancy,[14] and women with pregnancy-induced hypertension or preeclampsia differ in their fatty acid status from normotensive women.[15-17]

Hence, we hypothesized that specific fatty acid patterns exist that are related to GWG and hypertensive disorders during pregnancy. We aimed to assess whether plasma fatty acid patterns are associated with gestational weight gain and adequacy of GWG.[3] In addition, we aimed to assess whether these fatty acid patterns were associated with pregnancy complications related to GWG such as pregnancy-induced hypertension and preeclampsia.

Methods

Study design

This study was embedded in the Generation R Study, an ongoing population-based prospective cohort in Rotterdam, the Netherlands (enrolment 2002-2006). Details of the Generation R Study have been described in detail before.[18,19] All participants provided written informed consent. The study was approved by the Medical Ethics Committee of Erasmus Medical Center and was conducted according to the World Medical Association Declaration of Helsinki.

Population of analysis

In total, 9778 women participated in the Generation R Study. For the current study, women were eligible when they enrolled before 25 weeks of gestation (n=8663). We excluded

women of whom no plasma samples were available (n=1292) and women with incomplete information on plasma fatty acid profiles (n=372), leaving 6999 women with complete data on plasma fatty acid profiles for fatty acid pattern analyses. Subsequently, we excluded women with multiple pregnancies (n=73) and women who were lost to follow-up during pregnancy (n=1). Finally, we excluded women with missing GWG information (n=358), leaving 6567 women for the current analyses (**Supplementary Figure 3.2.1**).

Plasma fatty acids

Non-fasting venous blood samples were collected at a median gestational age of 20.5 weeks (90%-range 18.8-22.9). The fatty acid composition of plasma phosphoglycerides was analyzed at the Division of Metabolic Diseases and Nutritional Medicine, Dr. von Hauner Children's Hospital, Ludwig-Maximilians-University of Munich in 2010 by gas chromatography as described by Glaser et al.[20,21] The average coefficient of variation was 15.7%. Concentrations of individual fatty acids were expressed as weight percentages of total fatty acids.

Fatty acid patterns

We used 22 fatty acids to perform a principal component analyses (PCA).[10] Most of the fatty acids were correlated (**Supplementary Table 3.2.1**). Correlated fatty acids are grouped together with PCA and principal components (fatty acid patterns) were created. Based on the scree-plot, an Eigenvalue ≥2, and the distinctive character of the principal components, we identified three plasma fatty acid patterns. A Varimax rotation was applied on the PCA to improve the interpretability of those patterns. Factor loadings, which describe how strongly each individual fatty acid contributes to each fatty acid pattern, were calculated (**Table 3.2.1**). Using these factor loadings and individual fatty acid levels, each woman received an individual score on each of the fatty acid patterns which are z-scores with a mean of zero and a standard deviation (SD) of one. A higher z-score indicated that the individual fatty acid profile was closer to that identified fatty acid pattern.

Gestational weight gain and pregnancy outcomes

Women visited the Generation R research centre three times during pregnancy (at median gestational ages of 13, 20, and 30 weeks). During these visits women's weight was measured. GWG between 20-30 weeks of gestation was calculated by subtracting the weight that was measured at median 20 weeks of gestation (interquartile range (IQR) 20-21) from the weight at median 30 weeks of gestation (IQR 30-31) and was divided by follow-up duration to calculate an average weekly GWG. Subsequently, women were categorized as having inadequate, adequate, or excessive GWG based on recommendations from the US Institute of Medicine.[3] Women were categorized as having adequate GWG if their weekly GWG was between 440 and 580 g/week in underweight women (Body mass index (BMI) <18.5 kg/m^2), between 350 and 500 g/week in normal weight women (BMI 18.5-24.9 kg/m^2), between 230 and 330 g/week in overweight women (BMI 25-29.9 kg/m^2), and obese women (BMI ≥30 kg/m^2) had an adequate weekly GWG between 170 and 270 g/week.

In a subgroup (n=3308), information on self-reported maximum weight during pregnancy was available collected using a questionnaire 2 months postpartum, and GWG

between 20-39 weeks of gestation was calculated (median 39 (IQR 37-40) weeks). The self-reported highest weight and the measured weight at 30 weeks of gestation were highly correlated (r=0.86 (P <0.001)) and no systematic measurement error was detected.[22]

Table 3.2.1. Factor loadings of the individual fatty acids in fatty acid patterns

Individual fatty acids	Chemical name	Concen-tration[a]	Fatty acid patterns		
			'High n-6 PUFA'	'MUFA and SFA'	'High n-3 PUFA'
Saturated fatty acids					
Myristic acid	14:0	0.64	**0.23**	**0.30**	0.06
Palmitic acid	16:0	30.68	0.06	**0.88**	0.14
Margaric acid	17:0	0.36	-0.03	**-0.31**	0.03
Stearic acid	18:0	11.49	0.08	**-0.84**	-0.02
Mono-unsaturated fatty acids (cis)					
	15:1n-5	0.06	0.01	0.16	0.14
Palmitoleic acid	16:1n-7	0.68	**0.43**	**0.63**	0.16
Oleic acid	18:1n-9	10.30	0.19	**0.29**	**0.21**
Vaccenic acid	18:1n-7	1.46	0.00	**0.28**	0.08
Eicosenoic acid	20:1n-9	0.19	**-0.25**	**-0.31**	-0.09
Poly-unsaturated fatty acids n-3					
α-Linolenic acid (ALA)	18:3n-3	0.30	-0.13	-0.03	0.09
Eicosatrienoic acid	20:3n-3	0.10	0.13	-0.07	0.16
Eicosapentaenoic acid (EPA)	20:5n-3	0.44	**-0.33**	0.06	**0.69**
Docasapentaenoic acid (DPA)	22:5n-3	0.70	0.09	-0.02	**0.59**
Docosahexaenoic acid (DHA)	22:6n-3	4.67	**-0.39**	0.06	**0.67**
n-6					
Linoleic acid (LA)	18:2n-6	22.37	**-0.45**	**-0.45**	**-0.67**
γ-linolenic acid (GLA)	18:3n-6	0.08	**0.53**	0.10	0.19
Eicosadienoic acid	20:2n-6	0.52	-0.06	-0.04	**-0.69**
DH-γ-linolenic acid	20:3n-6	3.68	**0.44**	**0.42**	-0.19
Arachidonic acid (AA)	20:4n-6	9.72	**0.40**	-0.15	**0.30**
Adrenic acid	22:4n-6	0.42	**0.85**	0.03	-0.08
Osbond acid	22:5n-6	0.47	**0.82**	0.04	-0.17
n-9					
Mead acid	20:3n-9	0.12	**0.53**	0.12	**0.37**
Explained variance (%)			**14.4**	**12.7**	**12.2**

[a.] Median (percentage of total fatty acids (%)) in 6999 participants of the Generation R Study. The three fatty acid patterns explained together 39.2% of the variance in fatty acid patterns. The fatty acids that are considered to have a strong association with a fatty acid pattern (factor loading ≥0.2 or ≤-0.2) are shown in bold. Abbreviations: MUFA, mono-unsaturated fatty acids; n, omega; PUFA, poly-unsaturated fatty acids; SFA, saturated fatty acids.

Information on pregnancy-induced hypertension and preeclampsia were obtained from medical records. If pregnancy-induced hypertension or preeclampsia were suspected this was crosschecked with the original hospital records.[23] Pregnancy-induced hypertension was

defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg without proteinuria after 20 weeks of gestation in previously normotensive women.[24] Women were diagnosed with preeclampsia when the following two criteria were applicable: 1) systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg developed after 20 weeks of gestation in previously normotensive women; 2) presence of proteinuria (defined as two or more dipstick readings of ≥2+ or a 24h urine collection that contained ≥300 mg of protein).[24]

Covariates

At enrolment we used questionnaires to collect information on age, ethnicity, educational level,[25] household income, pre-pregnancy weight, parity, and folic acid supplementation. During each trimester of pregnancy women received questions on their smoking behaviour and alcohol consumption during pregnancy. An FFQ[26] was used to determine energy intake and diet quality based on four items of the Dutch Healthy Diet-index (DHD-index), namely fruit, vegetable, fibre and sodium.[27,28] Since fatty acid patterns may be a reflection of dietary fat intake, we did not include the components saturated fat intake and fish intake in the DHD-index. We used delivery reports to collect information on foetal sex. Information on birthweight and placental weight were collected from midwife and hospital registries at birth.[18]

During the visits at the research centre we measured women's height and with the self-reported pre-pregnancy weight we calculated pre-pregnancy BMI. Based on measured weights we calculated early GWG between 12-20 weeks of gestation. In addition, during the second and third visit foetal ultrasound examinations were performed and estimated foetal weights were calculated using the formula of Hadlock et al.[29]

Statistical methods

For non-response analyses, we compared the group of women that were included with the excluded women using Pearson's Chi-square tests for categorical variables and student t-tests for continuous variables.

Multivariable linear regression analyses were applied to assess the association of plasma fatty acid patterns with weekly GWG. We used multinomial and logistic regression analyses to evaluate the association with GWG adequacy (with having an adequate GWG as reference category vs. inadequate and excessive GWG) and pregnancy outcomes.

We added gestational ages at plasma sampling and visit to the research centre to the crude model. Covariates, which were selected based on literature, were added individually to the crude linear regression model and retained in the multivariable model when the effect-estimate changed with ≥5% for at least one fatty acid pattern. We used natural cubic splines to test for non-linear associations of fatty acid patterns with GWG.[30] Effect modification was evaluated for pre-pregnancy BMI, ethnicity, smoking, energy intake and foetal sex by adding an interaction term to the multivariable models and stratification was evaluated in case of a significant interaction term ($P<0.10$).

We performed several additional analyses. First, we included early GWG as covariate to the multivariable models (n=5131) because we hypothesized that the association of fatty

acid composition with GWG may be bidirectional. Second, to assess whether the association with GWG between 20-30 weeks of gestation was explained by the foetal component of GWG, we additionally adjusted the multivariable model for the change in estimated foetal weight between 20 and 30 weeks of gestation (n=6380). Likewise, in the subgroup with information on GWG between 20-39 weeks of gestation, we added birthweight and placental weight to the multivariable model (n=2478). Third, we additionally adjusted for energy intake and DHD-index to assess whether the observed associations were independent of dietary intake (n=4985). Finally, we evaluated if results were altered when excluding women with pre-existing comorbidities (n=300) and women with pregnancy complications (n=430) because these diseases may influence plasma fatty acid patterns and GWG.

To reduce bias due to missing information on confounders, we used multiple imputation to replace the missing values (**Supplementary Table 3.2.2**).[31] Ten different datasets were created and the analyses were performed in each dataset. These results were presented as pooled effect estimates. The statistical analyses were performed in SPSS version 21.0 (Corp., Armonk, NY, USA) and R version 3.1.2 (R Core Team (2015), R Foundation for Statistical Computing, Vienna, Austria).

Results

Study population

Baseline characteristics of the participating women are presented in **Table 3.2.2**. Seventy-three percent of women (n=4796) had a pre-pregnancy BMI below 25 kg/m^2 and 59% (n=3716) was of Western ethnicity. More than half of the women (n=3672 (56%)) had an excessive GWG, whereas 1490 women (23%) had an inadequate GWG, leaving 1405 women (21%) with an adequate GWG.

In a non-response analysis, we found that the women we included were older, higher educated and were more often of Western ethnicity than eligible women not included in the analyses, but not different in pre-pregnancy BMI, smoking behaviour or diet (**Supplementary Table 3.2.3**).

Fatty acid patterns

Three plasma fatty acid patterns were identified explaining 39% of the total variance in fatty acid concentrations, namely a '*High n-6 PUFA*' pattern, a '*Mono-unsaturated fatty acid (MUFA) and saturated fatty acid (SFA)*' pattern and a '*High n-3 PUFA*' pattern. The characteristics of these patterns are provided in **Table 3.2.1**.

Fatty acid patterns and gestational weight gain

After adjustment for sociodemographic and lifestyle factors, 1 SD higher score on the '*High n-6 PUFA*' pattern was associated with 42 g/week higher GWG (95%CI 36; 49) between 20-30 weeks of gestation, whereas 1 SD higher score on the '*High n-3 PUFA*' pattern was associated with 9 g/week lower GWG (95%CI -16; -2) (**Table 3.2.3**). The '*MUFA and SFA*' pattern was not associated with mid-pregnancy GWG (-2 g/week (95%CI -9; 5)). The associations attenuated

and were no longer significant when evaluating GWG between 20-39 weeks of gestation (**Table 3.2.3**).

We found that allowing a non-linear association of the '*High n-3 PUFA*' pattern improved the model fit significantly compared to a linear effect ($P_{for\ model}$ <0.001) indicating that women with high and low scores on the '*High n-3 PUFA*' pattern had higher mid-pregnancy GWG (**Supplementary Figure 3.2.2**). There was no indication for a non-linear association of the other fatty acid patterns with GWG (**Supplementary Figures 3.2.3 and 3.2.4**).

A higher score on the '*High n-6 PUFA*' pattern was associated with a lower prevalence of inadequate GWG (OR 0.78 (95%CI 0.72; 0.85) per SD), and with a higher prevalence of excessive GWG (OR 1.11 (95%CI 1.04; 1.19) per SD) relative to adequate GWG. Neither the '*MUFA and SFA*' nor the '*High n-3 PUFA*' pattern were associated with adequacy of GWG (**Supplementary Table 3.2.4**).

Fatty acid patterns and pregnancy outcomes

In the crude model, a higher prevalence of pregnancy-induced hypertension was found in women with higher scores on the '*MUFA and SFA*' pattern (OR 1.17 (95%CI 1.02; 1.34) per SD in fatty acid pattern) and the '*High n-3 PUFA*' pattern (OR 1.19 (95%CI 1.04; 1.36) per SD) (**Supplementary Table 3.2.4**). The '*High n-6 PUFA*' pattern was not associated with pregnancy-induced hypertension (OR 1.04 (95%CI 0.81; 1.18) per SD). However, after taking into account maternal sociodemographic and lifestyle factors none of the fatty acid patterns remained associated with pregnancy-induced hypertension. Also, no association was found of any fatty acid pattern with preeclampsia.

Table 3.2.2. Participant characteristics, the Generation R study, Rotterdam, the Netherlands (n=6567)[a]

Subject characteristics (n=6567)		**Original data**	**Imputed data[b]**
Age (years)		29.8 ± 5.2	No missing data
Ethnicity	Western	3716 (58.9)	3862 (58.8)
	Non Western	2598 (41.1)	2705 (41.2)
Educational level	Low and midlow	1571 (25.8)	1783 (27.1)
	Midhigh	3090 (50.7)	3313 (50.4)
	High	1436 (23.6)	1472 (22.4)
Household income	< 2200 Euros/month	2365 (45.1)	3345 (50.9)
	≥ 2200 Euros/month	2880 (54.9)	3222 (49.1)
Parity	0	3685 (56.6)	3708 (56.5)
	≥1	2831 (43.4)	2859 (43.5)
Weight categories	Underweight (BMI <18.5 kg/m^2)	241 (4.5)	245 (3.7)
	Normal weight (BMI 18.5-24.9 kg/m^2)	3652 (67.7)	4551 (69.3)
	Overweight (BMI 25-29.9 kg/m^2)	1039 (19.3)	1307 (19.9)
	Obesity (BMI ≥30 kg/m^2)	463 (8.6)	464 (7.1)
Alcohol consumption	Never during pregnancy	2783 (48.2)	3199 (48.7)
	Until pregnancy was known	798 (13.8)	894 (13.6)

Table 3.2.2. (continued) Participant characteristics, the Generation R study, Rotterdam, the Netherlands (n=6567)[a]

Subject characteristics (n=6567)		**Original data**	**Imputed data**[b]
Smoking	Continued throughout pregnancy	2191 (38.0)	2474 (37.7)
	Never during pregnancy	4250 (72.6)	4688 (71.4)
	Until pregnancy was known	517 (8.8)	675 (10.3)
	Continued throughout pregnancy	1085 (18.5)	1204 (18.3)
Folic acid supplementation	Started periconceptional	2011 (40.5)	2508 (38.2)
	Started first 10 weeks	1566 (31.5)	2058 (31.3)
	No supplementation	1391 (28.0)	2001 (30.5)
Energy intake (kcal/day)		2041 ± 568	Not imputed
DHD-index (maximum score: 40)		22 ± 6	Not imputed
Weekly GWG	12-20 weeks (g/week)	452 (250 – 651)	Not imputed
	20-30 weeks (g/week)	500 (300 – 671)	Not imputed
	20-39 weeks (g/week)	518 (359 – 680)	Not imputed
Pregnancy outcomes	Preeclampsia	132 (2.1)	Not imputed
	Pregnancy-induced hypertension	226 (3.6)	Not imputed
Gestational age at birth		39.9 (39.1 – 41.0)	39.9 (39.1 – 41.0)
Birthweight		3434 ± 538	Not imputed
Foetal sex, boys		3308 (50.4)	3308 (50.4)

[a.] Values represent n (%), mean ± SD, median (interquartile range). [b.] Number of participants might not add up to total number of subjects due to rounding of imputation. Missing values: ethnicity (3.9%), educational level (7.2%), household income (20.1%), parity (0.8%), weight categories (17.8%), alcohol consumption (12.1%), smoking (10.9%), folic acid supplementation (24.3%), energy intake (24.8%), DHD-index (24.8%), GWG between 12-20 weeks (21.9%), GWG between 20-39 weeks (49.6%), pregnancy outcomes (3.2%), gestational age at birth (0.0%), birthweight (0.5%), foetal sex (0.0%). Abbreviations: BMI: body mass index; DHD-index: Dutch Healthy Diet-index; GWG: gestational weight gain.

Table 3.2.3. Association of plasma fatty acid patterns with weekly gestational weight gain (n=6567)

	GWG (g/week) between 20 – 30 weeks of gestation (n=6567)		**GWG (g/week) between 20 – 39 weeks of gestation** (n=3308)	
	Crude[a]	***Multivariable***[b]	***Crude***[a]	***Multivariable***[b]
Plasma fatty acid patterns	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)
'High n-6 PUFA' (per SD)	**42 (35; 49)***	**42 (36; 49)***	20 (-7; 46)	24 (-3; 52)
'MUFA and SFA' (per SD)	-1 (-8; 6)	-2 (-9; 5)	-22 (-49; 6)	-23 (-51; 5)
'High n-3 PUFA' (per SD)	-3 (-10; 4)	**-9 (-16; -2)***	12 (-15; 39)	3 (-26; 31)

Results from multivariable linear regression analyses. Beta-coefficient reflects the change in weekly gestational weight gain (g/week) per standard deviation increase in plasma fatty acid pattern. [a.] Adjusted for gestational age at measurements and other fatty acid patterns. [b.] Crude model, additionally adjusted for maternal age, ethnicity, educational level, household income, pre-pregnancy BMI, parity, alcohol consumption during pregnancy, maternal smoking during pregnancy, folic acid supplementation, and foetal sex. * Statistical significant result (P-value <0.05). Abbreviations: CI, confidence interval; GWG, gestational weight gain; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; SD, standard deviation; SFA, saturated fatty acids.

Table 3.2.4. Association of plasma fatty acid patterns and pregnancy-induced hypertension and preeclampsia (n=6356)

	Pregnancy-induced hypertension (cases n=226)		**Preeclampsia** (cases n=132)	
	Crude[a]	***Multivariable***[b]	***Crude***[a]	***Multivariable***[b]
Plasma fatty acid patterns	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
'High n-6 PUFA' (per SD)	1.04 (0.81; 1.18)	1.03 (0.89; 1.19)	0.89 (0.75; 1.07)	0.88 (0.72; 1.06)
'MUFA and SFA' (per SD)	**1.17 (1.02; 1.34)***	0.99 (0.85; 1.14)	1.02 (0.85; 1.21)	1.02 (0.85; 1.23)
'High n-3 PUFA' (per SD)	**1.19 (1.04; 1.36)***	1.05 (0.90; 1.22)	0.95 (0.80; 1.13)	0.96 (0.79; 1.16)

Results from multivariable logistic regression. Odds ratios reflect the odds of having pregnancy-induced hypertension or preeclampsia per SD higher score on that fatty acid pattern. [a.] Adjusted for gestational age at fatty acid measurement, other fatty acid patterns, foetal sex, and gestational age at birth. [b.] Crude model additionally adjusted for maternal age, ethnicity, educational level, household income, pre-pregnancy BMI, parity, alcohol consumption during pregnancy, maternal smoking during pregnancy, and folic acid supplementation. * Statistical significant result (*P*-value <0.05). Abbreviations: CI, confidence interval; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; SD, standard deviation; SFA, saturated fatty acids.

Additional analyses

Because of significant effect modification, we stratified the analyses for ethnicity and pre-pregnancy BMI. Associations of fatty acid patterns with mid-pregnancy GWG were slightly stronger in non-Western women and in women with a pre-pregnancy BMI ≥25 kg/m^2 (**Supplementary Table 3.2.5**). Also, stratification of the analyses with pregnancy-induced hypertension by ethnicity showed that non-Western women with a higher score on the '*High n-3 PUFA*' pattern had a significantly higher prevalence of pregnancy-induced hypertension (OR 1.42 (95%CI 1.08; 1.87) per SD) whereas Western women did not (OR 0.91 (95%CI 0.76; 1.10) per SD) (**Supplementary Table 3.2.6**).

The association of fatty acid patterns with GWG, pregnancy-induced hypertension or preeclampsia hardly changed after additional adjustment for early GWG (**Supplementary Table 3.2.7**) or after additional adjustment for diet (*data not shown*). Additional adjustment for changes in foetal weight (**Supplementary Table 3.2.8**) or birthweight and placental weight (**Supplementary Table 3.2.9**) did not change the (non-significant) effect estimates with GWG. Also, the exclusion of women with pre-existing comorbidities or pregnancy complications did not alter our results (*data not shown*).

Discussion

Main findings

The results of this study showed that plasma phospholipid fatty acid patterns may be associated with GWG but not with pregnancy outcomes. The '*High n-6 PUFA*' pattern was associated with a higher prevalence of excessive GWG whereas the '*MUFA and SFA*' pattern was not associated with adequacy of GWG. In addition, evidence for a U-shaped association was found for the '*High n-3 PUFA*' pattern with GWG; both women with low and high

scores on this pattern had a higher GWG. Nonetheless this pattern was not associated with inadequate or excessive GWG.

Interpretation

GWG consists of maternal components, such as fat storage and blood volume, and foetal components, including foetal weight and placental weight.[13] The '*High n-6 PUFA*' pattern remained associated with higher GWG between 20-30 weeks of gestation after additional adjustment for estimated foetal growth. Hence, the higher GWG may originate from maternal components of GWG instead of foetal components. Although we did not collect information on the individual maternal components in our study, it might be higher body fat, because n-6 PUFAs have been suggested to be associated with higher adipose tissue development through promotion of preadipocyte differentiation.[35] In our population, the majority of women (56%) had an excessive GWG based on the IOM recommendations. This has also been reported in other observational studies.[36,37]

Observational and intervention studies in non-pregnant participants have suggested that a higher intake of n-3 PUFAs may be associated with reduced body fat, yet evidence is not conclusive.[38] In contrast, several studies have suggested that high levels of n-3 PUFAs in the maternal circulation may slightly increase birthweight, one of the components of GWG.[39,40] These differential effects of n-3 PUFAs on body fat versus birthweight may explain the U-shaped relation of the '*High n-3 PUFA*' pattern with GWG.

Results from observational studies have indicated that higher dietary saturated fat intake may be associated with higher GWG,[4,5] but this was not confirmed by the 'MUFA and SFA' pattern in our study.

We found no independent association of the fatty acid patterns with pregnancy-induced hypertension or preeclampsia, although results from observational studies have suggested that free fatty acids concentrations were higher in preeclamptic women than normotensive women[15] and that these differences were already present before the onset of preeclampsia.[16] Also, women with pregnancy-induced hypertension had different plasma phospholipid fatty acid concentrations during the last trimester of pregnancy than normotensive women.[17] The discrepancy between these studies[15-17] and our null results may be explained by the different approach of evaluating fatty acids (individual fatty acids (in mL or %) vs. fatty acid patterns) or due to differences in adjustment for confounding.

Strengths and Limitations

A strength of this study is the approach to evaluate the association of plasma fatty acid patterns with GWG and hypertensive complications in pregnancy, which takes into account the correlation between the individual fatty acids as well as differences in fatty acid metabolism.[10,32] Other strengths are its prospective population-based design, the large sample size, and the collection of numerous potential confounders. Also, the inclusion of women with different ethnic backgrounds can improve the generalizability of our results. Nevertheless, this multi-ethnicity led to a slightly lower explained variability in our study of the fatty acid patterns (39%) than reported in other studies (44–48%).[10,33]

Some issues should be taken into consideration when interpreting our results. First, we measured plasma fatty acids only once. Absolute and relative levels of fatty acids change over the course of pregnancy,[14,34] therefore we adjusted our models for gestational age at plasma fatty acid measurement. However, we do not know whether correlations between fatty acids, and consequently fatty acid patterns, change during pregnancy. This should be evaluated in future longitudinal studies using multiple plasma fatty acid measurements. Second, our population of analysis was a selection of the eligible women within the Generation R Study. Since the definition of the fatty acid patterns was a data driven approach, this may have affected the identified patterns in our population. Nevertheless, it is unlikely that the fatty acid patterns that we have defined would be differently associated with GWG. Finally, residual confounding may have influenced our results. Although we extensively adjusted for many socio-demographic and lifestyle confounding factors, we did not have information on for example physical activity levels.

Conclusions

The results from this large observational cohort study suggest that specific plasma fatty acid patterns at 20 weeks of pregnancy are associated with GWG, but not with pregnancy-induced hypertension or preeclampsia. To our knowledge, this is the first study reporting on fatty acid patterns in pregnancy. First, our results need replication in other cohorts to evaluate whether similar fatty acid patterns are identified in pregnancy and whether similar associations are found with GWG and pregnancy outcomes. If our results are replicated, fatty acid patterns in pregnancy may contribute to preventive strategies. For example, primary prevention may focus on modification of fatty acid profiles via dietary or lifestyle changes.[8,9] For secondary prevention strategies, assessing fatty acid profiles may be a tool to identify women at risk for inadequate or excessive gestational weight gain.[41]

Supplementary Material can be found online: http://hdl.handle.net/1765/80025

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Chapter 3.3

Maternal vitamin B12 in pregnancy and risk of preterm birth and low birth weight: A systematic review and individual participant data meta-analysis

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Abstract

Introduction: Vitamin (B12) deficiency in pregnancy is prevalent, and has been associated with lower birth weight (birth weight <2500 g) and preterm birth (length of gestation <37 weeks). Nevertheless, current evidence is contradictory and possible effects of B12 supplementation in pregnant women are not known.

Methods: We performed a systematic review and an individual participant data (IPD) meta-analysis to evaluate the associations between maternal serum or plasma B12 concentration in pregnancy and offspring birth weight and length of gestation. Studies published before August 2015 were identified from seven databases. Eligible studies provided IPD, and when IPD was not provided, relevant estimates from individual studies were included in the analyses. Two-step, random effects IPD meta-analyses were conducted. A priori subgroup analyses were explored.

Results: Of 606 unique references identified, 22 studies were eligible (11,993 observations). Eighteen studies were included in the meta-analysis (11,216 observations): Ten studies (8928 observations) provided IPD, and single-study estimates were obtained from eight remaining studies (2288 observations). No linear association was observed between maternal B12 status and birth weight. B12-deficiency (<148 pmol/L) in pregnancy was associated with an increased risk of giving birth to a low birth weight newborn (adjusted risk ratio (RR) 1.15 (95% confidence interval (CI) 1.01; 1.31)). Higher maternal B12 was strongly associated with a reduced risk of preterm birth: for one SD increase in B12 adjusted RR 0.89 (95% CI 0.82;0.97).

Discussion: Lower maternal B12 in pregnancy increased the risk of preterm birth. This finding supports the conduct of randomised controlled trials to evaluate whether vitamin B12 supplementation in pregnant women reduces the risk of preterm birth.

Introduction

Globally, preterm birth and low birth weight (LBW) cause over a third of the 2.9 million neonatal deaths each year, and prevention of these events is important to reduce under-five year mortality.[1,2] The aetiology of preterm birth, however, is complex, and few interventions have been successful in preventing it.[3]

Vitamin B12 (B12) is a vitamin with metabolic roles closely related to folate and homocysteine, and is found in animal-derived foods only.[4] It is important for the synthesis[5] and methylation of DNA,[6] and plays a role in the energy production of the cell.[7] It has been hypothesized that B12 may affect placentation and foetal growth.[8] B12-deficiency may affect up to three quarters of the pregnant population.[9]

Few supplementation-studies of B12 in pregnancy to assess possible effects on birth weight and length of gestation have been undertaken. However, a recent meta-analysis concluded that multiple-micronutrient supplementation may reduce the risk of LBW and the number of stillbirths, but not preterm birth or neonatal mortality.[10] Thus, a more targeted micronutrient supplementation practice may be warranted.

The aim of this systematic review and individual participant data (IPD) meta-analysis was to study whether maternal serum or plasma B12 levels in pregnancy may be associated with birth weight and length of gestation. Individual studies have reported conflicting results and have often been too small and heterogeneous to draw conclusions. We collected IPD and single-study estimates from eligible studies in order to pool the effects across all studies in a meta-analysis. The IPD approach also allowed for exploration of effects of confounding factors and evaluation of subgroup effects.

Methods

The systematic review and meta-analysis were reported according to the PRISMA guidelines,[11] and the protocol was registered at PROSPERO.[12]

Study inclusion criteria

We included studies that assessed the association between maternal B12 in serum or plasma during pregnancy and newborn birth weight or gestational age at delivery. Only studies of longitudinal cohort design were eligible for this review. To be eligible, information on birth weight had to be registered at birth and could not be retrospectively reported and length of gestation, in completed days or weeks, had to be estimated by either ultrasound or last menstrual period, or a combination of the two. Studies where B12 was measured after conception and prior to delivery were eligible. In addition, studies were excluded if they predominantly evaluated women or offspring with specific conditions other than B12-deficiency (e.g., preeclampsia or congenital malformations). Studies with fewer than 50 participants were not considered. Given the need to collaborate with authors of the original studies, we included only those studies published in 1998 or later.

Search methods

The electronic literature search was constructed by the first author (TR) and a librarian trained in medical database searches, and conducted in PubMed, Scopus, Web of Knowledge, EBSCO-host (CINAHL) and OvidSP (MEDLINE, EMBASE and GLOBAL HEALTH); last accessed August 2015. The search terms were adapted to each service provider and database, and were composed of a combination of the following terms (or related terms): B12 and pregnancy and birth weight or length of gestation. We added restriction terms excluding review articles, intervention studies and case reports, also excluding studies evaluating adults, children (other than infants), rodents, and patients with anaemia. We used a combination of controlled vocabulary terms and free text words. No language restriction was applied. **Supplementary Material 3.3** includes information on the exact search terms for each service provider and database. The reference lists of all studies read in full-text were hand searched to find additional eligible studies.

Data collection

The electronic literature search was carried out by the first author (TR). Duplicates were removed and the eligibility of all references was evaluated by screening of the titles and abstracts by the first author (TR). All potentially eligible studies were read in full-text and assessed for inclusion independently by two authors (TR and KRR). The hand search of the reference lists was done independently by two authors (TR and KRR or MJT). When several reports from the same study were found, we used the most complete report.

Risk of bias was independently assessed by two authors (TR and MJT) based on a modified version of the Newcastle-Ottawa Scale (range 0-7).[13] Disagreements were resolved by consulting a third reviewer (KRR). We defined high risk of bias as a score of four or less, and moderate to low risk of bias was defined as scores five through seven.

Authors from all eligible studies were contacted to obtain IPD, and each research group was approached at least three times. IPD was received without personal identification. For studies where IPD could not be shared, authors were asked to provide results from pre-specified reanalyses of their data. When neither IPD nor reanalyses could be retrieved, relevant estimates were extracted from the publications.

Variables

The main exposure of interest was vitamin B12 levels in maternal serum or plasma. We calculated trimester-specific standard deviation (SD) scores based on studies providing IPD and reanalysed aggregate data. Analyses were also performed for B12-deficiency pre-defined as <148 pmol/L,[14] and B12 tertiles constructed on the basis of included individual data; <148 pmol/L (tertile 1), 148-216 pmol/L (tertile 2), and >216 pmol/L (tertile 3).

The three pre-defined main outcomes were: birth weight as a continuous measure in grams, LBW (birth weight <2500 g) and small-for-gestational-age (SGA; birth weight SD score <10th centile).[1] Birth weight SD score was calculated using gestational age at delivery and sex-specific reference standards published by the INTERGROWTH 21st Project.[15] We assumed birth weight SD score to serve as a proxy of foetal growth, and SGA to serve as a proxy of restricted foetal growth. Outcomes related to length of gestation were gestational

age at delivery (days) and preterm birth (gestational age at delivery <37 weeks).

Three main confounders were identified based on a priori assumptions of confounding factors, availability of data and exploration of effect of covariates on outcome and exposure: maternal age (continuous), pre-pregnancy or pregnancy body mass index (BMI, continuous) and parity (nulliparous versus primiparous or multiparous). Maternal weight was used when information on BMI was unavailable. Also, we considered smoking habits (smoking versus not smoking during pregnancy) and highest completed education (completed high school, equal to 13 years of education, versus not completed high school).

Statistical analysis

We applied a two-step IPD meta-analysis with random effects to pool the results across studies, including aggregate data from individual studies when IPD was not available. All presented results are adjusted for maternal age, BMI/weight and parity (the "main model"), unless otherwise specified. Precision was assessed by 95% confidence intervals (CI).

Mean difference of the continuous outcomes birth weight (g), gestational age at delivery (days) and birth weight SD score (SD) were analysed by linear regression. To estimate risk ratios (RR), Poisson regression with robust error variance[16] was used to analyse the dichotomous outcomes LBW, SGA, and preterm birth.

A main multivariable model was applied adjusting for maternal age, BMI (or weight when BMI was unavailable) and parity. When IPD was not provided, we requested results from the following reanalyses of original studies: the association of B12 (SD score) with birth weight, gestational age at delivery, LBW and preterm birth; and the association of B12-deficiency with birth weight, LBW and preterm birth. Results were provided for both crude analyses, and two different multivariable analyses (adjusting for maternal age, BMI and parity; and adjusting for maternal age, BMI, parity and smoking habits). When neither IPD nor results from requested reanalyses were available, we extracted relevant results from the publications.

We stratified our analysis for the following a priori subgroup and sensitivity analyses: trimester of B12 measurement (four strata: 1st, 2nd, 3rd trimesters, and 1st and 2nd trimesters combined), country income category (high-income versus low- and middle-income countries, as defined by The World Bank),[17] risk of bias (high risk versus moderate or low risk of bias), and excluding each of the studies one by one. Additional sensitivity analyses that were carried out: overweight status (BMI ≥25 kg/m^2 versus BMI<25 kg/m^2), assay technique (radioimmunoassay, electroluminescence, microbiological), alternative multivariable models (e.g., a more saturated model including maternal education and smoking habits in addition to the main model), fixed effects model, Poisson regression with non-robust error variance, logistic regression model (dichotomous outcomes), and by excluding studies that only evaluated newborns born at term. We also conducted a post-hoc meta-analysis that evaluated how B12 was associated with maternal weight. Publication bias was explored using funnel plots. Heterogeneity between the studies was explored by computing the I^2 statistic, and was considered to be present when I^2 was greater than 30%. All statistical analyses were carried out using Stata SE version 13.1.

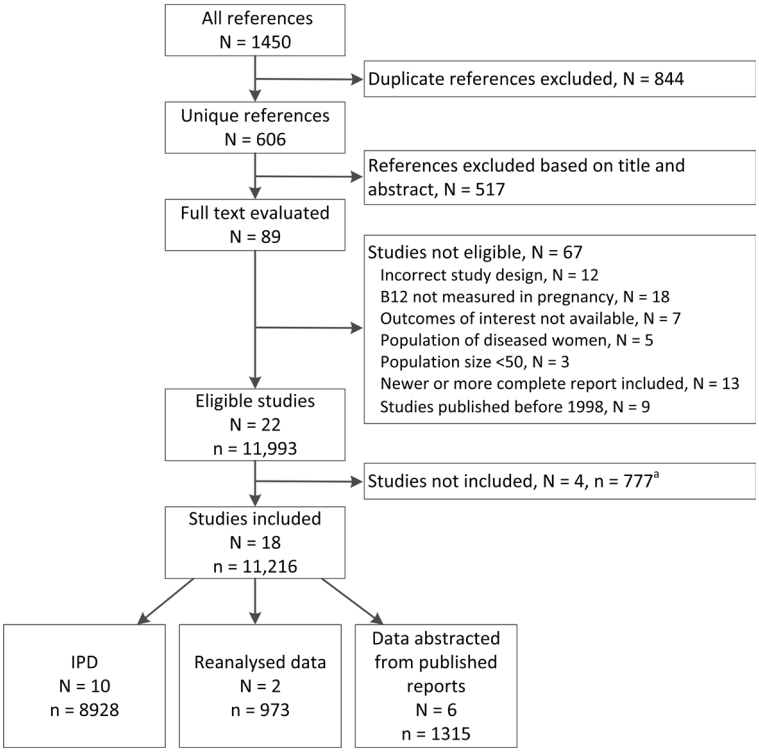
Results

Availability of data

The electronic literature search and hand search of the reference lists identified 606 unique references (**Figure 3.3.1**). Twenty-two studies met the eligibility criteria (11,993 observations) of which 14 studies reported estimates for the association between B12 in pregnancy and birth weight or length of gestation, and were included in the systematic review (10,563 observations).[18–31] Four additional studies did not report the associations of interest, but provided IPD or reanalysed results, and could be included in the meta-analyses.[32–35] Thus, 18 studies were included in the meta-analyses (11,216 observations), representing 94% of all eligible observations.

For the meta-analyses, ten studies provided IPD (8928 observations),[18–21,24,25,28,33–35] two studies provided results from reanalyses (973 observations),[31,32] and relevant information and estimates were extracted from the published reports of six studies (1315 observations) where IPD or reanalyses of original data were not provided.[22,23,26,27,29,30]

Figure 3.3.1 Flow chart of studies included in at least one of the meta-analyses of the association between B12 and birth weight or length of gestation



Abbreviations: B12, vitamin B12; IPD, individual participant data; N, number of studies; n, number of pregnancies. [a.] IPD or reanalyses not provided, and results could not be abstracted from published reports.

Details of eligible studies

Studies included in the meta-analyses are described in **Table 3.3.1**; details of the four eligible studies not included are presented in **Supplementary Table 3.3.1**.[36–39] Of the included studies, one was conducted in North America,[30] nine in Europe,[18,19,23,24,27,28,33–35] one in Africa,[26] one in Oceania,[22] and six in Asia.[20,21,25,29,31,32] The number of pregnancies studied ranged from 84 to 5641. B12 was measured during the first trimester in seven studies,[19,21,27–29,33,35] during the second trimester in 15 studies,[18–22,24,25,27–29,31–35] and during the third trimester in 12 studies.[18,20,21,23–26,28–32] Mean ± SD B12 concentrations in the first, second and third trimester were 219.8 ± 128.2, 187.8 ± 91.3 and 188.7 ± 82.5 pmol/L, respectively. Preterm deliveries were excluded from four studies.[23,27,29,34]

Key maternal and newborn characteristics of the included studies are presented in **Table 3.3.2.** Higher maternal weight was inversely associated with maternal B12, and one SD higher maternal BMI or weight was associated with an 11 pmol/L decrease in B12 (95% CI, -15; -7).

Systematic review

Birth weight/SGA

The association between B12 and birth weight or risk of SGA birth was reported in 14 of 22 eligible studies. Three studies reported a statistically significant association: in one study reported that birth weight was higher among B12-deficient women than among non-deficient women;[30] another study reported that only among women with gestational diabetes mellitus, lower B12 was associated with higher birth weight.[28] Conversely, a third study reported that lower values of B12 significantly increased the risk of SGA births.[21] In the remaining 11 studies, there was weak evidence of an inverse association in three studies,[23,24,29] and no association in eight studies.[18–20,22,25–27,31]

Length of gestation

Only two published reports reported on the association between B12 and length of gestation or preterm birth. The first study observed that higher B12 was associated with a longer length of gestation and a reduced risk of preterm birth, but the small sample size yielded low precision of the estimates.[20] The second study did not find evidence of an association between B12 and length of gestation.[19]

Risk of bias

Evaluation of the risk of bias showed that the scores ranged between three and seven, and that two studies were classified with high risk of bias (**Supplementary Table 3.3.2**).

Table 3.3.1 Characteristics of studies included in the meta-analysis

							Included in specific meta-analyses[a]					
Study	**Data**	**n**	**Country**	**Study years**	**B12 analysis method**	**Week of B12 measurement, range, median**	**Birth weight**	**LBW**	**SGA**	**Birth weight SD score**	**Length of gestation**	**Preterm birth**
Baker 2009[18]	IPD	290	The United Kingdom	2004-2007	RIA	27-43, 30	x	x	x	x	x	x
Bergen 2012[19]	IPD	5641	The Netherlands	2002-2006	ECL	5-18, 13	x	x	x	x	x	x
Bhate 2012[32]	Reanalysed data	214	India	2004-2006	Micro-biological	24-30, 28	x	x			x	x
Chen 2015[20]	IPD	988	Singapore	2009-2010	ECL	26-29, 27	x	x	x	x	x	x
Dayaldasani 2014[33]	IPD	187	Spain	2011	ECL	3-23, 10	x[b]	x[b]	x[b]	x[b]	x[b]	x[b]
Dwarkanath 2013[21]	IPD	344	India	2001-2003	ECL	T1: 5-19, 12 T2: 20-29, 24 T3: 30-39, 34	x	x	x	x	x	x
Furness 2013[22]	Data from publication	84	Australia	NA	ECL	18-20			x[c]			
Halicioglu 2012[23]	Data from publication	208	Turkey	2008	ECL	>37	x[d]					
Hay 2010[34]	IPD	149	Norway	1997	Micro-biological	17-19	x					
Hogeveen 2010[24]	IPD	363	The Netherlands	2002-2004	Micro-biological	27-38, 31	x[e]	x[e]	x[e]	x[e]	x[e]	x[e]
Kaymaz 2011[35]	IPD	103	Turkey	2007	ECL	11-14, 13	x	x			x	x
Krishnaveni 2014[25]	IPD	654	India	1997-1998	Micro-biological	22-35, 26	x	x	x	x	x	x
Mamabolo 2006[26]	Data from publication	219	South Africa	1999-2000	RIA	28-36			x[c]			

Table 3.3.1 (continued) Characteristics of studies included in the meta-analysis

Study	Data	n	Country	Study years	B12 analysis method	Week of B12 measurement, range, median	Included in specific meta-analyses[a]					
							Birth weight	LBW	SGA	Birth weight SD score	Length of gestation	Preterm birth
Relton 2005[27]	Data from publication	500	The United Kingdom	2000-2002	RIA	11.5 ± 5.8[f]				x[e]		
Sukumar 2011[28]	IPD	209	The United Kingdom	2005-2010	RIA (n=182), ECL (n=27)	0-37, 24	x	x	x	x	x	x
Takimoto 2007[29]	Data from publication	88	Japan	2001-2003	ECL	T1: 7-14 T3: 34-36	x[g]					
Wu 2013[30]	Data from publication	216	Canada	NA	RIA	Median 36	x[d]					
Yajnik 2008[31]	Reanalysed data	759	India	1994-1996	Micro-biological	T2: 18 ± 2[f]	x	x			x	x

Studies are referred to according to their citation number in the text. [a.] included in the analyses of the exposures B12 SD score and B12-deficiency, both crude and adjusted (maternal age, body mass index or weight, and parity), if not otherwise specified; [b.] does not contribute in the analyses of B12-deficiency (none of the participants were deficient); [c.] level of B12 in SGA versus non-SGA, crude analysis; [d.] birth weight among B12-deficient versus non-deficient, crude analysis; [e.] crude analysis; [f.] mean ± SD; [g.] adjusted analysis (maternal age, body mass index or weight, and parity). Abbreviations: ECL, electroluminescence; IPD, individual participant data; n, number of pregnancies; NA, not available; RIA, radioimmunoassay; SD, standard deviation; SGA, small-for-gestational-age; T1, 1st trimester; T2, 2nd trimester; T3, 3rd trimester.

Table 3.3.2 Maternal and newborn characteristics of studies included in the meta-analysis

Study	Maternal age (years), mean ± SD	Maternal BMI (kg/m²), mean ± SD	Para 0, n (%)	Vitamin B12 (pmol/L), mean ± SD	B12-deficient[a], n (%)	Birth weight (g), mean ± SD	LBW[b], n (%)	SGA[c], n (%)	Length of gestation (weeks), mean ± SD	Preterm birth[d], n (%)
Baker 2009[18]	18 ± 1	65 ± 14[e]	277 (96)	192 ± 84	93 (32)	3232 ± 534	26 (9)	33 (12)	39.7 ± 1.8	22 (8)
Bergen 2012[19]	30 ± 5	25 ± 5	3208 (57)	188 ± 93	2098 (37)	3418 ± 563	280 (5)	412 (7)	39.9 ± 1.8	268 (5)
Bhate 2012[32]	23 ± 3	20 ± 3	165 (71)	145 ± 84	148 (69)	2707 ± 411	49 (25)	NA	38.6 ± 2.6	18 (8)
Chen 2015[20]	31 ± 5	66 ± 12[e]	420 (43)	220 ± 79	161 (16)	3101 ± 449	76 (8)	86 (9)	38.6 ± 1.4	85 (9)
Dayaldasani 2014[33]	30 ± 6	26 ± 5	96 (51)	387 ± 123	0 (0)	3267 ± 526	11 (6)	12 (7)	38.8 ± 1.9	14 (8)
Dwarkanath 2013[21]	24 ± 4	53 ± 10[e]	203 (59)	205 ± 115[f]	100 (29)[f]	2771 ± 498	95 (28)	102 (30)	38.3 ± 1.7	47 (14)
Furness 2013[g, 22]	33 ± 7	27 ± 5	NA	234 ± 129	NA	3390 ± 789	NA	21 (25)[h]	38.8 ± 2.9	NA
Halicioglu 2012[g, 23]	28 ± 5	NA	NA	120[i]	99 (48)[j]	3357 ± 466	NA	NA	NA	NA
Hay 2010[34]	30 ± 4	65 ± 10[e]	67 (45)	294 ± 87	2 (1)	3727 ± 476	0 (0)	NA	NA	NA
Hogeveen 2010[24]	33 ± 4	NA	109 (30)	186 ± 69	120 (34)	3436 ± 545	18 (5)	19 (5)	39.5 ± 1.6	21 (6)
Kaymaz 2011[35]	27 ± 3	24 ± 4	45 (44)	152 ± 59	54 (52)	3241 ± 553	5 (5)	NA	38.4 ± 1.9	9 (9)
Krishnaveni 2013[25]	24 ± 4	24 ± 4	331 (51)	187 ± 100	264 (40)	2857 ± 475	126 (19)	202 (32)	39.0 ± 1.8	63 (10)
Mamabolo 2006[g, 26]	25 ± 7	27 ± 4	NA	175 ± 77	36 (16)[k]	3120 ± 550	NA	66 (30)[l]	NA	NA
Relton 2005[g, 27]	28 ± 6[m]	NA	NA (43)[m]	239 ± 97	NA	3430 ± 470[m]	NA	NA	NA	NA
Sukumar 2011[28]	31 ± 6	27 ± 6	68 (33)	168 ± 126	114 (55)	3381 ± 558	10 (5)	16 (8)	39.3 ± 1.7	9 (4)
Takimoto 2007[g, 29]	29 ± 5	21 ± 3	NA	405 ± 146[f]	13 (16)[n]	3120 ± 411	5 (5)	NA	39.6 ± 1.0	NA
Wu 2013[g, 30]	33 ± 4	NA	NA	224 ± 96	51 (24)	3486 ± 452	NA	NA	NA	NA
Yajnik 2008[31]	21 ± 4	18 ± 2	252 (31)	151 ± 78	447 (59)	2612 ± 392	230 (33)	NA	38.8 ± 2.1	87 (11)

[a.] B12 <148 pmol/L; [b.] birth weight <2500 g; [c.] birth weight SD score (i.e., accounting for length of gestation and sex) below 10th centile; [d.] length of gestation <37 weeks; [e.] kg (BMI not available); [f.] first measurement; [g.] data extracted from publication; [h.] serial tapering of growth in abdominal circumference and of estimated foetal weight below the 10th centile of an Australian growth chart; [i.] median (range not available); [j.] B12 ≤118 pmol/L; [k.] B12-deficiency not defined; [l.] lowest birth weight tertile (mean birth weight 2940 g) used as approximation of SGA for the purpose of this review; [m.] based on a larger study population than the subgroup with available B12 data included in this review (n=974-997); [n.] third trimester. Abbreviations: BMI, body mass index; LBW, low birth weight; NA, not available; SD, standard deviaton; SGA, small-for-gestational-age.

Meta-analysis of maternal B12 in relation to birth weight and LBW

In the meta-analysis, we found no evidence of any linear association between B12 and birth weight (**Figure 3.3.2**): The adjusted estimate was 5.1 g increase in birth weight per SD increase in B12 (95% CI -10.9; 21.0; I^2 = 30%).

Subgroup and sensitivity analyses are presented in **Supplementary Table 3.3.3.** Stratification by country income showed that while there was no association between B12 and birth weight in high-income countries, in low- and middle-income countries one SD higher B12 was associated with a 22.2 g higher birth weight (95% CI 2.1; 42.4; I^2 = 0%). Heterogeneity between the studies was explained largely by country income level and maternal BMI or weight. Excluding a study that used late-pregnancy BMI,[25] instead of pre-pregnancy or early pregnancy BMI/weight in the other studies, reduced the heterogeneity from I^2 = 30% to I^2 = 13% (*results not presented*). One study reported an association between B12 and birth weight that greatly deviated from the other studies.[29] Excluding this study did not change the effect estimate noteworthy, but resulted in a modest reduction in heterogeneity (from I^2 = 30% to I^2 = 21%; *results not presented*). Sensitivity analyses excluding each of the included studies one by one, and excluding studies only evaluating newborns born at term, did not meaningfully alter the association between B12 and birth weight (*results not presented*).

Results for categories of B12 supported our main results. Neither B12-deficiency nor B12 tertiles were associated with birth weight (**Supplementary Table 3.3.4**). B12-deficiency was associated with a 15% (95% CI 1, 31; I^2 = 5%) increased risk of LBW (**Supplementary Figure 3.3.1**). The funnel plot of B12 and birth weight indicated low risk of publication bias (**Supplementary Figure 3.3.2**).

Meta-analysis of maternal B12 in relation to length of gestation and preterm birth

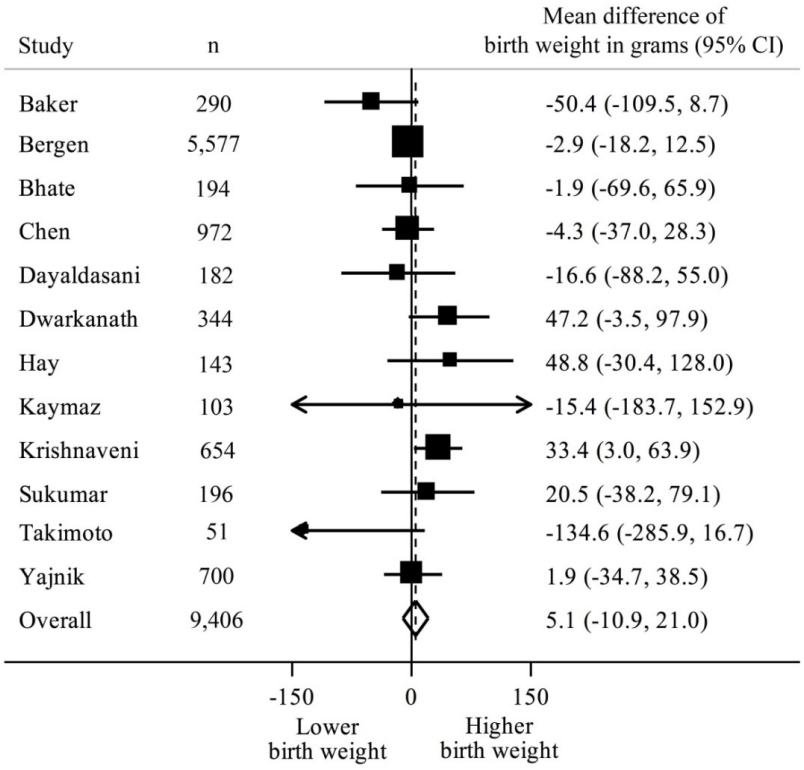
The analyses did not support a linear association between maternal B12 levels with length of gestation in days (0.1 days (95% CI -0.2; 0.3; I^2 = 0%) per SD increase of B12). However, one SD higher B12 was associated with an 11% (95% CI 3; 18; I^2 = 0%; **Figure 3.3.3**) reduced risk of preterm birth. The risk of preterm birth was particularly high in B12-deficient pregnancies (RR 1.21 (95% CI 0.99; 1.49; I^2 = 20%); **Figure 3.3.3**).

The association between B12 and preterm birth was similar within all subgroup and sensitivity analyses, although there was a loss of precision in these subgroup analyses due to smaller sample sizes (**Supplementary Table 3.3.5**).

Meta-analysis of maternal B12 in relation to birth weight SD score and SGA (indicators of foetal growth)

B12 was not associated with birth weight SD scores in the main analysis (**Supplementary Figure 3.3.3**). However, B12 was associated with birth weight SD score in low- and middle-income countries (0.08 birth weight SD score per 1 SD increase in B12 (95% CI 0.03; 0.14; I^2 = 0%)), whereas it was not in high-income countries (-0.02 SD (95% CI -0.05; 0.02; I^2 = 23%)). Women with B12-deficiency were not at higher risk of SGA births than non-deficient women (**Supplementary Figure 3.3.4**), and B12 levels were similar in SGA and non-SGA pregnancies (**Supplementary Table 3.3.6**).

Figure 3.3.2. Forest plot presenting the association between B12 and birth weight



Meta-analysis of studies of the association between vitamin B12 and birth weight after adjustment for maternal age, parity and body mass index or weight. Effect estimates are expressed as change in birth weight per one standard deviation increase of vitamin B12. Abbreviations: CI, confidence interval; n, number pregnancies.

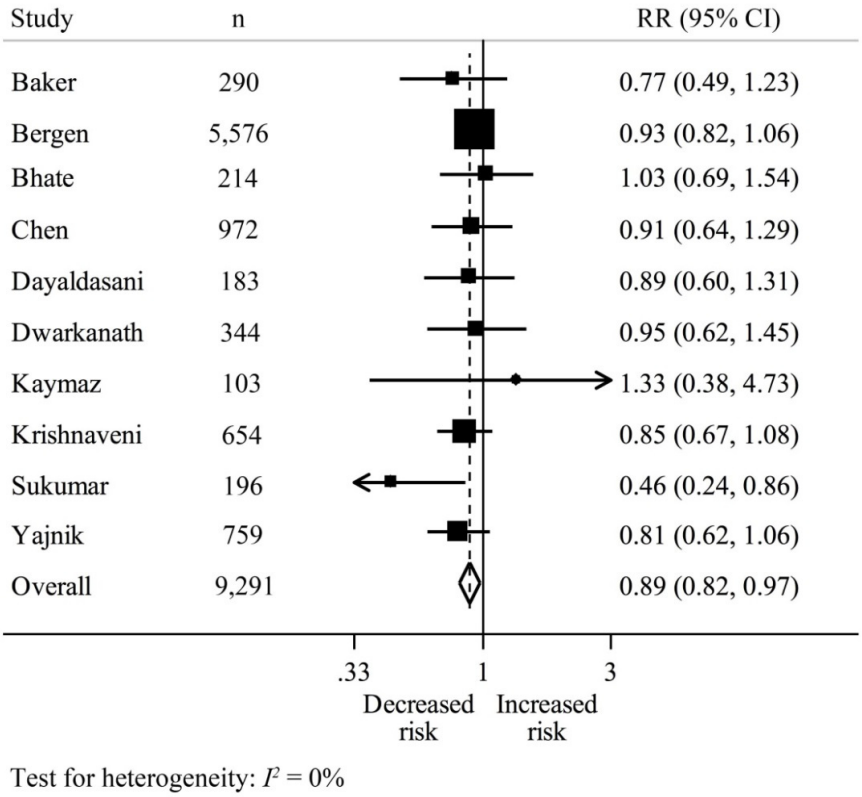
Discussion

The results from this systematic review and meta-analysis indicate that vitamin B12 levels in pregnancy are not associated with birth weight. However, our findings provide evidence that maternal B12 levels are associated with risk of preterm birth.

Strengths and limitations

A strength of this study is the use of IPD and reanalysed data. Due to substantial heterogeneity in the published analyses, a traditional meta-analysis could not answer our research questions. Incomplete or selective reporting may reduce the replicability of studies and distort the literature.[40]

Figure 3.3.3 Forest plot presenting the association between B12 and the risk of preterm birth



Meta-analysis of studies of the association between vitamin B12 and the risk of preterm birth after adjustment for maternal age, parity and body mass index or weight. Effect estimates are expressed as risk ratios of preterm birth per one standard deviation increase of vitamin B12. Abbreviations: CI, confidence interval; n, number pregnancies; RR, risk ratio.

By collecting IPD and requesting reanalyses from contributing studies, we were able to standardise the analyses across most of the included studies, thereby reducing heterogeneity and facilitating interpretation of results. Additionally, this strategy enabled conduction of subgroup and sensitivity analyses, along with more complete adjustment for important confounders. The lack of adjustment for confounders (especially maternal weight) may have contributed to the conflicting results on the association between B12 and birth weight reported in the systematic review section of this paper.

We included 94% of all eligible participants, permitting an unbiased summary of the published literature. Given the relative large number of included subjects, we also had increased power to evaluate findings reported with low precision in individual studies. We were able to test the stability of our findings with a broad range of sensitivity analyses.

Another strength was that our analyses were not post-hoc, but followed a detailed protocol. We performed a thorough literature search without language restrictions, and systematically reviewed all eligible studies.

There are also several limitations. Unpublished studies were not considered for this review, which could potentially skew the results. However, the funnel plot did not suggest publication bias. We were unable to include four eligible studies (777 observations, 6% of all observations) in our systematic review and meta-analysis. In these reports, no information on the association between B12 and birth weight and length of gestation was presented. Given the small number of observations, it is unlikely that inclusion of these remaining studies would have importantly influenced the main results.

B12 was the only biomarker evaluated, although we know that levels of this biomarker are often related to levels of several others (e.g., homocysteine, folate, methylmalonic acid). Potential collinearity and interactions among multiple biomarkers could not be taken into account. Also, our approximations of foetal growth and restricted foetal growth by use of gestational age and sex specific birth weight charts are suboptimal, as these outcomes should be estimated by use of serial ultrasound measurements.[41]

Mixing of effects is inherent in observational studies, and residual confounding cannot be ruled out. Reassuringly, we found little discrepancy in the pooled results of the adjusted main models as compared to the extended adjusted models (i.e. additionally adjusting for maternal education and smoking habits).

Possible explanation of findings

Low birth weight is either a result of preterm birth or by being born small at term.[42] While we found that there was an increased risk of preterm birth and LBW among B12-deficient women, there was little evidence that maternal B12 levels influenced offspring birth weight SD score or SGA status (proxies for foetal growth and restricted foetal growth, i.e. accounting for length of gestation and sex). It is, therefore, more likely that the observed higher risk for LBW in B12-deficient women can be explained by preterm birth rather than by reduced foetal growth.

Higher B12 was associated with higher birth weight in low- and middle-income countries, but not in high-income countries. However, four of the five studies included in the low- and middle-income group – contributing with 95% of the evaluated pregnancies – were performed in an Indian population. Therefore, generalisation of these results to low- or middle-income countries outside India should be treated with caution. Indian women generally have lower dietary intake of B12, due to a mainly vegetarian diet, making them susceptible to B12-deficiency.[43] Additionally, Indian newborns are among the smallest in the world, and 30% are born LBW.[42] Our findings may indicate that the pregnancies already at the greatest risk of giving birth to small newborns were the ones most vulnerable to low levels of B12.

The association between B12 and the risk of preterm birth was consistent across studies in both high-income and low- and middle-income countries, and generalisation to countries not studied may be feasible.

In line with our findings, maternal obesity has been associated with B12-deficiency

in several populations.[44,45] It is hypothesised that this association is due to altered fat distribution and metabolism in the overweight compared with normal weight.[44] Maternal weight is positively correlated with newborn weight,[46] and failure to adjust for maternal weight may thus underestimate a positive association between B12 and birth weight, and this was also evident from our sensitivity analyses.

Potential mechanism of action

B12 may potentially affect foetal growth through its close relationship with DNA synthesis and methylation, and the succinyl-coenzyme A pathway.[5–8] Additionally, low levels of B12 may induce an accumulation of homocysteine, resulting in hyperhomocysteinemia.[47] The same is true for low levels of folate, another B-vitamin.[47] Hyperhomocysteinemia is a well-known risk factor for vascular disease,[48] and has been increasingly associated with disorders related to placental function.[49] In a systematic review, hyperhomocysteinemia was found to increase the risk of SGA births.[50] Furthermore, in a recent Mendelian randomisation study, homocysteine was proposed to be causally related to foetal growth.[43] However, based on our findings, it seems unlikely that B12 plays a major role in the link between hyperhomocysteinemia and reduced birth weight.

Few studies have evaluated the role of B12 in predicting length of gestation. A case-control study evaluating Chinese women found that low pre-pregnancy levels of B12 were associated with an increased risk of preterm birth, but not LBW or SGA.[51] In other studies, hyperhomocysteinemia has been hypothesised to affect length of gestation through oxidative stress and placenta dysfunction.[52] This hypothesis has been validated in experimental studies on mice.[53]

It is possible that supplementation of B12 or folic acid, with a subsequent reduction of homocysteine, increases birth weight and length of gestation. However, a Cochrane review concluded that supplementation of folic acid during pregnancy did not reduce the risk of either preterm birth or LBW.[54] We have identified two randomised controlled trials (RCTs) of B12 supplementation during pregnancy that report on birth weight and length of gestation.[55,56] Both studies observed higher B12 plasma levels in the supplemented group compared with the control group, but no reduction in homocysteine levels. No differences were observed in birth weight or frequency of LBW births in the supplemented group compared with the control group in either study. In both studies, B12 supplementation did not affect length of gestation or risk of preterm birth (C. Duggan, personal communication).[56] However, the studies included only 36,655 and 6856 pregnancies, and were not powered to detect small but meaningful differences in preterm birth.

Context

There are 15 million preterm births and 20 million low birth weight births globally each year.[1] The greatest burden of LBW is found in South Asia, while the rate of preterm birth is highest in Africa.[1] Preterm birth is the leading cause of neonatal deaths.[1] In the era of The Millennium Development Goals (1990-2015), the post-neonatal under-five mortality rate was reduced by 58%.[2] The reduction in neonatal mortality was less pronounced (47%).[2] Prevention of preterm birth is thus a key strategy to reduce the number of neonatal deaths

and reach the new target of under-five year mortality of 25 per 1000 live births by 2030, down from 43 per 1000 in 2015, as proposed in the Sustainable Development Goals.[2]

Conclusion and implications for clinical practice and future research

The results of this systematic review with IPD meta-analyses suggest that maternal vitamin B12 level in pregnancy may be an important determinant of preterm birth. Our findings support the conduct of RCTs to evaluate whether maternal B12 supplementation in pregnancy reduces the risk of preterm birth.

Supplementary Material can be found online: http://hdl.handle.net/1765/80025

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Chapter 4

Maternal diet & Child outcomes

Chapter 4.1

Protein intake during pregnancy and offspring body composition at 6 years: The Generation R Study

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Abstract

Purpose: Intra-uterine exposure to protein may affect body composition and may increase the prevalence of childhood adiposity. Therefore, we examined whether protein intake during pregnancy is associated with offspring body composition at the age of 6 years and whether associations differ for animal protein and vegetable protein.

Methods: We included 2694 Dutch mother-child pairs participating in a prospective population-based cohort in Rotterdam, the Netherlands. Energy-adjusted protein was measured in pregnancy using a food-frequency questionnaire and analysed in quartiles. At a mean age of 6.1 ± 0.4 years, we measured children's body mass index (BMI), and fat-free mass index (FFMI) and fat mass index (FMI) using dual-energy X-ray absorptiometry. Outcomes were standardized for age and sex. BMI was used to classify children's overweight status.

Results: After adjustment for sociodemographic and lifestyle factors, a higher maternal protein intake was associated with a higher children's FFMI (difference 0.17 SD [95%CI 0.06; 0.28] for highest vs. lowest quartile of protein intake), but not with children's FMI. A higher maternal protein intake was also associated with children's BMI (difference 0.14 SD [95%CI 0.02; 0.26] highest vs. lowest quartile). Comparable associations were found for animal protein and vegetable protein. Maternal protein intake was not associated with children's overweight.

Conclusions and relevance: This study suggests that higher protein intake during pregnancy is associated with a higher fat-free mass in children at the age of 6 years, but not with their fat mass. Our results do not suggest specific recommendations regarding maternal protein intake during pregnancy to prevent overweight in the offspring.

Introduction

The prevalence of childhood overweight is increasing worldwide.[1] Many overweight children will stay overweight or become obese when reaching adulthood,[2] consequently increasing their risk of developing cardiovascular disease or type 2 diabetes later in life.[3]

Childhood obesity and body mass index (BMI) can be influenced by several determinants such as genetic factors, children's diet, and sedentary behaviour.[4-7] In addition to this, intra-uterine exposures, such as pre-pregnancy BMI and maternal diet, have been suggested to affect body composition of the offspring via foetal programming.[8,9] For example, higher maternal protein intake during pregnancy has been associated with body composition of the child, however the results of several observational studies were inconsistent. Some studies have reported no associations,[10,11] whereas others found that higher maternal protein intake was associated with an increased risk of the offspring becoming overweight,[12] or with a higher offspring lean mass.[13] The exact mechanisms through which maternal protein intake might influence children's body composition have not been clarified, but may involve changes in the release of growth hormones, or prenatal programming of child's appetite.[14,15]

The effect of maternal protein intake on childhood body composition might differ depending on the source of protein. For instance, whether protein is animal-derived or vegetable-derived, because they differ in amino acid composition.[16] However, studies on the effects of different maternal protein sources on childhood body composition are scarce. We hypothesized that the association between maternal protein intake and offspring body composition would depend on the source of protein.

Therefore, the aim of our study was to assess whether maternal protein intake during pregnancy was associated with children's body composition at the age of 6 years. Additionally, we assessed the differences in effect among protein sources (animal versus vegetable protein). Finally, we evaluated whether substitution of maternal protein for other macronutrients would influence these associations.

Subjects and methods

This study was embedded in the Generation R Study, a prospective population-based birth cohort (Rotterdam, the Netherlands). Details of the study have been described in detail previously.[17] All women provided written informed consent at enrolment between April 2002 and January 2006. The study was approved by the Medical Ethics Committee of Erasmus Medical Center Rotterdam and conducted according to the World Medical Association Declaration of Helsinki.

Study population

Out of 8976 women enrolled in the Generation R Study while pregnant, we restricted our analysis to women of Dutch ancestry (n=4101). Ancestry was self-reported and defined according to the classification of Statistics Netherlands.[18] We excluded women with missing dietary information (n=542), women with multiple pregnancies (n=53) or no live childbirth (n=24), and women who were lost-to-follow-up (n=3). In our population of analysis, we

included only mother-child pairs with available childhood body composition information at the age of 6 years (n=2694) (**Supplementary Figure 4.1.1**).

Maternal protein intake

Protein intake during pregnancy (i.e., total, animal, and vegetable protein) was assessed with a 293-item semi-quantitative food-frequency questionnaire (FFQ)[19] that women received at enrolment at median 13.4 (IQR 12.2 – 15.5) weeks of gestation. The FFQ covered the average dietary intake of a Dutch diet over the previous three months. The average daily intake of energy, protein, and other nutrients was calculated using the Dutch food-composition table 2006.[20] Validation of the FFQ against three instances of 24-hour dietary recall in 71 pregnant women of Dutch ancestry living in Rotterdam showed an intra-class correlation coefficient of 0.65 for energy-adjusted protein intake. There was no indication for systematic measurement error (**Supplementary Figure 4.1.2**).

Body composition measurements

Children visited the research centre at a mean (±SD) age of 6.1 ± 0.4 years. We measured height (using a Harpenden stadiometer) and weight (using an electronic personal scale (Seca®)) to calculate their BMI (kg/m^2). This BMI was also used to classify overweight status according to age- and sex-specific cut-offs.[21]

During this visit, body composition was measured by dual-energy X-ray absorptiometry (DXA) (iDXA; General Electrics-Lunar, 2008, Madison, WI, USA) following standardized procedures.[22] The DXA-scanner calculated fat, lean, and bone mass of the total body and of specific body regions, using enCORE software (version 13; GE Healthcare). Fat-free mass index (FFMI (kg/m^2); calculated as total fat-free mass divided by height squared) and fat mass index (FMI (kg/m^2); total fat mass divided by height squared) were calculated. Additional outcome measurements were lean mass index (kg/m^2, total fat-free mass minus total bone mass divided by height squared), total fat percentage (total body fat mass divided by total body mass times 100%), and android/gynoid fat mass ratio (android fat mass divided by gynoid fat mass). All outcomes were standardized for age and sex of the child.

Covariates

At enrolment, we collected information by questionnaire on maternal age, educational level, folic acid supplement use, and parity. Additionally, weight and height were measured at the research centre at enrolment to calculate BMI and a foetal ultrasound was performed to establish gestational age. Energy, fat, and carbohydrate intake during pregnancy were measured using the FFQ described previously. Smoking and alcohol use were assessed during each trimester by questionnaire and categorized into never users, stopped when pregnancy was known, and continued use during pregnancy. Gestational weight gain (g/week) was calculated by subtracting maternal weight at enrolment from the weight in early third trimester and divided by the follow-up duration (weeks).

At birth, we collected information on gestational age at birth, birthweight Z-score, sex, and hypertensive pregnancy complications (i.e. preeclampsia and pregnancy-induced hypertension) from delivery reports.[23] Preterm birth was defined as childbirth before 37

weeks of gestation. Breastfeeding practice at two months was assessed by a combination of delivery reports and questionnaires. Child protein and energy intake were measured using an FFQ at a median age of 12.9 (IQR 12.6 – 14.1) months in a subgroup of our population (n=1591). At the age of 6 years, information on screen time (<2 vs. ≥2 hours/day[24]) and participation in sports (yes/no) of the children was collected using a questionnaire.

Statistical methods

Maternal protein intake was adjusted for total energy using the nutrient residual method to evaluate the effect of maternal protein intake independent of energy intake and to reduce the magnitude of measurement error.[25] We categorized protein intake into quartiles and used the lowest quartile (Q1) as the reference category. Because of skewed distributions, total body fat percentage and android/gynoid fat mass ratio were natural-log transformed.

We used multivariable linear regression models to assess the associations of maternal total, animal, and vegetable protein intake with childhood body composition measurements. Multivariable logistic regression models were used for childhood overweight.

The analyses were performed with energy-adjusted protein intake during pregnancy. Analyses with animal protein intake were adjusted for vegetable protein intake, and vice versa (*Model 1*). Additionally for the outcomes total fat percentage and android/gynoid fat mass, *Model 1* included also height of the child. The decision to include confounders in the multivariable regression models (*Model 2*) was based on previous literature or a >10%-change of the effect estimate in *Model 1*. The following confounders were considered: maternal age, educational level, parity, smoking and alcohol consumption in pregnancy, folic acid supplementation, energy intake, gestational age at birth, breastfeeding, childhood sedentary time, and childhood physical activity. The confounders included are listed in the footnotes of the figures and tables. Potential intermediate factors were added to a separate multivariable model (*Model 3*), namely maternal BMI, gestational weight gain, hypertensive complications during pregnancy, and birthweight Z-score. Effect modification was evaluated for maternal BMI, gestational weight gain, and child sex, because the associations between maternal protein intake and childhood body composition may differ due to differences in protein intake or reporting as well as differences in childhood body composition.[26-28] In case of significant effect modification (p-value for interaction term <0.05), stratified analyses were performed.

To evaluate whether the observed associations were due to a higher protein intake rather than a lower intake of another macronutrient, we assessed if substituting protein with other macronutrients (e.g., carbohydrates and fat) had any effect on our results.[29] For example, the substitution model for replacing protein by carbohydrates included the macronutrients (in energy percent (E%)) protein, fat and alcohol, but not the macronutrient carbohydrate). As a result, the regression coefficients for protein from these models reflect the effect of replacing 1 E% from carbohydrates with 1 E% of protein.

To evaluate the robustness of our findings, several secondary analyses were performed. First, we further adjusted our models for protein intake of the children (n=1591). Second, we restricted analyses to women with a child born after 37 weeks of gestation, those without hypertensive complications in pregnancy, and to children with a normal birthweight

(which we defined as a gestational age- and sex-adjusted birthweight between ±2 SD). Also, we excluded siblings (n=185), and finally we excluded the covariate child height from the multivariable models since height might also be associated with obesity.[30]

To reduce bias due to missing data, missing covariates (0 – 17.7%) were imputed using multiple imputation which includes fully condition specification of the imputation. Ten imputed data sets were created and the analyses were performed in each dataset before the results were pooled by Rubin's rules[31] taking into account uncertainty with the prediction of missing data. Details on the imputation procedure are described in **Supplemental Table 4.1.1**. All statistical analyses were performed in SPSS version 21.0 (IBM Corp., Armonk, NY, USA).

Results

Subject characteristics

Maternal and child characteristics are presented in **Table 4.1.1**. The main sources of protein in our study population were dairy products, meat and meat products, and nuts and seeds (together explaining 60% of the variance in total protein intake). Mothers with a higher protein intake were, on average, older, had greater levels of education, more often non-smokers and used more frequently folic acid supplementation than those with a lower protein intake (**Supplementary Table 4.1.2**).

Table 4.1.1. Subject characteristics, the Generation R Study (n=2694)[a]

Maternal Characteristics (n=2694)		**Original data**	**Imputed data[b]**
Gestational age at enrolment (weeks)		13.4 (12.2 – 15.5)	No missing values
Age (years)		31.7 ± 4.2	No missing values
Maternal education (%)	Low and midlow (%)	11.9	12.0
	Midhigh (%)	53.0	53.0
	High (%)	35.1	35.1
Nulliparity (%)		61.9	61.8
Body mass index at enrolment (kg/m^2)		23.4 (21.6 – 26.0)	23.4 (21.6 – 26.0)
Gestational weight gain[c] (g/week)		503 ± 196	475 ± 204
Smoking during pregnancy	Never (%)	75.9	76.1
	Until pregnancy was known (%)	9.5	9.5
	Continued (%)	14.6	14.4
Alcohol during pregnancy	Never (%)	31.4	31.2
	Until pregnancy was known (%)	16.7	16.7
	Continued (%)	51.8	52.0
Alcohol consumption (g/day)		0.0 (0.0 – 0.7)	No missing values
Folic acid supplementation	No (%)	9.2	9.5
	Started < 10 wk of gestation (%)	90.8	90.5
Energy intake (kcal/day)		2153 ± 503	No missing values
Protein intake (g/day)	Total protein	80 ± 19	No missing values
	Animal protein	49 ± 14	No missing values
	Vegetable protein	31 ± 9	No missing values

Table 4.1.1. (continued) Subject characteristics The Generation R Study (n=2694)[a]

		Original data	Imputed data[b]
Protein intake (E%)	Total protein	15 ± 2	No missing values
	Animal protein	9 ± 2	No missing values
	Vegetable protein	6 ± 1	No missing values
Pregnancy and birth outcomes			
	Hypertensive complications (%)	7.3	Not imputed
	Gender, boy (%)	50.1	No missing values
	Birth weight (g)	3503 ± 541	3503 ± 540
	Gestational age at birth (weeks)	40.0 ± 1.7	No missing values
	Preterm birth (%)	4.2	No missing values
	Breastfeeding at 2 months (%)	69.8	68.0
Dietary intake of the children at 13 months of age			
	Energy intake (kcal/day)	1300 ± 342	Not imputed
	Protein intake (g/day)	41 ± 11	Not imputed
	Protein intake (E%)	13 ± 2	Not imputed
Children's characteristics at 6 years of age			
	Age (years)	6.1 ± 0.4	No missing values
	Playing sports (%)	50.0	49.9
	≥ 2h/day screen time (%)	19.9	20.8
	Height of the children (cm)	120 ± 6	No missing values
	Overweight/obese (%)	11.3	Not Imputed
	Body mass index (kg/m^2)	15.7 (15.0 – 16.6)	No missing values
	Fat mass index (kg/m^2)	3.6 (3.1 – 4.2)	Not imputed
	Fat-free mass index (kg/m^2)	11.9 ± 0.8	Not imputed
	Total fat percentage (%)	23 (21 – 27)	Not imputed
	Android/gynoid fat mass ratio	0.24 (0.21 – 0.27)	Not imputed

[a.] Values represent % for categorical variables, and for continuous variables mean ± SD or median (interquartile range); [b.] Percentages may not add up to 100% because of pooling of the imputed datasets; [c.] Weekly gestational weight gain (g/week) between enrolment around 13 weeks of pregnancy and early-third trimester (around 30 weeks). Missing values: maternal education (1.3%), nulliparity (0.1%), body mass index at enrolment (0.5%), gestational weight gain (17.6%), smoking during pregnancy (7.8%), alcohol during pregnancy (8.5%), folic acid supplementation (17.7%), hypertensive complications (3.2%), birthweight (0.1%), breastfeeding at 2 months (15.0%), energy and protein intake of the children at 13 months of age (40.9%), playing sports at the age of 6 years (6.3%), screen time at the age of 6 years (15.2%), overweight at the age of 6 years (0.2%), fat mass index (2.6%), fat-free mass index (2.6%), total fat percentage (2.6%), android/gynoid fat mass ratio (2.6%).

Protein intake during pregnancy and body composition in childhood

Children of mothers in the highest quartile (Q4) of protein intake had a 0.14 SD higher BMI (95%CI 0.02; 0.26) than children of mothers in the lowest quartile (Q1) (**Figure 4.1.1**). Both animal protein and vegetable protein intake were also associated with a higher childhood BMI (**Table 4.1.2**) after adjustment for confounders.

Total maternal protein intake was not associated with childhood overweight at the age of 6 years (OR 1.13 (95%CI 0.80; 1.59) Q4 vs. Q1), after adjusting for educational level, maternal alcohol and folic acid supplementation during pregnancy (p-value for trend =

0.31) and neither was the source of maternal protein intake associated with childhood overweight.

Higher protein intake during pregnancy was associated with a higher FFMI in children aged 6 years (difference 0.17 SD (95%CI 0.06; 0.28) for Q4 vs. Q1) (**Figure 4.1.1**), and effect estimates were similar for animal and vegetable protein (**Table 4.1.2**). Maternal total protein intake remained significantly associated with childhood FFMI (difference 0.15 SD (95%CI 0.04; 0.25) for Q4 vs. Q1, p-value for trend = 0.01) after additional adjustment of the potential intermediate factors maternal BMI, gestational weight gain, hypertensive complications, and birthweight (*Model 3*).

Maternal protein intake was not associated with childhood FMI (**Figure 4.1.1**). Vegetable protein intake was associated with lower FMI in *Model 1*, but this association did not remain after adjustment for lifestyle factors and sociodemographic background (**Table 4.1.2**). Conversely, higher maternal animal protein intake was only associated with higher childhood FMI after adjustment (**Table 4.1.2**). In **Supplementary Table 4.1.3**, we have added the results from the main analyses that were performed in a non-imputed dataset. These results largely overlapped with the 95% confidence intervals of the results obtained from the imputed datasets. In multivariable models, we did not find associations of maternal protein intake with body fat percentage or android/gynoid fat mass ratio (**Supplementary Table 4.1.4**).

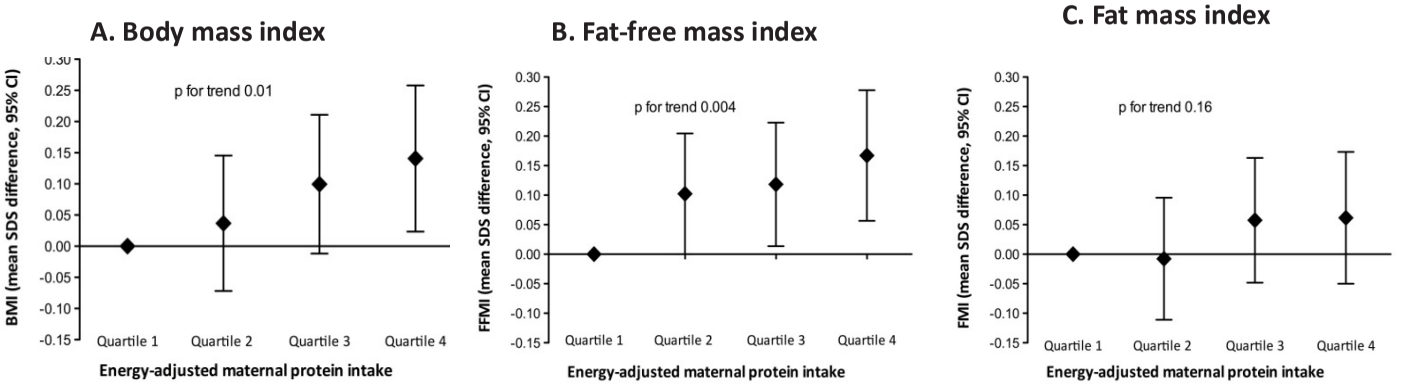
Secondary analyses

Because we observed significant influence of protein intake and maternal BMI on childhood BMI and childhood FFMI (p-value for interaction term <0.05), we stratified our analyses on maternal overweight status (strata: BMI <25 kg/m^2 and BMI ≥25 kg/m^2). The effect estimates between maternal protein intake and childhood BMI and FFMI were larger in mothers with a BMI ≥25 kg/m^2 than in mothers with a BMI <25 kg/m^2 (**Supplementary Table 4.1.5**).

We did not observe specific substitution effects when protein (E%) was exchanged for different types of macronutrients in the association with FFMI (**Table 4.1.3**). Additional adjustment for protein intake of the child at 14 months of age (n=1558; 59%), slightly attenuated the results with FFMI, but they remained statistically significant (**Supplementary Table 4.1.6**).

The association between maternal protein intake and childhood lean mass index was similar to those for FFMI (**Supplementary Table 4.1.7**). When we restricted the analyses to a healthy population (i.e. term birth, normal birth weight and mothers without hypertensive complications), there were no large differences in effect estimates (**Supplementary Table 4.1.8**). Excluding siblings from our population or excluding current height of the children from the analyses did not change the effect estimates (*data not shown*).

Figure 4.1.1. Associations of protein intake during pregnancy with different measures of offspring body composition at the age of 6 years



Mean difference (95% CI) in regression coefficients reflect the difference in age- and sex-specific SDS of childhood body mass index (panel A), fat-free mass index (panel B), and fat mass index (panel C) relative to the first quartile of energy-adjusted protein intake. Adjusted for maternal age, maternal education, maternal smoking and alcohol use during pregnancy, folic acid supplementation, maternal energy and carbohydrate intake, gestational age at birth, breastfeeding practice 2 months postpartum, and screen-time of the children at 6 years of age. Abbreviations: BMI, body mass index; CI, confidence interval; FMI, fat mass index; FFMI, fat-free mass index; SDS, standard deviation score.

Table 4.1.2. Association of maternal animal and vegetable protein intake during pregnancy with childhood body composition at the age of 6 years (n=2694)

	Childhood body mass index (SDS) (n=2694)		**Fat-free mass index** (SDS) (n=2624)		**Fat mass index** (SDS) (n=2624)	
	Model 1[a]	***Model 2***[b]	***Model 1***[a]	***Model 2***[b]	***Model 1***[a]	***Model 2***[b]
Animal protein intake[c]	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Quartile 1 (Low)	reference	reference	reference	reference	reference	reference
Quartile 2	0.03 (-0.07; 0.14)	0.09 (-0.02; 0.20)	0.10 (-0.00; 0.20)	**0.11 (0.01; 0.21)**	0.01 (-0.09; 0.11)	0.08 (-0.02; 0.19)
Quartile 3	0.04 (-0.07; 0.14)	**0.12 (0.01; 0.24)**	**0.11 (0.01; 0.21)**	**0.12 (0.01; 0.22)**	-0.02 (-0.12; 0.08)	0.09 (-0.01; 0.20)
Quartile 4 (High)	0.07 (-0.04; 0.18)	**0.19 (0.07; 0.31)**	**0.18 (0.08; 0.28)**	**0.20 (0.08; 0.31)**	-0.03 (-0.14; 0.07)	**0.12 (0.01; 0.24)**
p for trend	*0.23*	***0.002***	***0.001***	***0.001***	*0.45*	***0.048***
Vegetable protein intake[c]						
Quartile 1 (Low)	reference	reference	reference	reference	reference	reference
Quartile 2	-0.07 (-0.18; 0.04)	0.02 (-0.09; 0.12)	0.06 (-0.04; 0.16)	0.06 (-0.04; 0.16)	-0.10 (-0.20; 0.01)	0.01 (-0.09; 0.11)
Quartile 3	-0.03 (-0.13; 0.08)	0.10 (-0.01; 0.22)	**0.14 (0.04; 0.24)**	**0.15 (0.04; 0.25)**	**-0.11 (-0.21; -0.01)**	0.06 (-0.04; 0.17)
Quartile 4 (High)	-0.03 (-0.14; 0.08)	**0.12 (0.00; 0.24)**	**0.22 (0.12; 0.32)**	**0.22 (0.11; 0.33)**	**-0.19 (-0.30; -0.09)**	0.01 (-0.10; 0.13)
p for trend	*0.75*	***0.02***	***<0.001***	***<0.001***	***0.001***	*0.61*

Results from multivariable linear regression analyses, based on imputed data. The regression coefficients (95% CI) reflect the difference in age- and sex-specific SDS of childhood body mass index, fat-free mass index and fat mass index relative to the first quartile of energy-adjusted protein intake. Trend tests were conducted by using the quartiles of protein intake as a continuous variable in the model. The associations of total protein intake with childhood body mass index, fat-free mass index and fat mass index are shown in Figure 1; changes in effect estimates after adjustment for confounders was comparable to the results presented in this Table. [a.] *Model 1*: Vegetable and animal protein intake were additionally adjusted for each other; [b.] *Model 2: Model 1* further adjusted for maternal age, educational level, smoking and alcohol use and folic acid supplementation during pregnancy, energy and carbohydrate intake during pregnancy, gestational age at birth, breastfeeding 2 months postpartum and screen time of the children at 6 years of age; [c.] Energy-adjusted protein intake using the nutritional residual method. Abbreviations: CI, confidence interval; SDS, standard deviation score.

Table 4.1.3. Substitution of maternal protein intake with other macronutrients and its ssociation with childhood fat-free mass index at the age of 6 years (n=2624)[a]

Fat-free mass index (SDS) n=2624	
	β (95% CI)
Total Protein (E%)	
Substitution for carbohydrate	**0.03 (0.02; 0.05)**
Substitution for monosaccharides and disaccharides	**0.03 (0.02; 0.05)**
Substitution for polysaccharides	**0.03 (0.01; 0.04)**
Substitution for fat	**0.04 (0.02; 0.06)**
Substitution for saturated fat	**0.03 (0.01; 0.05)**
Substitution for unsaturated fat	**0.04 (0.02; 0.06)**
Substitution for alcohol	0.04 (-0.03; 0.11)
Animal protein (E%)	
Substitution for carbohydrate	**0.03 (0.01; 0.05)**
Substitution for monosaccharides and disaccharides	**0.03 (0.01; 0.05)**
Substitution for polysaccharides	**0.03 (0.01; 0.05)**
Substitution for fat	**0.04 (0.02; 0.06)**
Substitution for saturated fat	0.02 (-0.00; 0.05)
Substitution for unsaturated fat	**0.04 (0.02; 0.06)**
Substitution for alcohol	0.03 (-0.03; 0.10)
Vegetable protein (E%)	
Substitution for carbohydrate	**0.07 (0.03; 0.11)**
Substitution for monosaccharides and disaccharides	**0.09 (0.04; 0.14)**
Substitution for polysaccharides	**0.09 (0.03; 0.15)**
Substitution for fat	**0.08 (0.04; 0.12)**
Substitution for saturated fat	**0.09 (0.04; 0.13)**
Substitution for unsaturated fat	**0.10 (0.05; 0.16)**
Substitution for alcohol	**0.07 (0.00; 0.15)**

[a.] The effect estimates can be interpreted as difference in fat-free mass index per exchange of 1 E% from protein or sources of protein with an isocaloric amount of another macronutrient, while keeping the other macronutrients constant. Analysis were adjusted for maternal age, educational level, smoking and alcohol use and folic acid supplementation during pregnancy, gestational age at birth, breastfeeding 2 months postpartum, and screen time of the children at 6 years of age. Abbreviations: CI, confidence interval; E%, energy percent; SDS, standard deviation score.

Discussion

The results of this observational study indicate that higher protein intake during pregnancy is associated with higher fat-free mass in the offspring at the age of six years, but not with fat mass. These associations were similar for animal and vegetable protein and we did not observe any specific substitution effect of maternal protein for other macronutrients.

Our results suggest that the higher BMI in children of mothers with a higher protein intake was driven by a higher fat-free mass in the offspring rather than a higher fat mass. This implies that BMI, a method frequently used to assess adiposity, is in children an inaccurate measurement of excess fat mass, a finding which has been addressed by Freedman et al.[32] In addition, we found that maternal protein intake was not associated with childhood fat mass

after taking into account differences in maternal lifestyle and sociodemographic factors, a finding in line with results from previous cohort studies.[11,13]

Our finding that children of mothers with a higher protein intake had a higher fat-free mass could not be explained by maternal lifestyle and socioeconomic characteristics, nor could it be explained by maternal BMI, gestational weight gain, hypertensive complications, birth weight, or by infant protein intake. Furthermore, the association was no different when excluding bone mass from the analyses (lean mass index). These results are in line with those of Brion et al.[13] who performed an observational study in 5534 mother-child pairs, which found that higher maternal protein intake was associated with higher fat-free mass, but not with fat mass, in the offspring at the age of 10 years. Furthermore, this study showed that maternal but not paternal protein intake was associated with children's fat free mass, suggesting intrauterine effects. Conversely, another smaller study (n=264) reported no association of maternal protein intake with fat-free mass in the offspring at the age of 16 years.[11]

We found differential associations of maternal protein intake with childhood BMI and FFMI in overweight versus non-overweight women. A higher childhood fat mass has been reported in women who are obese during pregnancy,[33] whereas this has not been found for childhood fat-free mass.[34] This differential finding in overweight versus non-overweight women may be due to differences in glucose levels, insulin sensitivity, and growth hormones.[35,36] Another explanation could be that the level of residual confounding (e.g., related to physical activity levels) is different for overweight women from that of non-overweight women.

We did not observe consistent differential effects for maternal animal or vegetable protein intake. A previous study that investigated the association between different sources of maternal protein intake and body composition in the offspring reported that animal, but not vegetable, protein intake during pregnancy was associated with higher BMI in the offspring.[12] However, this association was only found in female offspring.[12] Whether specific sources of maternal protein do in fact influence body composition differently requires further study. We did not observe any specific macronutrient substitution effect, which indicates that it does not matter whether maternal protein intake is increased at the expense of fat, or carbohydrate, or specific subtypes of these macronutrients.

Maternal protein intake might influence childhood body composition through several mechanisms. Protein intake is needed for the regulation and accretion of muscle mass, which is a major component of fat-free mass.[37] In line with our results, a study in pigs showed that a higher maternal protein intake during pregnancy led to a higher lean but not fat mass in the offspring.[38] Further analyses of skeletal muscle of the piglets revealed that the effect on muscle mass may be due to both increased myogenesis and muscular differentiation. Further potential mechanisms that could influence child growth may be changes in secretion of growth hormones,[14] or prenatal programming of children's appetite.[15]

Strengths and limitations

Strengths of this study are the prospective population-based design, the large sample size, the postnatal follow-up of the offspring through 6 years of age, and the collection of

numerous confounding factors. A further strength is the detailed information we collected with regard to body composition measurements, since DXA has a high accuracy of measuring fat mass and other soft-tissue body composition components.[39,40]

However, some limitations should be considered when interpreting our results. A limitation of our study is the measurement of protein intake using an FFQ, which is not very precise. However, FFQs have been shown to be accurate in ranking participants according to their intake,[41] and energy-adjustment may have reduced the magnitude of measurement error.[25] Also there might be measurement error of the anthropometric measurements (i.e., BMI during pregnancy) and other covariates. However, since these are measured before the objective outcome measurement (i.e., body composition of the child) this measurement error is most likely non-differential and not leads to differential associations between dietary protein intake during pregnancy and body composition of the child. Since we had no data on maternal physical activity during pregnancy, residual confounding due to maternal physical activity levels could influence our results. A third limitation is the restriction to women of Dutch ancestry in our analyses within this multi-ethnic prospective cohort study. While the inclusion of other ethnicities could have led to differential misclassification of dietary intake,[42] the restriction with regard to ethnicity may reduce the external validity of our results. Also, the Generation R Study consisted of a higher percentage of women with higher socioeconomic status than those that were eligible to participate.[17] However, such a selection bias has not been found to influence exposure-outcome associations.[43]

Conclusion

We found that higher protein intake during pregnancy is associated with higher childhood fat-free mass, but not with childhood fat mass. The associations did not differ for vegetable versus animal protein and the associations were not explained by maternal lifestyle or sociodemographic factors. Also, it did not matter whether protein intake was substituted for maternal fat or carbohydrate intake. Our results do not implicate specific recommendations on maternal protein intake during pregnancy to prevent overweight in children, however it may be relevant for discussions on the influence of healthy diet during pregnancy on offspring lean mass. Further research is needed to identify the underlying mechanisms related to the observed associations (i.e. potential pathways related to different amino acids).

Supplementary Material can be found online: http://hdl.handle.net/1765/80025

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Chapter 4.2

Maternal dietary patterns during pregnancy and offspring cardiometabolic health at age 6 years: The Generation R Study

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Abstract

Background & aims: Maternal nutrition during pregnancy might be important in influencing offspring cardiometabolic health. However, research has focused mostly on specific nutrients or total energy, and possible effects of whole diet are unclear. We aimed to assess the associations between different dietary patterns during pregnancy and offspring cardiometabolic health among 2592 mother-child pairs from Generation R, a prospective population-based cohort study from foetal life onwards in Rotterdam, the Netherlands.

Methods: Maternal diet was assessed in early pregnancy with a food-frequency questionnaire. We identified three *a posteriori*-dietary patterns, namely a 'Vegetable, fish and oil', 'Nuts, soy and high-fibre cereals' and 'Margarine, snacks and sugar'-pattern. An *a priori*-pattern was created based on the 'Dutch Healthy Diet Index'. Cardiometabolic health (pulse wave velocity, blood pressure, insulin, HDL-cholesterol and triglycerides) was measured at the child's age of 6 years.

Results: In the crude models, the 'Vegetable, fish and oil', 'Nuts, soy and high-fibre cereals' and 'Dutch Healthy Diet Index' seemed beneficial, as higher adherence to these patterns was significantly associated with lower blood pressure and lower pulse wave velocity. After adjustment for other sociodemographic and lifestyle factors, most associations disappeared, except for lower pulse wave velocity with the 'Vegetable, fish and oil'-dietary pattern (-0.19 SD (95%CI -0.33; -0.06), highest quartile of adherence vs. lowest quartile). No associations were found between maternal dietary patterns and offspring blood lipids or insulin levels.

Conclusions: Our results suggest that there are no consistent independent associations of maternal dietary patterns with offspring cardiometabolic health at 6 years.

Introduction

Cardiometabolic diseases in adults have been linked to exposures during early life.[1] One of the consequences of malnutrition during pregnancy is low birth weight, and this may predispose higher risk of cardiometabolic diseases later in life.[2,3] However, the Hungerwinter study showed that maternal malnutrition was associated with offspring health without affecting size at birth.[4] In addition, micronutrient status during pregnancy has been related to cardiometabolic outcomes in the offspring, also independent of child's birth weight.[5] This suggests that total energy intake and foetal growth restriction are not the only pathways in predisposing these children to a higher risk of chronic disease, but that a direct effect of maternal diet might exist.[6,7]

Severe energy restriction during pregnancy is suggested to influence offspring health.[2] However, what the optimal diet is during pregnancy for adequate child health is still an unresolved question.[8] In addition, human studies on intrauterine exposures and later cardiometabolic health mainly focused on birth weight, and studies on the role of maternal diet are scarce and inconsistent.[8,9]

Last decades, research in nutritional epidemiology started focusing on overall diet instead of individual nutrients or foods, to take the interactions within diet into account.[10,11] Furthermore, studies based on dietary patterns are helpful in translating results from nutritional epidemiology to food-based dietary guidelines.[12]

A priori-dietary patterns are usually defined based on dietary guidelines and expert advice, and thus generally reflect a diet that is related to health outcomes.[10] *A posteriori*-dietary patterns are data-driven and thus reflect actual dietary patterns within specific study populations. We examined the associations of different types of *a posteriori*-derived and *a priori*-defined dietary patterns during pregnancy with cardiometabolic health in offspring at the age of 6 years.

Materials and methods

Design

The present study was embedded within the Generation R Study, a population-based cohort study from foetal life onwards that has been previously described in detail.[13] The study was conducted following the World Medical Association Declaration of Helsinki and was approved by the Medical Ethics Committee at Erasmus University Medical Center. Written consent was obtained from all participants.

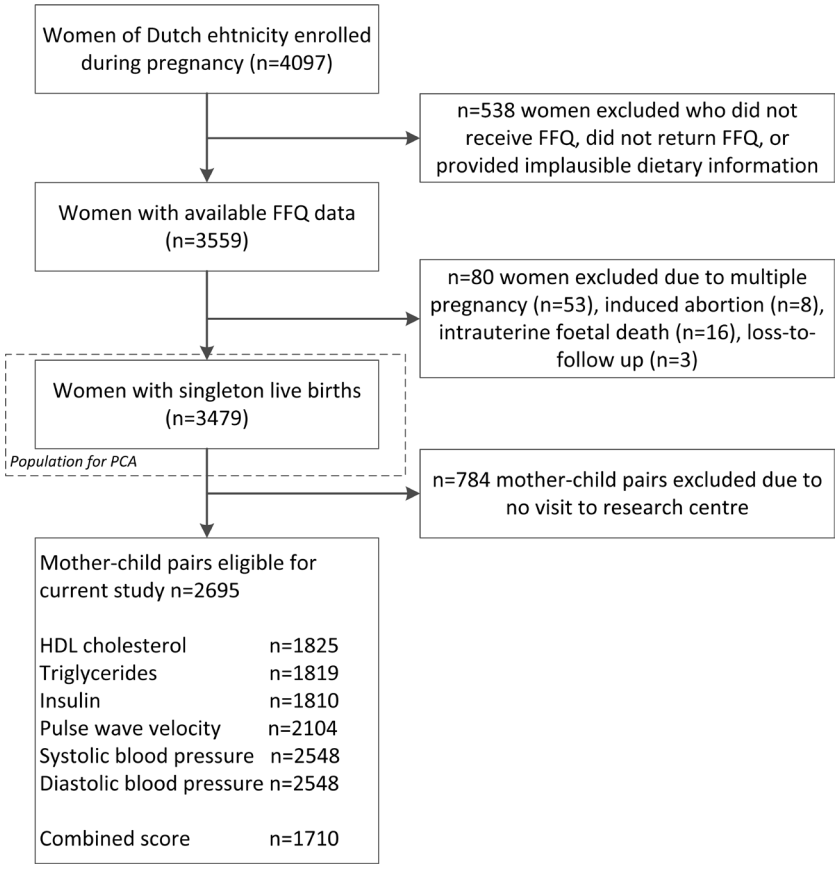
Population

A flowchart of the selection process of the study population is shown in **Figure 4.2.1**. Since cultural differences could influence dietary patterns and the food-frequency questionnaire (FFQ) was designed for a Dutch population, we included only mothers of Dutch national origin. Dietary patterns were determined in the 3479 mothers with dietary data available and a singleton live birth. At the child's age of 6 years, 2689 children visited the research centre. Since not all children had all measurements done, population for analysis ranged from 1710 to 2548 (**Figure 4.2.1**).

Dietary assessment

Diet in early pregnancy (median 13.4 weeks of gestation, 95%-range 9.9-22.8) was assessed with an adapted version of the semi-quantitative 170-item FFQ from Klipstein-Grobusch et al.[14] In addition, for the purpose of this study, the FFQ was complemented with additional food items and the final FFQ consisted of 293 food items. This FFQ was validated with three 24h-recalls in a group of Dutch pregnant women in Rotterdam (n=71), who were visiting the community midwife practices. The intraclass correlation coefficients (ICCs) for energy-adjusted macronutrient intake were between 0.48 and 0.68 (*unpublished data*).

Figure 4.2.1: Population for analysis



Abbreviations: FFQ, food-frequency questionnaire; PCA, principal component analysis.

A priori-dietary patterns

An *a priori*-dietary pattern was defined based on the previously constructed 'Dutch Healthy Diet Index'(DHD-index) developed by van Lee et al.[15] The DHD-index comprises of ten components: physical activity, vegetable, fruit, dietary fibre, fish, saturated fat (SFA), *trans*-fat (TFA), consumption occasions with acidic drinks and foods sodium and alcohol, which

represent the 2006 Dutch dietary guidelines.[16] For the purpose of this study, we omitted the components 'physical activity', 'consumption of acidic drinks and foods' and 'TFA' since this data was not collected during pregnancy.

We also excluded the alcohol component, because women who consume one unit of alcohol per day would still receive the maximum score for the DHD-index, while during pregnancy any alcohol consumption is discouraged because of the known adverse effects on the foetus.[17]

The scores for the remaining six DHD-index components ranged between 0 and 10 points, resulting in a total summed score ranging between 0 and 60 points. Higher scores correspond to a higher level of adherence to the Dutch dietary guidelines and therefore a healthier diet.

Table 4.2.1. Factor loadings of food groups in dietary patterns of the women during pregnancy (n=3479)

Food group	**'Vegetable, fish and oil' dietary pattern**	**'Nuts, soy and high-fibre cereals' dietary pattern**	**'Margarine, snacks and sugar' dietary pattern**
Potatoes and other tubers	0.05	**-0.53**	**0.21**
Vegetables	**0.78***	0.17	-0.03
Fruits	0.13	**0.37**	0.02
Dairy products – high fat	**0.26**	**-0.26**	**0.29**
Dairy products – low fat	-0.15	**0.29**	0.16
Cereals – high fibre	**0.24**	**0.43***	**0.36**
Cereals – low fibre	**0.23**	-0.16	**0.25**
Meat and meat products	0.08	**-0.54**	**0.33**
Fish and shellfish	**0.45***	**0.24**	-0.11
Eggs and egg products	**0.27**	0.05	0.19
Vegetable oils	**0.74***	0.08	-0.12
Margarine and butter	-0.06	-0.03	**0.61***
Sugar and confectionary and cakes	-0.11	0.13	**0.56***
Snacks	0.05	0.08	**0.40***
Coffee and tea	**0.28**	**0.34**	0.10
Sugar-containing beverages	-0.14	**-0.28**	**0.29**
Light soft drinks	0.13	**0.28**	0.02
Alcoholic beverages	**0.35**	-0.00	-0.04
Condiments and sauces	0.05	-0.09	**0.39**
Soups and bouillon	0.19	-0.02	0.15
Nuts, seeds and olives	0.03	**0.64***	**0.30**
Soy products	0.01	**0.39***	-0.10
Legumes	**0.44**	-0.02	0.07
Explained % of variance	**10.9%**	**8.0%**	**6.9%**

Food groups with bold numbers are considered to have a strong association (factor loading ≥ 0.2 or ≤ -0.2) with a dietary pattern. The three highest positive factor loadings per dietary pattern are shown with an asterisk (*) and are used to label the pattern. The three dietary patterns together explained 25.8% of the total variance in maternal dietary intake.

A posteriori-dietary patterns

Principal Component Analysis (PCA)[11] was used in order to determine *a posteriori*-dietary patterns. First, the 293 individual food items were reduced to 23 food groups (**Table 4.2.1**). This division was based on the Dutch National Food Consumption Survey classification,[18] but some adjustments towards this division have been made in order to better capture specific nutrients (e.g., dividing cereals into low and high-fibre cereals). All factors (i.e., dietary patterns) with an eigenvalue of ≥1.5 were extracted. To improve interpretation of the dietary patterns, the Varimax rotation was used.[19] Subsequently, a factor loading was calculated for each single food group, which illustrates the extent to which each food group is correlated with the specific dietary pattern. The three highest factor loadings per dietary pattern were used to label the dietary pattern (**Table 4.2.1**). For each mother, regression-based scores were extracted and used as adherence scores for these dietary patterns. Subsequently, the adherence scores of the population for analysis (n=2695) were categorized into quartiles.

Cardiometabolic risk factors

At age 6 years, all children were invited to our dedicated research facility at the Sophia's Children Hospital. While the children were lying, systolic and diastolic blood pressure (SBP and DBP) were measured at the right brachial artery for four times with one-minute intervals, using the validated automatic phygmomanometer Datascope Accutor Plus TM (Paramus, NJ, USA). Mean SBP and DBP were calculated, with exclusion of the first measurement. Carotid-femoral pulse wave velocity (PWV) was assessed using the automatic Complior SP device (Complior;Artech Medical, Pantin, France) with participants in supine position. Non-fasting blood samples were drawn by antecubital venipuncture. Insulin, HDL cholesterol (HDL-c), and triglyceride concentrations were measured with enzymatic methods (using a Cobas 8000 analyser, Roche, Almere, The Netherlands). Quality control samples demonstrated intra-assay and inter-assay coefficients of variation ranging from 0.69 to 1.57%. Body fat was measured by Dual-energy X-ray absorptiometry (DXA) scans (iDXA; General Electric, 2008, Madison, WI, USA). Percentage body fat (BF%) was calculated as 100%*[total body fat mass(g)]/[total body mass (fat mass+lean mass+bone mass)(g)]. Body fat percentage was analysed as part of a separate study focused on body composition, and is therefore not presented. Age- and sex-specific SD scores were created for all outcomes based on the total Generation R population with available measurements. Insulin was not normally distributed and was therefore transformed with square root transformation before standardizing.

In addition to the individual cardiometabolic outcomes, we calculated a continuous cardiometabolic risk factor score. Following examples of previously defined metabolic syndrome scores for children,[20] we included BF%, blood pressure (including DBP and SBP), and serum levels of HDL-c, triglycerides, and insulin. We summed the age- and gender-specific SD-scores of these five variables, as proposed previously for paediatric populations.[20] Hence, the cardiometabolic risk factor score was calculated as: SDS BF% + 0.5*SDS SBP + 0.5*SDS DBP + SDS triglycerides + (-1*SDS HDL-c) + SDS insulin.

Covariates

Information regarding paternal age, maternal age, pre-pregnancy BMI (self-reported pre-pregnancy weight divided by height measured at intake (squared)), education (low vs. high), family income (<2200 vs. >2200 Euros per month), parity (nulliparous vs. multiparous), maternal smoking and alcohol during pregnancy (never, until pregnancy was known, or continued throughout pregnancy), folic acid supplementation (never, started during first 10 weeks, or started periconceptional) and stress during pregnancy (Global Severity Index) was obtained from prenatal questionnaires sent in different trimesters. Information regarding breastfeeding of the child (never, partially breastfed in the first 4 months, or exclusively breastfed for 4 months) was collected by a combination of delivery reports and postnatal questionnaires. Other postnatal questionnaires included information on watching television (hours/day) at 2 years and participation in sports (yes/no) at 6 years. Diet at 1 year was assessed with an FFQ and a diet quality score was created.[21]

Statistical analyses

The dietary patterns were analysed categorically (in quartiles) as well as linearly (per SD score). All associations were first assessed in a crude model. Additionally, potential confounders were entered individually into a linear regression model of dietary patterns and the cardiometabolic risk factor score, and were included in all models when they induced a change in effect estimate of at least 5% for any dietary pattern. Hence, the same multivariable models were used for all exposures and outcomes.

To prevent bias due to missing data, we used multiple imputation[22] to replace missing values on covariates. Analyses were performed in each of the 10 imputed data sets separately, and final results were pooled.

We performed several sensitivity analyses. We repeated the analysis excluding mothers with pre-pregnancy comorbidities (hyperlipidaemia, type 2 diabetes or hypertension) (n=48), and mothers who vomited daily or a few days per week (n=321), as this might alter the effect of maternal diet. Furthermore, additional adjustment was performed for maternal pregnancy complications (gestational diabetes, pregnancy-induced-hypertension, pre-eclampsia), maternal total energy intake and child weight at age 6 years, as they could be possible intermediates in the relationship between maternal diet and offspring cardiometabolic health. Also, we additionally adjusted for child diet quality at age 1 year in the subgroup in which child diet was assessed (n=1591). Additional analyses were also performed with the *a priori*-'Dutch Healthy Diet Index'-dietary pattern including the component of alcohol intake.

We tested for possible interactions between dietary patterns and maternal pre-pregnancy BMI, child birth weight-for-gestational-age and child gender by adding an interaction term to the multivariable model, because we considered these variables as potential effect modifiers. To avoid chance findings (type I errors) due to multiple testing, we corrected all p-values for the number of independent tests (i.e. number of dietary patterns). Thus, we used a p-value of 0.05/4=0.0125 as significance level. All statistical analyses were performed using SPSS Statistics 21.0.

Results

Study population

Table 4.2.2 shows the characteristics of the study population. Mothers were on average 31.7 years old (SD 4.2) at enrolment, and most mothers were nulliparous (61.9%). Most mothers used folic acid supplements (57.8% periconceptional, 33.1% started in first weeks) and 75.9% never smoked during pregnancy, but 51.8% of the mothers continued alcohol drinking during pregnancy. Mothers who were not included in the analysis were on average lower educated, had a lower income, more often smoked during pregnancy, but less often consumed alcohol during pregnancy (**Supplementary Table 4.2.1**).

A posteriori-dietary patterns were a 'Vegetable, fish and oil'-dietary pattern, a 'Nuts, soy and high-fibre cereals'-dietary pattern and a 'Margarine, snacks and sugar'-dietary pattern (**Table 4.2.1**). Mean score on the *a priori*-'Dutch Healthy Diet Index'-dietary pattern was 31.8 (SD 7.7), on a theoretical scale from 0 to 60. None of the mothers received the maximum score.

Table 4.2.2. Characteristics of the participants (n=2695)[a]

Maternal characteristics		
Age (years)		31.7 ± 4.2
Pre-pregnancy BMI (kg/m^2)		23.3 ± 3.9
Gestational age at enrolment (weeks)		13.4 (9.9 – 22.8)
Educational level	Primary or secondary	1019 (37.8%)
	Higher	1640 (61.7%)
Household income	<2200 Euros/month	591 (23.8%)
	>2200 Euros/month	1893 (76.2%)
Parity	0	1665 (61.9%)
	≥1	1026 (38.1%)
Smoking	Never during pregnancy	1886 (75.9%)
	Until pregnancy was known	236 (9.5%)
	Continued during pregnancy	363 (14.6%)
Alcohol consumption	Never during pregnancy	776 (28.8%)
	Until pregnancy was known	413 (15.3%)
	Continued during pregnancy	1278 (51.8%)
Folic acid supplement use	No	203 (9.2%)
	Started first 10 weeks	734 (33.1%)
	Started periconceptional	1281 (57.8%)
Total energy intake (kcal/day)		2153 ± 503
Stress during pregnancy		0.12 (0.00 – 0.77)
Child characteristics		
Gender (% boys)		1351 (50.1%)
Birth weight (grams)		3503 ± 541
Gestational age at births (weeks)		40.0 ± 1.7

[a.] Values are means ± SD, absolute numbers (valid percentages) or medians (95% range). Missing values were 373 (13.8%) for pre-pregnancy BMI, 36 (1.3%) for educational level, 211 (7.8%) for household income, 4 (0.1%) for parity, 210 (7.8%) for smoking, 228 (8.5%) for alcohol consumption, 477 (17.7%) for folic acid supplement use, 284

(10.5%) for stress during pregnancy and 3 (0.001%) for birth weight. Abbreviations: BMI, body mass index; FFQ, food-frequency questionnaire; kcal, kilocalories.

Maternal dietary patterns and offspring cardiometabolic outcomes

Table 4.2.3 shows the associations between maternal dietary patterns and offspring metabolic outcomes. There were no significant associations between any of the *a posteriori*-dietary patterns or the *a priori*-dietary pattern during pregnancy and child HDL-cholesterol, triglyceride levels or insulin levels at age 6 years.

Table 4.2.4 shows the associations of maternal dietary patterns with offspring cardiovascular outcomes at age 6. There were no significant associations between any of the *a posteriori*-dietary patterns or the *a priori*-dietary pattern during pregnancy and systolic or diastolic blood pressure of the child at age 6 after adjustment for confounders. Also, the 'Nuts, soy and high-fibre cereals'-dietary pattern and 'Margarine, snacks and sugar'-dietary pattern were not significantly associated with pulse wave velocity of the child. However, a higher adherence score on the *a posteriori*-'Vegetable, fish and oil'-dietary pattern was associated with a lower pulse wave velocity of the child at age 6 (SD -0.19 (95%CI-0.33; -0.06), highest vs. lowest quartile). Also, a higher score for the modified version of the *a priori*-'Dutch Healthy Diet index' was associated with a lower pulse wave velocity of the child, but this was significant only in the third quartile.

Table 4.2.5 shows the associations between the different maternal dietary patterns with the combined cardiometabolic risk factor score at age 6. In the crude model, only the third quartile of the *a posteriori*-'Vegetable, fish and oil'-dietary pattern was significantly associated with a lower cardiometabolic risk factor. In the multivariable model, there were no significant associations between any of the *a posteriori*-dietary patterns or the *a priori*-dietary pattern during pregnancy with the cardiometabolic risk factor score in offspring at 6 years of age.

Additional analyses *(data not shown)*

Additional analyses for the *a priori*-'Dutch Healthy Diet Index'-dietary pattern including the alcohol component did not change the results. Also, the results did not change after the exclusion of mothers with pre-pregnancy comorbidities or mothers who vomited daily or a few days per week. Furthermore, additional adjustment for maternal pregnancy complications, total energy intake, child diet quality at age 1 year or child weight at age 6 years had no effect on the results.

We observed no significant interactions between any of the dietary patterns and maternal pre-pregnancy BMI, child birth weight for gestational age, or child gender (p-interaction all non-significant).

Table 4.2.3. Association of maternal dietary patterns with metabolic outcomes at age 6 years[a]

	HDL-cholesterol (n=1825)		**Triglycerides** (n=1819)		**Insulin** (n=1810)	
	Crude	***Adjusted***	***Crude***	***Adjusted***	***Crude***	***Adjusted***
			'Vegetable, fish and oil'-dietary pattern			
Q1 (low)	Reference	Reference	Reference	Reference	Reference	Reference
Q2	0.09 (-0.04; 0.22)	0.05 (-0.08; 0.19)	-0.01 (-0.15; 0.12)	0.02 (-0.12; 0.15)	0.00 (-0.13; 0.13)	-0.02 (-0.15; 0.12)
Q3	0.07 (-0.06; 0.20)	0.02 (-0.12; 0.15)	-0.10 (-0.23; 0.04)	-0.05 (-0.19; 0.09)	0.01 (-0.12; 0.14)	0.02 (-0.11; 0.16)
Q4 (high)	-0.00 (-0.13; 0.12)	-0.07 (-0.20; 0.07)	-0.07 (-0.20; 0.06)	-0.01 (-0.15; 0.14)	0.04 (-0.09; 0.17)	0.05 (-0.09; 0.19)
Per SD	*-0.02 (-0.06; 0.03)* *p=0.48*	*-0.04 (-0.09; 0.01)* *p=0.09*	*-0.02 (-0.06; 0.03)* *p=0.46*	*0.01 (-0.04; 0.06)* *p=0.76*	*0.01 (-0.04; 0.05)* *p=0.76*	*0.01 (-0.04; 0.06)* *p=0.66*
			'Nuts, soy and high-fibre cereals'-dietary pattern			
Q1 (low)	Reference	Reference	Reference	Reference	Reference	Reference
Q2	-0.12 (-0.25; 0.00)	-0.15 (-0.28; -0.02)	0.13 (-0.01; 0.26)	0.15 (0.01; 0.29)	0.17 (0.04; 0.30)	0.15 (0.01; 0.28)
Q3	-0.01 (-0.14; 0.11)	-0.04 (-0.18; 0.09)	0.08 (-0.05; 0.21)	0.10 (-0.04; 0.24)	0.10 (-0.03; 0.23)	0.08 (-0.05; 0.22)
Q4 (high)	-0.00 (-0.13; 0.12)	-0.02 (-0.16; 0.12)	0.08 (-0.05; 0.21)	0.12 (-0.03; 0.27)	0.08 (-0.05; 0.21)	0.07 (-0.08; 0.21)
Per SD	*0.02 (-0.02; 0.07)* *p=0.31*	*0.02 (-0.03; 0.07)* *p=0.37*	*0.03 (-0.02; 0.07)* *p=0.27*	*0.04 (-0.01; 0.10)* *p=0.12*	*0.02 (-0.03; 0.06)* *p=0.48*	*0.01 (-0.04; 0.07)* *p=0.63*
			'Margarine, snacks and sugar'-dietary pattern			
Q1 (low)	Reference	Reference	Reference	Reference	Reference	Reference
Q2	0.07 (-0.06; 0.19)	0.07 (-0.07; 0.21)	-0.12 (-0.25; 0.01)	-0.10 (-0.24; 0.05)	-0.02 (-0.15; 0.11)	-0.01 (-0.15; 0.13)
Q3	0.03 (-0.10; 0.15)	0.04 (-0.13; 0.21)	-0.10 (-0.24; 0.03)	-0.07 (-0.24; 0.11)	-0.05 (-0.18; 0.07)	-0.04 (-0.21; 0.13)
Q4 (high)	-0.11 (-0.23; 0.02)	-0.09 (-0.31; 0.13)	-0.12 (-0.25; 0.02)	-0.06 (-0.29; 0.17)	0.05 (-0.08; 0.17)	0.07 (-0.15; 0.29)
Per SD	*-0.03 (-0.08; 0.01)* *p=0.14*	*0.01 (-0.09; 0.10)* *p=0.92*	*-0.04 (-0.08; 0.01)* *p=0.13*	*-0.01 (-0.11; 0.09)* *p=0.82*	*0.01 (-0.03; 0.06)* *p=0.56*	*0.03 (-0.07; 0.12)* *p=0.60*

Table 4.2.3.(continued) Association of maternal dietary patterns with metabolic outcomes at age 6 years[a]

	'Dutch Healthy Diet index'-dietary pattern					
Q1 (low)	Reference	Reference	Reference	Reference	Reference	Reference
Q2	0.05 (-0.08; 0.17)	0.04 (-0.09; 0.17)	0.07 (-0.06; 0.20)	0.08 (-0.05; 0.21)	0.04 (-0.09; 0.17)	0.03 (-0.10; 0.16)
Q3	-0.01 (-0.14; 0.11)	-0.02 (-0.15; 0.11)	0.10 (-0.03; 0.23)	0.11 (-0.03; 0.24)	0.09 (-0.04; 0.22)	0.09 (-0.04; 0.23)
Q4 (high)	-0.01 (-0.14; 0.11)	-0.02 (-0.15; 0.11)	0.05 (-0.08; 0.18)	0.06 (-0.08; 0.19)	0.07 (-0.05; 0.20)	0.08 (-0.06; 0.21)
Per SD	*0.01 (-0.04; 0.05)* *p=0.70*	*0.01 (-0.04; 0.05)* *p=0.85*	*0.01 (-0.03; 0.06)* *p=0.62*	*0.01 (-0.04; 0.06)* *p=0.68*	*0.03 (-0.01; 0.08)* *p=0.18*	*0.03 (-0.02; 0.08)* *p=0.19*

[a.] Values are regression coefficients (95% confidence interval) and reflect differences in age-and-gender-specific SD-scores of the outcomes for quartiles 2 to 4, as compared to quartile 1 (lowest adherence to the dietary pattern). The P values represents P values for linear trend tests (per SD increase of maternal adherence to the dietary pattern). * p<0.0125. Multivariable model: adjusted for maternal age at intake, gestational age at dietary assessment, folic acid use, smoking and alcohol during pregnancy, maternal educational level, family income, parity, maternal pre-pregnancy BMI, maternal stress during pregnancy, child gender, breast feeding, watching television at age 2 years, participation in sports at age 6 years and height at age 6 years.

Table 4.2.4. Association of maternal dietary patterns with cardiovascular outcomes at age 6 years[a]

	Pulse wave velocity (n=2104)		**Systolic BP** (n=2548)		**Diastolic BP** (n=2548)	
	Crude	***Adjusted***	***Crude***	***Adjusted***	***Crude***	***Adjusted***
			'Vegetable, fish and oil'-dietary pattern			
Q1 (low)	Reference	Reference	Reference	Reference	Reference	Reference
Q2	-0.09 (-0.21; 0.04)	-0.09 (-0.22; 0.04)	-0.04 (-0.15; 0.07)	-0.02 (-0.13; 0.09)	-0.06 (-0.17; 0.05)	-0.02 (-0.14; 0.09)
Q3	-0.10 (-0.23; 0.02)	-0.11 (-0.24; 0.02)	**-0.18 (-0.28; -0.07)***	-0.11 (-0.22; 0.01)	**-0.15 (-0.26; -0.04)***	-0.09 (-0.20; 0.03)
Q4 (high)	**-0.19 (-0.31; -0.07)***	**-0.19 (-0.33; -0.06)***	-0.11 (-0.21; 0.00)	-0.03 (-0.14; 0.09)	-0.13 (-0.24; -0.03)	-0.05 (-0.17; 0.06)
Per SD	*-0.05 (-0.10; -0.01) p=0.02*	*-0.05 (-0.10; -0.01) p=0.03*	*-0.03 (-0.07; 0.01) p=0.13*	*0.00 (-0.04; 0.04) p=0.96*	*-0.03 (-0.07; 0.01) p=0.10*	*0.00 (-0.04; 0.04) p=0.98*
			'Nuts, soy and high-fibre cereals'-dietary pattern			
Q1 (low)	Reference	Reference	Reference	Reference	Reference	Reference
Q2	-0.03 (-0.16; 0.10)	-0.01 (-0.15; 0.12)	-0.02 (-0.13; 0.09)	0.01 (-0.10; 0.12)	0.06 (-0.05; 0.17)	0.09 (-0.02; 0.21)
Q3	-0.08 (-0.20; 0.05)	-0.06 (-0.19; 0.08)	-0.04 (-0.15; 0.07)	0.01 (-0.11; 0.12)	0.01 (-0.10; 0.12)	0.06 (-0.06; 0.17)
Q4 (high)	-0.02 (-0.15; 0.11)	-0.01 (-0.15; 0.13)	-0.13 (-0.24; -0.02)	-0.08 (-0.20; 0.04)	-0.04 (-0.15; 0.07)	0.01 (-0.11; 0.13)
Per SD	*-0.02 (-0.07; 0.02) p=0.34*	*-0.02 (-0.07; 0.03) p=0.37*	***-0.05 (-0.09; -0.01) p=0.01***	*-0.03 (-0.07; 0.01) p=0.14*	*-0.02 (-0.05; 0.02) p=0.43*	*0.00 (-0.04; 0.05) p=0.96*
			'Margarine, snacks and sugar'-dietary pattern			
Q1 (low)	Reference	Reference	Reference	Reference	Reference	Reference
Q2	-0.08 (-0.21; 0.04)	-0.10 (-0.24; 0.04)	-0.05 (-0.15; 0.06)	-0.03 (-0.15; 0.09)	-0.01 (-0.12; 0.09)	-0.01 (-0.13; 0.11)
Q3	0.07 (-0.05; 0.19)	0.03 (-0.14; 0.19)	0.03 (-0.08; 0.13)	0.03 (-0.12; 0.17)	0.02 (-0.09; 0.13)	0.01 (-0.14; 0.15)
Q4 (high)	-0.01 (-0.13; 0.12)	-0.08 (-0.30; 0.14)	-0.01 (-0.12; 0.10)	0.01 (-0.18; 0.19)	-0.02 (-0.13; 0.09)	-0.04 (-0.23; 0.15)
Per SD	*0.02 (-0.02; 0.07) p=0.31*	*0.02 (-0.08; 0.11) p=0.71*	*0.01 (-0.03; 0.04) p=0.78*	*0.01 (-0.07; 0.09) p=0.75*	*-0.00 (-0.04; 0.04) p=0.86*	*-0.01 (-0.10; 0.07) p=0.73*

Table 4.2.4. (continued) Association of maternal dietary patterns with cardiovascular outcomes at age 6 years[a]

	Pulse wave velocity (n=2104)		**Systolic BP** (n=2548)		**Diastolic BP** (n=2548)	
	Crude	***Adjusted***	***Crude***	***Adjusted***	***Crude***	***Adjusted***
			'Dutch Healthy Diet index'-dietary pattern			
Q1 (low)	Reference	Reference	Reference	Reference	Reference	Reference
Q2	-0.01 (-0.13; 0.12)	-0.01 (-0.14; 0.11)	-0.10 (-0.21; -0.01)	-0.08 (-0.19; 0.03)	-0.05 (-0.15; 0.06)	-0.03 (-0.14; 0.08)
Q3	**-0.17 (-0.29; -0.05)***	**-0.18 (-0.30; -0.05)***	-0.11 (-0.20; 0.01)	-0.06 (-0.16; 0.05)	-0.03 (-0.14; 0.08)	0.01 (-0.10; 0.12)
Q4 (high)	-0.09 (-0.21; 0.04)	-0.09 (-0.22; 0.04)	-0.04 (-0.15; 0.06)	-0.01 (-0.12; 0.10)	0.03 (-0.08; 0.14)	0.06 (-0.05; 0.17)
Per SD	*-0.05 (-0.10; -0.01)* *p=0.02*	*0.05 (-0.10; -0.01)* *p=0.03*	*-0.02 (-0.05; 0.02)* *p=0.45*	*0.00 (-0.04; 0.04)* *p=0.99*	*0.01 (-0.03; 0.05)* *p=0.60*	*0.02 (-0.02; 0.06)* *p=0.23*

[a.] Values are regression coefficients (95% confidence interval) and reflect differences in age-and-gender-specific SD-scores of the outcomes for quartiles 2 to 4, as compared to quartile 1 (lowest adherence to the dietary pattern). The P values represents P values for linear trend tests (per SD increase of maternal adherence to the dietary pattern). p<0.0125. Multivariable model: adjusted for maternal age at intake, gestational age at dietary assessment, folic acid use, smoking and alcohol during pregnancy, maternal educational level, family income, parity, maternal pre-pregnancy BMI, maternal stress during pregnancy, child gender, breast feeding, watching television at age 2 years, participation in sports at age 6 years and height at age 6 years. Abbreviation: BP, blood pressure.

Table 4.2.5. Association of maternal dietary patterns with cardiometabolic risk factor score at age 6 years[a]

	Cardiometabolic risk factor score (n=1710)	
	Crude	***Adjusted***
	'Vegetable, fish and oil'- dietary pattern	
Q1 (low)	Reference	Reference
Q2	-0.13 (-0.26; 0.00)	-0.06 (-0.19; 0.08)
Q3	**-0.21 (-0.34; -0.08)***	-0.08 (-0.22; 0.05)
Q4 (high)	-0.16 (-0.29; -0.03)	-0.01 (-0.15; 0.13)
Per SD	*-0.04 (-0.08; 0.01)* *p=0.11*	*0.19 (-0.03; 0.07)* *p=0.45*
	'Nuts, soy and high-fibre cereals'- dietary pattern	
Q1 (low)	Reference	Reference
Q2	0.11 (-0.03; 0.24)	0.17 (0.03; 0.30)
Q3	0.03 (-0.11; 0.16)	0.11 (-0.03; 0.25)
Q4 (high)	-0.04 (0.17; 0.09)	0.08 (-0.07; 0.22)
Per SD	*-0.03 (-0.08; 0.02)* *p=0.22*	*0.11 (-0.04; 0.06)* *p=0.68*
	'Margarine, snacks and sugar'- dietary pattern	
Q1 (low)	Reference	Reference
Q2	-0.09 (-0.22; 0.04)	-0.03 (-0.17; 0.11)
Q3	-0.08 (-0.21; 0.05)	0.00 (-0.17; 0.17)
Q4 (high)	-0.00 (-0.13; 0.13)	0.12 (-0.10; 0.35)
Per SD	*0.00 (-0.05; 0.05)* *p=0.98*	*0.05 (-0.04; 0.15)* *p=0.27*
	'Dutch Healthy Diet index'- dietary pattern	
Q1 (low)	Reference	Reference
Q2	0.01 (-0.12; 0.15)	0.05 (-0.08; 0.18)
Q3	-0.01 (-0.13; 0.13)	0.06 (-0.07; 0.19)
Q4 (high)	0.02 (-0.11; 0.15)	0.08 (-0.05; 0.21)
Per SD	*-0.01 (-0.06; 0.04)* *p=0.70*	*0.13 (-0.03; 0.06)* *p=0.58*

[a.] Values are regression coefficients (95% confidence interval) and reflect differences in age-and-gender-specific SD-scores of the outcomes for quartiles 2 to 4, as compared to quartile 1 (lowest adherence to the dietary pattern). The P values represents P values for linear trend tests (per SD increase of maternal adherence to the dietary pattern). * p<0.0125. Multivariable model: adjusted for maternal age at intake, gestational age at dietary assessment, folic acid use, smoking and alcohol during pregnancy, maternal educational level, family income, parity, maternal pre-pregnancy BMI, maternal stress during pregnancy, child gender, breast feeding, watching television at age 2 years, participation in sports at age 6 years and height at age 6 years.

Discussion

Summary of main findings

In a population-based prospective cohort from foetal life onwards, we observed no consistent associations of dietary patterns (*a posteriori* and *a priori*) with cardiometabolic risk factors individually or combined as a score after adjusting for potential confounders including sociodemographic and lifestyle factors. The highest quartile of adherence on the *a posteriori*-'Vegetable, fish and oil'-dietary pattern, and the third quartile of adherence to the *a priori*-'Dutch Healthy Diet index', were significantly associated with a lower pulse wave velocity of the child at the age of 6 years. We observed no associations between the 'Nuts, soy and high-fibre cereals'- or the 'Margarine, snacks and sugar'-dietary patterns with offspring pulse wave velocity.

Interpretation and comparison with other studies

Based on the developmental origins of health and disease hypothesis,[23] it could be expected that diet during pregnancy can have a long-term effect on the offspring's cardiometabolic profile, such as blood pressure, lipid profile and insulin sensitivity. Our results do not demonstrate this potential effect at an early age (6 years). Perhaps the effect could occur at a later age, however the age of our population does not permit us to evaluate these effects in subsequent age periods.

Previous research on the effects of maternal nutrition on offspring cardiometabolic health focused mostly on total energy, macronutrients, or micronutrients, and literature on overall diet is lacking. Studies on maternal diet often use birth weight as a marker of infant health.[3] However, observations from the Dutch famine have shown that malnutrition during pregnancy could affect offspring cardiometabolic health at middle-age, without influencing birth weight.[24] Furthermore, many studies used indirect measures of nutritional status, such as anthropometric measurements.[25] However, nutritional status is much more complex and much remains unknown about optimal diet during pregnancy for child health.[9] Thus, studies on the effects of overall maternal diet on cardiometabolic health in offspring are necessary.

The only significant relationship that we observed was on pulse wave velocity. Higher adherence to the *a posteriori*-'Vegetable, fish and oil'-dietary pattern during pregnancy was associated with a lower pulse wave velocity in the offspring. The third quartile of *a priori*-'Dutch Healthy Diet index' was also significantly associated with a lower pulse wave velocity, while the highest quartile was not. This may be a chance finding, but it may also suggest that there is not a linear dose-response relationship. However, the association of the 'Vegetable, fish and oil'-dietary pattern with lower pulse wave velocity was significant in the highest quartile of adherence, and results from this pattern may indicate that a dose-response relationship does exist. Although statistically significant, it is not known whether this effect will also be clinically significant. Pulse wave velocity is a measure arterial stiffness, and a large meta-analysis in adults has shown that participants with a high pulse wave velocity had a higher risk for cardiovascular events.[26] As there are indications that the atherosclerotic process begins much earlier in life, lower pulse wave velocity in children is thus suggested to

be beneficial for long-term cardiometabolic health, but this has not yet been demonstrated in this age-group.

Nevertheless, we did not observe consisted associations with other cardiometabolic outcomes, or the combined cardiometabolic risk factor score. The lack of consistent associations between maternal dietary patterns and cardiometabolic health in our study can be explained in several ways.

First, the original hypothesis on the effects of maternal nutrition on child health is based on studies that observed extreme malnourishment,[2,24] and many of the studies that found associations between maternal diet and infant outcomes were done in nutritionally at-risk populations.[8] Studying extremes might make it easier to detect associations, but it could also be that the associations only exist in extreme undernutrition or overnutrition. Our population has a selection towards a healthy population of pregnant women and perhaps the effect of maternal dietary patterns on offspring cardiometabolic health is just not relevant in our population. Also, the suggested relation between maternal diet and offspring health came to the attention because increased incidence of cardiometabolic diseases. Cardiometabolic diseases occur late in life, and it might be that the variation in outcome in children is too small and all within a range of healthy. While currently we found no effect, it could be that effects become visible when children are older and outcomes are more deviated towards cardiometabolic risk. In addition, it may be argued that the outcome measures used in adults are not sensitive enough to assess cardiometabolic risk early in life.

Second, if epigenetic mechanisms may influence cardiometabolic health, it may be the case that dietary effects are mainly caused by for specific dietary components influencing gene expression or DNA methylation. For example, epigenetic effects have been described for methyl-donor nutrients, iron, zinc and flavonoids.[7] Although dietary patterns analyses can be useful for creating food-based dietary guidelines,[12] the downside of studying dietary patterns may be that effects of specific nutrients influencing epigenetic mechanisms may be diluted.[11] If associations between maternal diet and child cardiometabolic health are fully driven by certain nutrients, analyses using dietary patterns might not detect these effects, which might explain why we did not find clear associations.

We studied both *a priori*- and *a posteriori*-dietary patterns during pregnancy. *A posteriori*-patterns are not hypothesis-driven but data-driven, but the hypothesis is important when defining food groups.[11] If food groups are too heterogeneous or contain food products with opposite effects on health, they may not be useful in predicting disease risk. Therefore, we created the food groups based on a possible relation with the outcome, for example we separated high fat dairy products from low fat dairy products, and separated cereals based on their fibre content. However, we were restricted by the design of the FFQ and were therefore for example not able to separate vegetables based on their folate content, which would have been interesting in light of the previously mentioned epigenetic programming effects.[7]

A priori-dietary patterns are hypothesis-oriented and the food groups should be correctly chosen for that purpose.[27] The Dutch Healthy Diet index was developed based on Dutch guidelines,[15] which we slightly modified based on our available data and for the use during pregnancy. Although we removed the alcohol component from the score because

of known adverse effects of alcohol on the foetus, the *a priori*- dietary pattern was mostly developed based on hypothesized effects for health of the mother herself, and not for fetal health. Nevertheless, to our knowledge, there are currently no dietary indexes for the use during pregnancy that are designed based on offspring health outcomes.

Strengths and limitations

Dietary patterns can capture the totality of diet but also overall lifestyle, since dietary patterns cluster with other lifestyle behaviours as well.[28] Nevertheless, it is important to take sociodemographic and lifestyle factors into account that can confound the relation between maternal diet and child cardiometabolic health, including also vitamin supplementation.[27] We had detailed information about periconceptional folic acid supplement use and many other possible confounders, and we observed that adjustment for these factors had large effects on our results for diastolic and systolic blood pressure, but only a little effect on the association with pulse wave velocity. Nevertheless, a limitation may be that residual confounding might still be present due to lack of information on potential confounding factors, such as physical activity and sedentary behaviour during pregnancy.

We used a self-administered FFQ to measure diet during pregnancy, which is considered an appropriate method to assess average dietary intake over an extended period of time in epidemiological studies.[29] However, as a consequence of self-reported dietary intake, measurement error might exist. Although the ICCs for nutrients were similar to other studies,[30] previous studies on nutrient intake have suggested that this may lead to an underestimation of the true relation between diet and health outcomes.[31] Nonetheless, the latter may not be applicable to dietary pattern analysis since this does not include absolute intake of nutrients or foods but underlying patterns of food intake. Unfortunately, we were not able to validate our dietary patterns in this population but several studies have shown that dietary pattern analysis using PCA can be a valid and reproducible method.[32-35]

Conclusions

In conclusion, low birth weight or severe maternal malnutrition has been related to cardiometabolic diseases in later life,[3,24] but the effects of overall dietary patterns during pregnancy with offspring health outcomes are unknown. In our population-based prospective cohort study, we examined the associations of different *a priori*- and *a posteriori*-dietary patterns during pregnancy with offspring cardiometabolic health. We found no independent associations between different maternal dietary patterns during pregnancy with blood lipids, blood pressure or insulin in offspring, but higher adherence to an *a posteriori*-'Vegetable, fish and oils'-dietary pattern was associated with lower pulse wave velocity at offspring age 6. Further studies are needed to enable recommendations for maternal dietary patterns that are optimal for child cardiometabolic health.

Supplementary Material can be found online: http://hdl.handle.net/1765/80025

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Chapter 5

General discussion

The aim of this thesis was to gain more insight into the role of maternal nutrition during pregnancy on health outcomes of women and their children. First, we evaluated the associations of maternal dietary intake during pregnancy on maternal health (e.g., gestational weight gain (GWG), blood pressure and the occurrence of hypertensive complications). Second, we studied the associations of plasma biomarkers in pregnancy with GWG and the occurrence of hypertensive complications, as well as birth outcomes including birth weight and gestational age. Finally, we studied the associations of maternal dietary intake during pregnancy with child health, which included offspring cardiometabolic health and body composition 6 years after birth.

In this chapter we summarize our main findings, we evaluate the main methodological considerations of the methods that we used, we discuss potential implications for prevention, maternal care and public health, and we give recommendations for future research.

Main findings

Maternal nutrition in pregnancy and maternal health

In a systematic review of published studies we demonstrated that higher energy intake during pregnancy is associated with higher absolute GWG as well as with excessive GWG, whereas this remained unclear for the energy-providing macronutrients fat, carbohydrate and protein (**Chapter 2.1**). Nevertheless, specific subtypes of macronutrients (e.g., saturated fat) may be associated with GWG. The results did not indicate that the association between macronutrient intake and GWG differed between low-, middle-, and high-income countries. It is important to note that many studies did not sufficiently adjust for important confounders such as maternal BMI and most of the included studies were of relative low quality. Our findings in this systematic review are in line with studies that have been performed in general populations which also suggest that weight gain largely depends on dietary intake of energy and less on specific macronutrient composition.[1] Yet, a meta-analysis of 18 studies found that change in energy intake during pregnancy was not correlated with the amount of GWG.[2] This latter study however focused on the increase in energy intake during pregnancy and not on absolute energy intake.

Because macronutrients are not consumed in isolation and the intake of specific food sources of macronutrients might indicate the characteristics and quality of the diet,[3] we assessed whether maternal dietary patterns were associated with GWG in the Generation R Study (**Chapter 2.2**). Overall, no consistent associations were found for the identified dietary patterns with GWG. Nevertheless in normal weight women, the *a posteriori-derived* 'Vegetable, oil and fish' pattern, a relatively healthy dietary pattern, was associated with higher weekly GWG in early pregnancy, whereas the 'Margarine, sugar and snacks' pattern was associated excessive GWG. The 'Nuts, high-fibre cereals and soy' pattern was associated with slightly slower weight gain. In contrast, the *a priori-defined* 'Dutch Healthy Diet index' pattern was not associated with any GWG measurement. The 'Dutch Healthy Diet index' pattern was developed to measure adherence to the Dutch guidelines for a healthy diet. We found that none of the women received the maximum score on adherence to these Dutch guidelines suggesting that there is room for improvement for dietary habits during

pregnancy.

GWG is a physiological process in pregnancy but mainly gaining inadequate or excessive weight have been associated with adverse pregnancy and birth outcomes such as gestational diabetes, preterm birth, large-for-gestational-age newborns as well as with the development of obesity in both mother and child.[4]

Aspects of maternal nutrition have been previously associated with the development of blood pressure and the occurrence of hypertensive complications in pregnancy.[5,6] A high dietary acid load, characterized by high protein intake and low potassium intake amongst others, may induce a mild metabolic acidosis and consequently increase blood pressure.[7,8] Maternal dietary acid load as measured by net endogenous acid production, potential renal acid load and animal protein/potassium ratio, was not associated with development of blood pressure during pregnancy, although women with a higher vegetable protein/potassium ratio had a slightly lower diastolic blood pressure (**Chapter 2.3**). Maternal dietary acid load was also not associated with the occurrence of hypertensive complications in pregnancy. Dietary acid load is closely linked to dietary quality; a typical Western diet (e.g., high in meat and processed foods) has a high dietary acid load, whereas a prudent diet (e.g., high in vegetables and fruits) has a low dietary acid load.[9] Therefore, our findings may reflect the association between a healthy dietary pattern, rather than dietary acid load specifically, and development of blood pressure during pregnancy.[6]

Maternal nutritional biomarkers in pregnancy and maternal health and birth outcomes

Neither maternal fish intake nor plasma polyunsaturated fatty acids (PUFAs) in pregnancy were associated with plasma levels of the angiogenic factors placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) during pregnancy or in cord blood (**Chapter 3.1**). Dysregulated maternal levels of angiogenic factors have been linked to placental dysfunction.[10] Consequently, maternal levels of angiogenic factors been associated with adverse maternal outcomes (e.g., pre-eclampsia) as well as with adverse birth outcomes (e.g., foetal growth restriction).[11,12] Maternal fish intake as well as maternal n-3 PUFA supplementation during pregnancy have been associated with higher birth weight and lower risk of preterm birth.[13,14] Hence, levels of angiogenic factors during pregnancy may be not an intermediate factor in the association between maternal fish intake during pregnancy and birth outcome.

We evaluated the associations between plasma fatty acid patterns and GWG in the Generation R Study (**Chapter 3.2**). First, we identified three fatty acid patterns, namely, a 'High n-6 PUFA' pattern, a 'Monounsaturated (MUFA) and saturated fatty acid (SFA)' pattern, and a 'High n-3 PUFA' pattern. The 'High n-6 PUFA' pattern was associated with excessive GWG, whereas the 'MUFA and SFA' pattern was not. Interestingly, additional analysis revealed that the 'MUFA and SFA' pattern was associated with higher occurrence of large-for-gestational-age newborns, whereas the 'High n-6 PUFA' pattern was not (**Table 5.1**). These differential findings indicate that the fatty acid patterns are differently associated with components of GWG (e.g., maternal fat gain and foetal growth). We also found that women with low and high scores on the 'High n-3 PUFA' pattern had higher weekly GWG and gave

birth to children with higher birth weights than women with average scores. This 'High n-3 PUFA' pattern was however not associated with excessive GWG or large-for-gestational-age newborns. None of the maternal fatty acid patterns were associated with the occurrence of hypertensive complications during pregnancy. This finding is in line with our null findings of maternal PUFA levels with angiogenic factors that were presented in **Chapter 3.1.**

Table 5.1 Association of plasma fatty acid patterns with small-for-gestational age and large-for-gestational age new-borns (n=6534)

	Small-for-gestational age (n=652)	**Appropriate-for-gestational age** (=5222)	**Large-for-gestational age** (n=652)
Plasma fatty acid patterns	OR (95%CI)	OR (95%CI)	OR (95%CI)
'High n-6 PUFA' (per SD)	1.08 (0.99; 1.17)	reference	0.97 (0.89; 1.06)
'MUFA and SFA' (per SD)	**0.90 (0.83; 0.98)**	reference	**1.11 (1.02; 1.21)**
'High n-3 PUFA' (per SD)	1.04 (0.96; 1.14)	reference	0.99 (0.91; 1.08)

Results from multinomial logistic regression. Odds ratio reflects the difference in odds to have a small-for-gestational-age or large-for-gestational-age newborn as compared with appropriate-gestational-age newborn. Adjusted for gestational age at fatty acid measurement, other fatty acid patterns, foetal sex, gestational age at birth, maternal age, ethnicity, educational level, household income, pre-pregnancy BMI, parity, alcohol consumption during pregnancy, maternal smoking during pregnancy, and folic acid supplementation. Abbreviations: CI, confidence interval; MUFA, monounsaturated fatty acids; OR, odds ratio; PUFA, polyunsaturated fatty acids; SD, standard deviation; SFA, saturated fatty acids.

In an individual participant data (IPD) meta-analysis that included more than 11,000 pregnant women, we found that maternal vitamin B12 levels during pregnancy were not associated with birthweight or with the occurrence of small-for-gestational age newborns (**Chapter 3.3**). Yet, higher maternal vitamin B12 levels during pregnancy were strongly associated with decreased relative risk for preterm delivery. These results were consistent across low-, middle-, and high-income countries and across maternal weight categories. We studied the associations of maternal vitamin B12 levels because supplementation studies on vitamin B12 are scarce and studies have suggested that multi-micronutrient supplementation may improve birth outcomes (e.g., reduce the occurrence of small-for-gestational-age newborns).[15] Furthermore, supplementation of folate, which has a metabolic role closely related to vitamin B12 has been shown to improve birth outcomes such as reducing the occurrence of small-for-gestational-age newborns.[16] Low maternal vitamin B12 levels during pregnancy may also be associated with suboptimal child health outcomes later in life, including impaired growth and brain development as well as occurrence of anemia.[17]

Maternal nutrition in pregnancy and child health outcomes

We found that children of women with a higher protein intake during pregnancy had a higher fat-free mass at the age of 6 years and a higher body mass index than children of women with a low protein intake, but no differences were found in their fat mass (**Chapter 4.1**). Additionally, the source of protein or substitution of protein by fat or carbohydrate did not markedly influence our results, whereas the results slightly attenuated after additional

adjustment for protein intake of the child. These results endorse the foetal programming hypothesis that intra-uterine exposures may have long-term health consequences for the offspring,[18] although longer follow-up duration and repeated measurements are needed to further investigate the influence of intra-uterine protein exposure. The role of protein intake in the development of adiposity may depend on the exposure window because we found that children of mothers with high protein intake during pregnancy did not have higher fat mass at the age of 6 years, whereas an intervention study showed that children with a high protein intake in infancy had increased the prevalence of childhood obesity at the age of 6 years.[19]

Maternal dietary patterns during pregnancy were not consistently associated with cardiometabolic health outcomes in their children at the age of 6 years after taking into account sociodemographic and lifestyle factors of the mother (**Chapter 4.2**). More specifically, we found that the *a posteriori-derived* 'Margarine, sugar and snacks' pattern, a relatively unhealthy dietary pattern, was not associated with any cardiometabolic marker in the offspring. In contrast, the 'Vegetable, oil and fish' pattern, the 'Nuts, high-fibre cereals and soy' pattern, and the *a priori-defined* 'Dutch Healthy Diet index' pattern were associated with lower blood pressure and pulse wave velocity. However, only the association between the 'Vegetable, oil and fish' pattern with lower pulse wave velocity remained statistically significant after additional adjustments for maternal sociodemographic and lifestyle factors. These maternal dietary patterns during pregnancy were also not associated with offspring body composition at the age of 6 years independently of sociodemographic and lifestyle factors in an previous study.[20] This may suggest that the role of shared sociodemographic and lifestyle factors may be more important than intra-uterine exposures.

Methodological considerations

Study design

The Generation R Study is a large population-based prospective cohort and participating women were on average higher educated and had a higher household income than women who did not participate.[21,22] This selection bias could have influenced our results when associations that are studied would markedly differ between participating and not-participating women. Results from two large cohorts in the Nordic countries showed that associations found in participants were similar to the associations found in non-participants.[23,24] Accordingly, we expect that selection bias did not influence the exposure-outcome associations studied in this thesis. Another source of bias may be loss-to-follow-up of participants over time. Six years after pregnancy, the general follow-up rates of the Generation R study exceeded 80%,[21] and almost 66% of the Dutch women enrolled during pregnancy visited the Generation R research centre with their children for cardiometabolic and body composition measurements. These women differed in their baseline characteristics (e.g., higher maternal education, household income and lower levels of maternal smoking) when compared to women who did not participate in the follow-up measurement. If the association between maternal diet during pregnancy and childhood cardiometabolic health and body composition would be different between those with and without follow-up, it may

have influenced the results in our thesis. However, it may be speculated that the incomplete follow up has resulted in an underestimation of the exposure-outcome association because the childhood health outcomes may be worse in those who were lost-to-follow-up than participants with a follow-up visit.

We also included the results of two systematic reviews in this thesis, of which one was included a meta-analysis of individual participant data. Systematic reviews and meta-analyses are considered high quality evidence.[25] However, this does not only depend on the quality of the systematic review itself, but predominantly on the quality of the individual studies included. The intervention and observational studies included in the systematic review on GWG (**Chapter 2.1**) were on average of relative low quality and heterogeneous in design, data collection and adjustment for confounding. This may have resulted in less precise or even biased effect estimates due to residual confounding, incorrect statistical analyses and misclassification (non-differential and differential) of exposure and outcome. However, the conclusions of this systematic review did not change when restricting to high quality studies. Nevertheless, more high quality studies with low risk of bias are needed to strengthen the conclusions of our review. Although we were not able to evaluate potential publication bias, it is likely that we did not include all eligible studies due to selective reporting of statistically significant findings.[26]

An option to reduce heterogeneity between observational studies is to combine all individual participant data (IPD) of the studies included, because this method permits the use of common definitions, allows for adjustment for the same variables, and provides the opportunity to explore subgroups analysis on an individual participant level. Furthermore, IPD meta-analysis has the ability to address questions not included in the original publication.[27] However, heterogeneity cannot be fully eliminated in IPD meta-analysis because differences in population characteristics and measurement methods may remain. In our main analysis of the IPD meta-analysis (**Chapter 3.3**), heterogeneity still remained (an I^2 of 30%) which was mainly explained by country income level and by maternal BMI. Sensitivity analyses showed that maternal vitamin B12 levels in pregnancy were associated with slightly higher birth weight in middle- or low-income countries, but not with birth weight in high-income countries. Consequently, further studies to evaluate the influence of maternal vitamin B12 on birth outcomes may be conducted in a middle- or low-income country rather than in a high-income country.

Nutritional assessment

For the work presented in this thesis, dietary intake was predominantly assessed using food-frequency questionnaires (FFQs). Using FFQs in epidemiological studies has several advantages, because this method is easy to complete by participants and less expensive than food diaries or extensive interviews. Accordingly, FFQs are feasible to be applied in large population-based studies such as the Generation R Study. FFQs can evaluate longer-term usual dietary intake, which may be more important for development of diseases than dietary intake on one particular day.[28] Although absolute nutrient intake cannot be precisely estimated with an FFQ, research has shown that the intake of participants can be sufficiently ranked based on the information filled in by the FFQ.[29] In addition, other birth cohort studies

have also used FFQs to assess dietary intake during pregnancy permitting comparability of results.[30,31]

An important limitation of the FFQ is that dietary intake is self-reported in a retrospective design, hence the reported diet may be an incorrect representation of the usual diet.[32] The reported values of dietary intake are subject to measurement error.[29] This measurement error can be a non-differential or a differential misclassification of the exposure but we assumed that it would be mainly non-differential because maternal dietary intake during pregnancy was assessed prior to most of the outcome measurements. This may have led to an underestimation of the observed exposure-outcome associations.[32] Consequently, we may not have had enough participants to observe an association between maternal dietary patterns during pregnancy and offspring cardiometabolic health taking into account potential misclassification. Alternatively, measurement error of dietary intake could depend on the health outcome and thus be a differential misclassification of the exposure, resulting in biased effect estimates that can either underestimate or overestimate the effect size. For example in the studies assessing the associations between maternal diet and GWG, misreporting of dietary intake may have been differential because GWG depends on pre-pregnancy BMI of the women and some studies have shown that misclassification of dietary intake may be related to BMI.[33,34] Nonetheless, additional adjustment of the dietary patterns for BMI using the residual method[35] did not affect our results which suggested that misclassification of dietary intake was also in this case most likely to be non-differential*(data not shown)*. To reduce the magnitude of the measurement error of dietary intake, we adjusted our dietary variables for total energy intake because studies have shown that measurement error increases with increase in energy intake.[35] For example, participants who over-report their total energy intake are more likely to over-report their energy intake from individual foods. As a result of using energy-adjusted dietary intake, the precision of exposure-outcome associations can be improved.[35] A second reason to use energy-adjusted nutrients is to account for potential confounding or mediation by total energy.[35]

The FFQ in the Generation R Study was assessed only in early pregnancy. Consequently, estimation of dietary intake may have been less precise than with repeated measurements and we were not able to take into account changes in dietary intake during pregnancy. However, several studies have shown that the overall dietary pattern of women only changed little before, during or after pregnancy.[36-38]

The Generation R Study has included women of many different ethnic backgrounds with various dietary habits. The FFQ that we used was originally designed to evaluate dietary habits of Dutch adults[39] and was modified for the Generation R Study to include dietary items relevant in pregnancy. However, this modification may not have been sufficient to adequately cover dietary habits of women of non-Dutch background. To reduce the likelihood of differential misclassification of dietary intake by including all women, we restricted our analysis in the **Chapters 2** and **4** to women of Dutch ancestry. Hence, this may reduce the generalizability of our findings.

We used several approaches to explore dietary intake, namely macronutrient intake, food groups, biomarkers, dietary algorithms and dietary patterns, which all have their advantages and disadvantages:

Macronutrients – Evaluating dietary intake of the energy-providing macronutrients (e.g., fat, carbohydrate, protein) has the advantage that associations can be directly related to knowledge on biological activity of that macronutrient. Yet, macronutrient subtypes (e.g., saturated fat versus polyunsaturated fat) may differ in their biological activity and may as a result be differently associated with health outcomes. Therefore, evaluating total fat intake may dilute opposite effects of fat subtypes and thereby result in an overall null finding. In addition, the effect of substituting macronutrients should be evaluated, because associations between nutrition and disease have been shown to depend on the type of substitution model (e.g., replacing saturated fat for polyunsaturated fat versus replacing for monounsaturated fat).[40] Unfortunately, only few studies evaluated substitution effects of macronutrients or macronutrient subtypes in our systematic review (**Chapter 2.1**).

Food groups – Nutrients are consumed as part of a nutrient-matrix in foods. Studying foods provides the opportunity to study potential synergic effects of nutrients. Additionally, foods groups are easier to interpret for the general public and results may be useful for public health communication. On the other hand, if associations are found with health outcomes, it remains unclear which component of the food group may explain these associations. Also, studying food groups may dilute the effects of individual components, and associations can be confounded by related food groups and sociodemographic factors. For example, fish contains favourable components (such as n-3 PUFAs, vitamin D, folate and iodine) but also environmental pollutants that have been shown to negatively influence foetal health,[41] which may have diluted the association between maternal fish intake during pregnancy and levels of angiogenic factors during pregnancy and in cord blood.

Dietary algorithms – Dietary acid load has been shown being able to influence acid-base balance in the body.[42] In addition, the dietary acid load calculations net endogenous acid production (NEAP) and dietary potential renal acid load (dPRAL) have been validated against urine pH and renal acid excretion in adults.[43,44] However, these dietary algorithms have not yet been validated in pregnancy. One may assume that due to hemodynamic adaptations and changes in the renal system during pregnancy,[45] the relation between these algorithms and urine pH and renal acid excretion may be different during gestation.

Overall diet – Overall diet can be evaluated using *a posteriori*-derived dietary patterns (such as dietary patterns derived from principal component analysis) and *a priori*-defined dietary patterns (such as the Dutch Healthy Diet-index[46]). Dietary pattern analysis takes into account the correlations between foods groups.[47] It may also detect stronger effects than the effects of individual food items, although opposite effects of food items within a dietary pattern may not be identified. *A posteriori*-derived dietary patterns are population specific but due to food group selection, dietary patterns identified within a population may differ. For example, different *a posteriori*-derived dietary patterns have been derived in the Generation R Study due to differently selected food groups.[6,20] In addition to the *a posteriori*-derived dietary patterns, we also used an adapted version of the Dutch Healthy Diet-index.[46] This *a priori*-defined dietary pattern was based on the Dutch recommendations for a healthy

diet. We have adapted the Dutch Healthy Diet-index for pregnancy by excluding the alcohol component, however it has not been studied whether this index sufficiently covers the dietary requirements in pregnancy. For example, it is likely that the cut-off values of recommended vegetable and fruit intake may be too low because of increased requirements of folate and other vitamins for optimal foetal health.[48]

Nutrition biomarkers – For the projects described in **Chapter 3** we used maternal biomarkers of dietary intake, namely plasma fatty acid and vitamin B12 levels, which are concentration biomarkers.[28] Concentration biomarkers are related to dietary intake but do not refer to absolute dietary intake.[28] For example plasma fatty acid levels are not only a reflection of dietary intake of these fatty acids, but also of absorption, fatty acid precursors, metabolism, and of elongation and desaturation enzymes amongst others.[28] Second, nutrition biomarkers may still be subject to residual confounding by other dietary factors. For example, vitamin B12 is found in animal-derived food products and may therefore be a marker of other nutrients in animal products such as protein, or iron. Furthermore, vitamin B12 may also be a reflection of other factors, because socioeconomic factors have been associated with vitamin B12 levels in observational studies.[49] Therefore, it may be argued that vitamin B12 may be a risk marker for preterm birth, but may not be causally related to preterm birth. At last, although dietary biomarkers may be less prone to measurement error than self-reported measures of dietary intake, for example the average coefficient of variation was 15.7% for plasma fatty acids, suggesting some misclassification of these biomarkers. The latter may be assumed to be non-differential misclassification that may have led to bias towards the null.

Gestational weight gain

To measure GWG in the Generation R Study we used measured weights during different gestational ages and we used self-reported pre-pregnancy weight and maximum weight in pregnancy. However, self-reporting weight is prone to measurement error which has been shown to be predominantly an underestimation of true weight, particularly in overweight and obese women.[50] Nonetheless, results of the Bland Altman plots did not indicate systematic measurement error of self-reported pre-pregnancy weight in the Generation R Study. Consequently, random measurement error of pre-pregnancy weight will most likely have resulted in loss of precision.

We used several methods to quantify gestational weight gain (e.g., weekly GWG, development of gestational weight during pregnancy, and adequacy of GWG). This allowed us to evaluate different aspects of GWG and to assess differential influences of maternal nutrition during pregnancy on period-specific GWG, because some studies have suggested that early GWG is already associated with adverse pregnancy and birth outcomes including the occurrence of gestational diabetes and large-for-gestational-age newborns.[51,52]

The development of adverse pregnancy and birth outcomes might be influenced by the extent to which maternal components, such as body fat and intravascular volume, contribute to GWG. Unfortunately, it is difficult to adequately measure change in body composition during pregnancy and to make a distinction between maternal and foetal components.[53]

Therefore, our results on diet and GWG preclude conclusions on specific components of GWG, such as gain in maternal fat mass.

Confounding and causality

The majority of the results presented in this thesis attenuated after taking into account maternal lifestyle factors and sociodemographic factors. This could be expected because several lifestyle factors have been found to cluster[54,55] and are strongly associated with sociodemographic factors.[56] Data on physical activity and sedentary behaviours during pregnancy was unfortunately not collected for the Generation R Study and this could have resulted in residual confounding in for example the association between maternal diet and GWG. Furthermore, information on maternal smoking and alcohol use was collected using questionnaires. This may have resulted in misclassification of these lifestyle factors that may have led to residual confounding.[57]

Since our results were predominantly derived from observational studies, the establishment of causality between the exposures and outcomes cannot be studied in this thesis. For example, we found a strong association between maternal vitamin B12 levels in pregnancy and preterm birth after adjustment for several confounding factors. However, both exposure and outcome are influenced by adiposity, dietary intake, sociodemographic factors, and other factors that may be causally related to the outcome. As alternative to an intervention study, causality could be explored by performing a Mendelian randomization of vitamin B12 with preterm birth.[58,59] Also, causality between macronutrient intake and GWG may also be explored using Mendelian randomization since several common genetic variants have been associated with carbohydrate and fat intake.[60]

Implications for public health and suggestions for future research

Pregnancy is a life changing event for a woman and her family and is characterized by ample metabolic, physiological and psychological changes, which all together frame a potential window of opportunity to evaluate health status and improve and maintain health through the implementation of dietary and lifestyle changes and adequate monitoring. The studies presented in this thesis suggest that dietary intake during pregnancy may influence maternal adaptation to pregnancy, birth outcomes, and child health but this depends on the type of outcome and dietary exposure. Although the effect sizes that we found were small, they can be relevant on a population level.

Gestational weight gain

Gestational weight gain is the sum of weight gained in multiple maternal and fetal components and can be calculated by subtracting pre-pregnancy weight from weight at delivery and can thus easily be calculated. For interpretation of absolute GWG, it is important to take into account the gestational age at measurement and the maternal BMI before pregnancy, because GWG increases with gestational age and will be higher in women

with lower pre-pregnancy BMI.[4,61] Currently, women in the Netherlands are not routinely weighed during pregnancy and they do also not receive weight targets during antenatal care. Nonetheless, it may be beneficial to routinely monitor GWG in practice and to advise women on GWG since the majority of women in the Generation R Study gained weight outside the GWG recommendations (i.e. inadequate or excessive GWG).[4] We also observed that more overweight and obese women gained weight outside the recommendations than women with a normal weight. Before regular weighing and GWG targets should be implemented in the Dutch antenatal care system, an intervention study should first evaluate whether regular weighing and advise on GWG reduces the occurrence of excessive GWG as well as subsequently adverse pregnancy and birth outcomes.[62] The recommendations that we currently use have been designed by the US Institute of Medicine,[4] therefore, a study should evaluate whether these US recommendations are also associated with the lowest risk of adverse pregnancy, birth and child health outcomes in a Dutch population of pregnant women.

Excessive GWG in early pregnancy is a strong predictor for excessive total GWG. Hence, it may be sensible to enrol women with early excessive GWG in an intervention program to limit further excessive GWG. Intervention studies targeting maternal diet and physical activity to limit GWG have been successful in reducing total GWG, although no differences were reported on pregnancy outcomes as yet.[63]

Not all women in the Netherlands receive information preconceptionally or in early pregnancy regarding gestational weight gain, physical activity and quality of dietary intake. This could be initiated by the health care professional but women may also receive electronic Health (eHealth) or mobile Health (mHealth) to stimulate adherence to a healthy lifestyle throughout pregnancy.[64] However, effectiveness of such interventions in reducing adverse outcomes needs further study.

Maternal dietary intake during pregnancy

Women are recommended to slightly increase their energy intake during the second and third trimester of pregnancy.[48,65] These recommendations are based on a gradual increase of basal metabolic rate, but they may not sufficiently account for a decrease in physical activity that may occur in pregnancy.[66] In a summary of previous research we found that women with higher energy intake gained more weight during pregnancy (**Chapter 2.1**). These results imply that it may not be recommended for women to largely increase their energy intake during pregnancy. Further studies should establish optimal energy intake dependent on basal metabolic rate as well as physical activity levels of pregnant women.

The results on adherence to the Dutch recommendation for a healthy diet that were presented in this thesis suggest that maternal diet in pregnancy was suboptimal. When we further evaluated the adherence to the individual items of the Dutch Healthy Diet index, we found that none of the women had a maximum score on the Dutch Healthy Diet index, which includes amongst others items on fruit, vegetable, and fish and intake.

Consequently, the current intake of micronutrients during pregnancy may be inadequate for optimal foetal development. Moreover, it has been shown in some studies that women may not be aware that their diet is suboptimal.[67,68] Accordingly, counselling before and during

pregnancy to optimize dietary quality is paramount. Effectiveness of dietary counselling could be evaluated in a cluster-randomized RCT in which midwife practices are randomized to either intervention (e.g., counselling of pregnant women to adhere to the Dutch dietary recommendations) or to routine care (e.g., no counselling). In both groups dietary quality and dietary quantity should be monitored and information on pregnancy and birth outcomes should be collected.

Biomarkers

Vitamin-B12 deficiency during pregnancy is prevalent, affecting up to 69% of the women in one of the studies included in the IPD meta-analysis (**Chapter 3.3**).[69] This percentage may be lowered by supplementing women with vitamin B12 during pregnancy.[70,71] Whether vitamin B12 supplementation reduces the risk of preterm birth, is not known yet. However, given the fact that vitamin B12 deficiency is common in pregnancy, pregnant women may benefit from early diagnosis and supplementation. In addition, future studies should evaluate causality of the association between vitamin B12 and preterm birth by performing a Mendelian randomization or a randomized placebo-controlled designed study. Alternatively, vitamin B12 levels may be used to identify women at risk for preterm delivery as a primary preventive strategy. For example, women with vitamin-B12 deficiency may receive during pregnancy more information on early signs and symptoms of preterm labour and extra ultrasound examinations may be performed to detect shortening of cervical length than women with adequate vitamin B12 levels.

Dietary acid load and fatty acid patterns have not been used previously in relation to pregnancy outcomes. Also, the reproducibility of both measures in pregnancy has yet to be explored in future studies. In addition to dietary assessment, pregnant women may collect 24-hour urine samples in future studies to measure urinary pH as well as urinary nitrogen and urinary potassium as indicators of acid load to clarify whether dietary acid load may be important in blood pressure development and pregnancy-induced hypertensive disorders. Also, the stability of other maternal biomarkers such as plasma fatty acid patterns should be evaluated by measuring fatty acids at multiple time points during pregnancy to assess potential associations more accurately.

Child health

We evaluated the associations between maternal diet during pregnancy with offspring cardiometabolic health and body composition at the age of 6 years. At this age, the variation in cardiometabolic markers and body composition may not be large and range within a normal spectrum. This may explain why we did not find consistent associations of maternal diet with children's cardiometabolic health. It has been suggested that cardiometabolic markers track from childhood to adulthood.[72] Differences may therefore appear at later ages when risk markers become more variable between individuals. To further explore the association between maternal diet during pregnancy and children's health outcomes, measurements should be repeated during multiple follow-up visits within the Generation R Study as well as in other cohort studies.

Foetal programming may explain the associations that we found between maternal

diet and child health outcomes (**Chapter 4.1**).[18] Alternatively, these associations might be explained by shared environment and lifestyle factors rather than due to intra-uterine dietary exposure. To further explore this, paternal dietary factors could be evaluated additionally to maternal dietary factors in the association with child health outcomes. Alternatively, a randomized controlled trial with long-term follow-up of the offspring may be a better method to evaluate causal effects of intra-uterine dietary exposure on child health outcomes in a well-nourished population. However, if a randomized controlled trial is considered to evaluate the effect of diets with different protein concentrations during pregnancy on child body composition, such a study should be carefully designed since the Harlem trial reported increased preterm births and neonatal deaths in women who received high protein supplementation.[73]

The results presented in **Chapter 4.1** suggest that BMI may not be a good indication for adiposity in all children, because no distinction can be made between fat-free mass and fat mass.[74] Consequently, children may be incorrectly classified as being adipose based on their BMI. In clinical practice BMI is still used in children because it is easy to use and inexpensive. However, other measures that make a distinction of fat mass and fat-free mass or fat mass distribution such as dual-energy X-ray absorptiometry (DXA), skinfold thickness and waist circumference could be considered in order to evaluate a child's nutritional status more in detail.[75]

Main conclusion

The results presented in this thesis provide insight in several aspects of maternal diet in a well-nourished population and its association with health outcomes in women and their children. More specifically, we observed that higher energy intake, some specific dietary patterns and plasma fatty acid patterns were associated with gestational weight gain. In addition, maternal vitamin B12 levels were associated with pre-term birth and children of women with high protein intake had a higher body mass index and fat-free mass index at the age of 6 years. Our results also imply that sociodemographic and lifestyle factors play an important role and partly explain some associations between maternal diet and health outcomes. Our results highlight that majority of women have an excessive or inadequate gestational weight gain and that maternal diet during pregnancy is suboptimal.

Therefore, our findings can be important for primary and secondary prevention strategies highlighting the need of identification of women at risk for suboptimal health outcomes in themselves and their children. This can be done by monitoring maternal weight, dietary intake and lifestyle during pregnancy.

To further unravel the role of maternal diet during pregnancy in maternal and child health, future studies on diet during pregnancy should focus on dietary quality as well as on the effects of diet modification during pregnancy in a controlled setting. In addition to this, future studies should require more detailed and repeatedly measured exposures, confounders, and outcomes in women and their children. Finally, pregnant women should be advised not to gain excessive gestational weight and 'Eating for Two' should refer to a nutrient-dense diet and not to an energy-dense diet.

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Chapter 6

Summary & Samenvatting

Summary

Adverse pregnancy and birth outcomes such as pre-eclampsia and preterm birth are prevalent worldwide and are important causes of maternal and perinatal mortality and morbidity. To reduce the occurrence of these adverse outcomes, risk factors should be identified that could be modified in pregnancy. Such modifiable risk factors may be maternal nutrition and gestational weight gain. However, not all aspects of maternal diet during pregnancy have been studied in relation with pregnancy and birth outcomes. In addition, previous studies have reported conflicting findings. Maternal nutrition during pregnancy can also affect health of the child later in life, although these effects have been predominantly shown in malnourished populations. Evidence on the association between maternal diet during pregnancy in well-nourished populations and outcomes regarding child health has been conflicting and requires future study. The aim of this thesis was to gain more insight into the role of maternal nutrition during pregnancy on health outcomes of women and their children (**Chapter 1**). Thereby, we summarized current scientific literature in systematic reviews and we performed several observational studies that were embedded in the Generation R Study, an ongoing prospective population-based birth cohort in the city of Rotterdam.

Chapter 2 describes one systematic review and two prospective cohort studies related to the association between maternal dietary intake during pregnancy and gestational weight gain, the development of maternal blood pressure and hypertensive complications in pregnancy. In **Chapter 2.1**, we studied the association of energy and macronutrient intake during pregnancy with gestational weight gain. We collected all published scientific literature on this subject until August 2015. Fifty-six relevant articles were identified, which were summarized in a systematic review. The results from this review provided evidence that higher energy intake in pregnancy is related with higher gestational weight gain. However, only 11 out of 56 studies that we included were of high quality and the roles of individual macronutrients in gestational weight gain remained unclear and may depend on the specific type of macronutrient studied. In **Chapter 2.2**, we focused on the relation between maternal dietary patterns and gestational weight gain. We studied four dietary patterns, namely 1) a Dutch Healthy Diet-index' pattern, 2) a 'Vegetable, oil and fish' pattern, 3) a 'Nuts, high-fiber cereals and soy' pattern, and 4) a 'Margarine, sugar and snacks' pattern. The 'Dutch Healthy Diet-index' was based on Dutch recommendations for a healthy diet (an *a priori-defined* dietary pattern). The three other dietary patterns were identified in the Generation R Study using principal component analysis (*a posteriori-derived* dietary patterns). The results from this study suggested that the 'Vegetable, oil and fish' pattern may increase gestational weight gain in early pregnancy but has no influence on gestational weight gain later in pregnancy. Our results also suggest that the other dietary patterns seem to have limited influence on gestational weight gain. In **Chapter 2.3**, we evaluated the relation between maternal dietary acid load and changes in blood pressure and hypertensive complications in pregnancy. Our results showed that maternal dietary acid load was not consistently associated with a change in blood pressure during pregnancy after taking into account maternal lifestyle and sociodemographic factors. However, we did find that women with a higher vegetable protein/potassium ratio had a slightly lower diastolic blood pressure. Dietary acid load had

no role in the occurrence of hypertensive complications in pregnancy.

Chapter 3 contains two prospective cohort studies and one individual participant data meta-analysis. These studies evaluated the association of maternal nutritional biomarkers in pregnancy with circulating angiogenic factors, gestational weight gain, hypertensive complications in pregnancy and birth outcomes. More specifically in **Chapter 3.1**, we studied the associations of maternal fish consumption and polyunsaturated fatty acids (PUFAs) in pregnancy with circulating angiogenic factors in Dutch pregnant women. We observed no consistent relation between fish consumption and angiogenic factors in pregnancy and cord blood. Similarly, circulating PUFAs were also not consistently associated with the angiogenic factors. In **Chapter 3.2**, we studied the associations of maternal fatty acid patterns with gestational weight gain and the occurrence of hypertensive complications in pregnancy. Three maternal fatty acid patterns were identified namely 1) a pattern that was high in omega-6 PUFAs, 2) a pattern that consisted of monounsaturated fatty acids and saturated fatty acids, and 3) a pattern that was high in omega-3 PUFAs. The patterns that were high in omega-6 PUFAs and omega-3 PUFAs were associated with gestational weight gain, whereas the pattern that was characterized by monounsaturated fatty acids and saturated fatty acids was not related with gestational weight gain. No fatty acid pattern increased the occurrence of hypertensive complications in pregnancy. In **Chapter 3.3**, we evaluated the associations of maternal vitamin B12 concentrations in pregnancy with birth outcomes in an individual participant data meta-analysis. We included 18 cohort studies, with in total more than 11,000 participants. We found that vitamin B12 concentrations in maternal blood were not associated with birth weight. Nevertheless, pregnant women with high vitamin B12 levels had lower occurrence of preterm birth.

In **Chapter 4**, we assessed the associations of maternal dietary intake during pregnancy with children's cardiometabolic health and body composition at school age in the Generation R Study. In **Chapter 4.1**, we studied the relation between maternal protein intake in pregnancy and childhood body composition at the age of 6 years. The results of this study suggested that higher protein intake during pregnancy is associated with higher children's weight at the age of 6 years. However, this higher weight was due to a higher children's fat-free mass, and not due to a higher fat mass. Children of mothers with high protein intake in pregnancy did not have more often overweight than children of mothers with lower protein intake. We found similar results for protein from animal and vegetable sources. In **Chapter 4.2**, we assessed the association between maternal dietary patterns during pregnancy and children's cardiometabolic health at the age of 6 years. Our results suggest that the associations between maternal dietary patterns during pregnancy and children's cardiometabolic health where explained by sociodemographic and lifestyle factors. This implies that maternal sociodemographic and lifestyle factors may be significant determinants of child health.

On the basis of this thesis it can be concluded that maternal nutrition during pregnancy seems to have a role in health outcomes of women and their children (**Chapter 5**). Furthermore, the results suggest that maternal sociodemographic and lifestyle factors explain part of the associations between maternal nutrition in pregnancy and health outcomes. The results of this thesis may be important for prevention of adverse health

outcomes in women and their children as well as for identification of pregnant women at risk for suboptimal pregnancy and birth outcomes. To further explore the role of maternal nutrition in pregnancy on maternal and child health, future studies should be of high quality with detailed assessments of maternal nutrition, health outcomes, and sociodemographic and lifestyle factors. In addition to this, studies should focus on the effects of dietary quality as well as on effects of dietary interventions in pregnancy to improve health outcomes in women and their children.

Samenvatting

Ongunstige zwangerschaps- en geboorte-uitkomsten, zoals pre-eclampsie ('zwangerschaps-vergiftiging') en vroeggeboorte, komen wereldwijd frequent voor en zijn belangrijke veroorzakers van morbiditeit en mortaliteit bij zwangere vrouwen en hun kinderen. Om het aantal ongunstige zwangerschaps- en geboorte-uitkomsten te kunnen reduceren, is het belangrijk dat er risicofactoren worden geïdentificeerd die tijdens de zwangerschap aangepast zouden kunnen worden. Voorbeelden van factoren die mogelijk van invloed zijn op zwangerschaps- en geboorte-uitkomsten zijn het voedingspatroon van de (toekomstige) moeder en de mate van gewichtstoename tijdens de zwangerschap. Eerdere studies hebben tot dusver tegenstrijdige resultaten gerapporteerd over de rol van maternale voeding op zwangerschaps- en geboorte-uitkomsten. De voeding van de zwangere lijkt ook de gezondheid van haar kind op latere leeftijd te kunnen beïnvloeden, al is dit verband voornamelijk gevonden in studies die in ondervoede populaties zijn uitgevoerd. Zo hadden kinderen van moeders die tijdens de hongerwinter in 1944 zwanger waren, op latere leeftijd vaker hart- en vaatziekten dan kinderen van moeders die net voor of na de hongerwinter zwanger waren. Studies in normaal gevoede populaties hebben echter minder consistente onderzoeksresultaten gevonden en daarom is verder onderzoek nodig. Het doel van dit proefschrift was om meer inzicht te krijgen wat de rol is van maternale voeding tijdens de zwangerschap in gezondheidsuitkomsten van zwangere vrouwen en hun nageslacht (**Hoofdstuk 1**). Om meer inzicht te krijgen, hebben we de wetenschappelijke literatuur samengevat in systematische literatuuronderzoeken; deze studies zijn te vinden in Hoofdstuk 2.1 en Hoofdstuk 3.3. Tevens hebben we studies uitgevoerd binnen het Generation R Onderzoek. Dit is een langlopende populatiestudie in de regio Rotterdam, waarbij zwangere vrouwen en hun kinderen vanaf de vroege zwangerschap worden gevolgd. Tijdens de zwangerschap vulden de vrouwen vragenlijsten in over onder andere hun voedingsgewoonten, foliumzuurgebruik, rookgedrag en alcohol consumptie. Daarnaast werd herhaaldelijk de foetale groei gemeten, evenals de maternale bloeddruk en het lichaamsgewicht. Ook werd er tijdens de zwangerschap meerdere malen bloed afgenomen waaruit onder andere concentraties van vetzuren, specifieke groeifactoren (zoals de angiogene factoren PlGF en sFlt-1) en vitamine B12 bepaald werden. Na de bevalling verzamelden we gegevens over de zwangerschapsduur, het geboortegewicht en het vóórkomen van zwangerschapshypertensie en pre-eclampsie. De kinderen bezochten op 6-jarige leeftijd het onderzoekscentrum van Generation R. Hier bepaalden we onder andere hun lichaamssamenstelling en bloeddruk, alsmede de cholesterol- en insulinewaarden in het bloed.

In **Hoofstuk 2** beschrijven we één systematisch literatuuronderzoek en twee studies die zijn uitgevoerd binnen het Generation R Onderzoek. We onderzochten in dit hoofdstuk de relatie tussen maternale voeding tijdens de zwangerschap en maternale gewichtstoename, maternale bloeddrukverandering en het ontstaan van zwangerschapshypertensie en pre-eclampsie. In **Hoofdstuk 2.1** bestudeerden we het verband tussen maternale inname van energie en macronutriënten (namelijk eiwitten, vetten en koolhydraten) tijdens de zwangerschap en de mate van maternale gewichtstoename. Hiervoor hebben we alle

wetenschappelijke literatuur over dit onderwerp verzameld. In totaal vonden we 56 artikelen. Op basis van de resultaten van deze studies konden we concluderen dat vrouwen met een hoge energie-inname tijdens de zwangerschap een grotere gewichtstoename hadden. We vonden geen duidelijk verband tussen de inname van eiwitten, vetten of koolhydraten en de hoeveelheid gewichtstoename in de zwangerschap. We vonden wel aanwijzingen dat de hoeveelheid gewichtstoename mogelijk afhankelijk is van het subtype van het macronutriënt; de inname van verzadigd vet is bijvoorbeeld mogelijk anders geassocieerd met de grootte van gewichtstoename dan de inname van meervoudig onverzadigd vet. De geïdentificeerde artikelen waren echter van relatief slechte kwaliteit, waarbij er bijvoorbeeld nauwelijks rekening werd gehouden met het lichaamsgewicht voor de zwangerschap en de lichaamsbeweging van de zwangeren; slechts 11 van de 56 artikelen waren van hoge kwaliteit. In **Hoofdstuk 2.2** onderzochten we de relatie tussen het voedingspatroon van de moeder en haar gewichtstoename tijdens de zwangerschap. We bestudeerden vier verschillende voedingspatronen, namelijk 1) een patroon dat gebaseerd is op de Nederlandse richtlijnen voor gezonde voeding uit 2006, 2) een patroon dat voornamelijk bestaat uit een hoge inname van groenten, olie en vis, 3) een patroon met voornamelijk consumptie van noten, vezelrijke ontbijtgranen en soja producten, en 4) een voedingspatroon gekarakteriseerd door een hoge inname van margarine, suikers en snacks. De laatste drie voedingspatronen hebben we geïdentificeerd op basis van de meest vóórkomende voedingsgewoonten van de Nederlandse vrouwen in het Generation R Onderzoek. De resultaten van deze studie suggereerden dat vrouwen met een voedingspatroon dat voornamelijk bestond uit groenten, olie en vis een grotere gewichtstoename hadden in de eerste 13 weken van de zwangerschap, maar niet later in de zwangerschap. Onze resultaten suggereerden daarnaast dat de andere voedingspatronen weinig invloed lijken te hebben op totale gewichtstoename in de zwangerschap. In **Hoofdstuk 2.3** bestudeerden we het verband tussen 'dietary acid load' van de zwangere en bloeddrukverandering tijdens de zwangerschap alsmede het optreden van zwangerschapscomplicaties, zoals hypertensie en pre-eclampsie. 'Dietary acid load' weerspiegelt de verhouding tussen de inname van zuurvormende voedingsstoffen (zoals bijvoorbeeld dierlijke eiwitten) en de inname van alkalische voedingsstoffen waaronder inname van kalium, magnesium en calcium. Er is gesuggereerd dat de 'dietary acid load' de zuurbase balans in het lichaam kan beïnvloeden en deze zowel kan verhogen als verlagen. Onze resultaten lieten zien dat de 'dietary acid load' tijdens de zwangerschap geen consequent verband vertoonde met bloeddrukverandering tijdens de zwangerschap, nadat we hadden gecorrigeerd voor verschillen in maternale leefstijl en sociaaleconomische factoren. We vonden wel dat vrouwen met een hoge ratio tussen inname van plantaardige eiwitten en kalium inname een iets lagere diastolische bloeddruk hadden dan vrouwen met een lagere ratio. Wij vonden geen verband tussen 'dietary acid load' en het ontstaan van zwangerschapshypertensie of pre-eclampsie.

Hoofdstuk 3 bestaat uit twee studies die binnen het Generation R Onderzoek zijn uitgevoerd en uit één systematisch literatuur onderzoek. In dit hoofdstuk onderzochten we het verband tussen bloedconcentraties van voedingsparameters en groeifactoren, maternale gewichtstoename, zwangerschapshypertensie, pre-eclampsie en geboorte-uitkomsten. In

Hoofdstuk 3.1 hebben we binnen het Generation R Onderzoek het verband bestudeerd tussen maternale vis inname en de groeifactoren PlGF en sFlt-1. Tevens onderzochten we het verband tussen bloedconcentraties van meervoudig onverzadigde vetten (vetzuren) tijdens de zwangerschap en de genoemde groeifactoren. Deze groeifactoren spelen een belangrijke rol bij de vorming van bloedvaten. We vonden geen consistent verband tussen maternale vis consumptie en concentraties van groeifactoren tijdens de zwangerschap. We observeerden ook geen consistent verband tussen bloedconcentraties van meervoudig onverzadigde vetten en de groeifactoren. **Hoofdstuk 3.2** beschrijft een studie waarbij de verbanden tussen vetzuurpatronen in het maternale bloed en gewichtstoename in de zwangerschap worden onderzocht, evenals het ontstaan van zwangerschapshypertensie en pre-eclampsie. Allereerst identificeerden we drie vetzuurpatronen in het maternale bloed, namelijk 1) een vetzuurpatroon gekarakteriseerd door omega-6 meervoudig onverzadigde vetten, 2) een vetzuurpatroon dat voornamelijk bestaat uit enkelvoudig onverzadigde vetten en verzadigde vetten, en 3) een vetzuurpatroon dat gekarakteriseerd wordt door omega-3 meervoudig onverzadigde vetten. De resultaten van dit onderzoek lieten zien dat er een verband was tussen de vetzuurpatronen die gekarakteriseerd werden door omega-6 en omega-3 meervoudig onverzadigde vetten en gewichtstoename tijdens de zwangerschap. We vonden geen verband tussen het vetzuurpatroon van enkelvoudig onverzadigde vetten en verzadigde vetten en gewichtstoename tijdens de zwangerschap. We vonden ook geen verband tussen de drie vetzuurpatronen en het ontstaan van zwangerschapshypertensie of pre-eclampsie. In **Hoofdstuk 3.3** onderzochten we het verband tussen vitamine B12 concentraties tijdens de zwangerschap en zwangerschaps- en geboorte-uitkomsten in een systematisch literatuuronderzoek. Voor deze studie combineerden we de gegevens van deelnemers aan 18 verschillende cohort studies, waardoor we in totaal de gegevens van meer dan 11.000 zwangeren hadden onderzoeken. We vonden geen verband tussen vitamine B12 concentraties tijdens de zwangerschap en geboortegewicht. Wel vonden we dat vrouwen met hoge vitamine B12 concentraties tijdens de zwangerschap minder vaak een vroeggeboorte hadden dan vrouwen met lage vitamine B12 concentraties.

Hoofdstuk 4 bestaat uit twee studies die zijn uitgevoerd binnen het Generation R Onderzoek, waarin we het verband onderzochten tussen voeding van de moeder tijdens de zwangerschap en gezondheidsuitkomsten van de kinderen op 6-jarige leeftijd. In **Hoofdstuk 4.1** bestudeerden we het verband tussen eiwitinname van de zwangeren en de lichaamssamenstelling van hun kinderen rond 6-jarige leeftijd. De resultaten lieten zien dat de kinderen van moeders met een hoge eiwitinname tijdens de zwangerschap zwaarder waren dan kinderen van moeders met een lage eiwitinname. Als we echter keken naar verschillen in lichaamssamenstelling van deze kinderen, zagen we dat dit hogere gewicht kwam door een hogere vetvrije massa en dat er geen was verschil in vetmassa. Tevens zagen we geen verschil in het vóórkomen van overgewicht tussen kinderen van moeders met een hoge of met een lage eiwitinname tijdens de zwangerschap. We vonden geen verschillen tussen dierlijke of plantaardige eiwitten. In **Hoofdstuk 4.2** onderzochten we of de eerder besproken voedingspatronen tijdens de zwangerschap van invloed kunnen zijn op de cardiometabole gezondheid van kinderen op 6-jarige leeftijd. Voor het bepalen van

de cardiometabole gezondheid bestudeerden we onder andere verschillen in bloeddruk, cholesterol en insuline van de kinderen. Onze resultaten lieten zien dat de verbanden tussen voedingspatronen tijdens de zwangerschap en de cardiometabole gezondheid van kinderen grotendeels verklaard werden door verschillen in maternale leefstijl zoals roken en foliumzuur gebruik tijdens de zwangerschap en sociaaleconomische positie. Deze bevinding suggereert dat maternale leefstijl en sociaaleconomische positie belangrijke factoren zijn voor de gezondheid van kinderen.

Op basis van dit proefschrift kan geconcludeerd worden dat de voeding van zwangeren een rol kan spelen in gezondheidsuitkomsten van zwangere vrouwen en hun kinderen (**Hoofdstuk 5**). De resultaten suggereren wel dat het verband tussen maternale voeding en gezondheidsuitkomsten zoals bijvoorbeeld verandering van bloeddruk in de zwangerschap en cardiometabole gezondheid van kinderen deels verklaard wordt door de leefstijlfactoren en sociaaleconomische positie van de zwangere vrouw. De resultaten van dit proefschrift zijn belangrijk om ongunstige zwangerschaps- en geboorte-uitkomsten te verminderen. Daarnaast kunnen onze resultaten mogelijk bijdragen aan een betere identificatie van zwangeren die een verhoogd risico hebben op ongunstige zwangerschaps- en geboorte-uitkomsten, door bijvoorbeeld vrouwen met lage vitamine B12 concentraties frequenter poliklinisch te controleren. Om de rol van maternale voeding tijdens de zwangerschap in gezondheidsuitkomsten van zwangere vrouwen en hun kinderen nog beter te begrijpen, moeten toekomstige observationele en interventie studies van hoge kwaliteit zijn en uitgevoerd worden met gedetailleerde metingen van maternale voeding, gezondheidsuitkomsten, sociaaleconomische positie en leefstijl factoren. Daarnaast zouden toekomstige studies gericht moeten zijn op de kwaliteit van de voeding. Tevens zouden interventie studies het effect moeten bestuderen van veranderingen van maternale voeding tijdens de zwangerschap op gezondheidsuitkomsten in vrouwen en hun kinderen.

Chapter 7

Appendices

I. Abbreviations
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I. Abbreviations

24hR	24-hour recall
AA	arachidonic acid
ALA	alpha-linolenic acid
ANOVA	analysis of variance
B12	vitamin B12
BF%	body fat percentage
BMI	body mass index
CI	confidence interval
DBP	diastolic blood pressure
df	degrees of freedom
DHA	docosahexaenoic acid
DHD-index	Dutch Healthy Diet-index
dPRAL	dietary potential renal acid load
DXA	dual-energy X-ray absorptiometry
E%	energy percent
EPA	eicosapentaenoic acid
FA	fatty acid
FMI	fat mass index
FFMI	fat-free mass index
FFQ	food-frequency questionnaire
FR	food record
GI	glycemic index
GWG	gestational weight gain
HDL-c	HDL cholesterol
IPD	individual participant data
IQR	interquartile range
LA	linoleic acid
LBW	low birth weight

MUFA	monounsaturated fatty acids
n	omega
n	number of participants
NA	not available
NEAP	net endogenous acid load
NR	not reported
OR	odds ratio
PCA	principle component analysis
PCB	polychlorinated biphenyls
PlGF	placental growth factor
pp	postpartum
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PUFA	polyunsaturated fatty acid
PWV	pulse wave velocity
Q	quartile
RCT	randomized controlled trial
RR	risk ratio
SBP	systolic blood pressure
SD	standard deviation
SDS	standard deviation score
SFA	saturated fatty acid
sFlt-1	soluble fms-like tyrosine kinase-1
SGA	small-for-gestational-age
SR	self-reported
suppl	supplementation
T	trimester or pregnancy
TFA	*trans*-fat
VEGF	vascular endothelial growth factor
Vi	vitamin supplementation
y	year

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III. Publications and manuscripts

1. Bautista Niño PK*, **Tielemans MJ***, Schalekamp-Timmermans S, Steenweg-de Graaff J, Hofman A, Tiemeier H, Jaddoe VWV, Steegers EAP, Felix JF, Franco OH. Maternal fish consumption, fatty acid levels and angiogenic factors: The Generation R Study. *Placenta, 2015 Oct; 36(10):1178-84*

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IV. About the author

Myrte Janien Tielemans was born on the 14th of November 1985 in Helvoirt, the Netherlands. She obtained her gymnasium diploma at Gymnasium Beekvliet, Sint-Michielsgestel, in 2004. Subsequently she started her medical education at Maastricht University, in the eponymous city of Maastricht. She received her Master of Science degree in Medicine in 2010, after which she worked as a resident in Obstetrics and Gynaecology (ANIOS) at the Orbis Medical Center in Sittard. Myrte started her PhD program in 2012 at the Department of Epidemiology and the Department of Obstetrics and Gynaecology, Erasmus MC in Rotterdam. She was supervised by prof.dr. Oscar Franco, prof.dr. Eric Steegers, dr. Jessica Kiefte-de Jong, and dr. Janine Felix. Her research was performed within ErasmusAGE, an academic center for aging research across the life course, and included systematic reviews of the literature, as well as original data studies with data from the Generation R Study, a large population-based cohort study from foetal life onwards. This work resulted in her PhD thesis entitled: 'Eating for Two in Pregnancy: Health outcomes in pregnant women and their children'. During her PhD program, Myrte completed a postgraduate Master of Science in Clinical Epidemiology at the Netherlands Institute for Health Sciences (2015). She also received the Juriy Wladimiroff Research Award 2015 that was awarded by the Department of Gynaecology and Obstetrics of the Erasmus MC. In January 2016, Myrte started her residency in Medical Microbiology (AIOS) at the Department of Medical Microbiology and Infectious Diseases, Erasmus MC in Rotterdam.

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Master of Science in Health Sciences, Clinical Epidemiology		
Study design	2013	4.3
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Clinical epidemiology	2014	5.7
Methodologic topics in epidemiologic research	2013	1.4
Biostatistical methods II: Classical regression models	2013	4.3
Principles of research in medicine	2013	0.7
Clinical decision analysis	2013	0.7
Methods of public health research	2013	0.7
Health economics	2013	0.7
Markers of prognostic research	2013	0.7
The practice of epidemiologic analysis	2013	0.7
Nutrition & physical activity (University of Cambridge, UK)	2013	1.4
Maternal and child health	2013	0.9
Women's health	2013	0.9
Intervention research and clinical trials	2014	0.9
History of epidemiologic ideas	2014	0.7
Logistic regression	2014	1.4
Advanced medical writing and editing	2014	0.7
Courses for the quantitative researcher	2014	1.4
Pharmaco-epidemiology	2015	0.7
Repeated measurements in clinical studies	2015	1.4
Missing values in clinical research	2015	0.7
Causal inference	2015	0.7
General academic skills		
Endnote, Medical Library, Erasmus MC	2012	0.3
Systematic literature retrieval, Medical Library, Erasmus MC	2012	0.6

	Year	Workload (ECTS)
Basic introduction to SPSS, Molecular Medicine, Erasmus MC	2013	0.5
Integrity in scientific research course, Erasmus MC	2013	2.0
Radiation hygiene and protection level 5R, Erasmus MC	2013	0.7
Didactic skills training, Eduplaza, Erasmus MC	2014	0.5
Biomedical English writing and Communication, Erasmus MC	2014	2.0
Seminars and workshops		
Workshop Nutritional Epidemiology, ErasmusAGE	2012	0.6
Seminars at the department of Epidemiology, Erasmus MC	2012-2015	1.0
2020 Epidemiology meetings, Erasmus MC	2012-2015	1.0
ErasmusAGE research meetings, Erasmus MC	2012-2015	1.0
Generation R growth and development meetings	2012-2015	1.0
Generation R research meetings, Erasmus MC	2012-2015	0.5
Nutritional Epidemiology research meetings (SIGN-E), Erasmus MC	2012-2015	0.5
PhD day, Erasmus MC	2013	0.3
Meeting WeVo (werkgroep voedingsgewoonten), Utrecht	2014	0.3
NAV Public lecture: '*Voedingsonderzoek op een tweesprong; voedingsstoffen of voedingsmiddelen*?', Utrecht	2015	0.3
Presentations and attended conferences		
Developmental Origins of Health and Disease (DOHaD), Satellite Meeting, Rotterdam	2012	0.6
NWO Nutritional Science Days, Deurne *Oral presentation*	2014	1.0
Diabetes in Pregnancy (DIB) congress, Berlin *Poster presentation*	2015	2.0
Annual Juriy Wladimiroff Research meeting, Erasmus MC *Oral presentation*	2015	1.0
European Congress of Epidemiology, Maastricht *Poster presentation*	2015	1.0
European Nutrition Conference (FENS), Berlin *Poster presentation*	2015	1.0

	Years	Workload (ECTS)
Other		
Peer review of articles for scientific journals: *British Journal of Nutrition, Reproductive Health, and BJOG*	2014-2016	0.5

2. Teaching

Teaching		
Supervising working groups Principles of research medicine and epidemiology, NIHES	2015	0.5
Lectures on pregnancy and subsequent women's health in the course Maternal and child health, Leiden University College, The Hague	2015	1.0
Supervising students		
Paula Bautista Niño, MSc thesis Epidemiology, NIHES Project title: '*First-trimester maternal fish consumption and plasma levels of angiogenic factors during pregnancy: The Generation R study*'	2013	2.0
Lotte Beekenkamp, BSc student, Leiden University College Project title: '*Single parenthood and composition of dietary intake during pregnancy: The Generation R Study*'	2015	1.0
Vincent Jen, medical student Erasmus MC Project title: '*Intake of sugar-containing beverages during early pregnancy and offspring's birth weight and body composition at the age of 6 years*'	2015	2.0

VI. Dankwoord

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